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Ultrasonographic Appearance and Echo-pattern Characterization of Donkeys' Internal Reproductive Organs

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ABSTRACT

The current study was achieved to designate the accessory sex glands, ampullae and urethra of 25 healthy male donkeys using the ultrasonography. Animals were classified according to their age into young peri-mature (i.e. ≤2 years; n=7) and old mature (>3 years; n=18). The examination was done per rectum by using Magic 2200 scanner with a 6 MHz linear transrectal transducer. The position of each gland was allocated, and length and width were measured. The dimensions of prostate gland lobes and ampulla ductus deferens significantly differed between the examined groups. The echo-pattern analysis of vesicular gland and ampulla ductus deferens, in terms of mean pixel value (MPV), pixel intensity (PI) and pixel number (PN), significantly differed between premature and mature donkeys. Age was significantly correlated with all sex glands measurements. There was a substantial confident relationship between MPV and PI and the dimensions of vesicular and ampulla ductus deferens. In the meantime, PN was clearly negatively correlated with vesicular gland length and width. In conclusions, ultrasound is a feasible non-invasive diagnostic tool that could be used to characterize the accessory glands along the course of the pelvic urethra of male donkeys reared in Egypt. The addressed measures as well as acoustic physiognomies of the secondary sex organs are considered a promising guide in fertility potential prediction and/or the discrimination of the pathological conditions of male reproductive organs.

Introduction

Donkeys are still well deserving of the name 'beasts of burden', despite the increase in mechanization throughout the world (Pearon *et al*., 1999). In Egypt, donkeys are used mainly in working life, either for carrying loads, riding and cultivations due to its high disease resistance, and cheap to purchase and to care. Although national estimates of donkey populations are seldom accurate, they illustrate broad trends. The world donkeys´ population is estimated to be 44 million; most of them are maintained for work (Starkey and Fielding, 1997).

The jack (male donkey) has many reproductive similarities to the horse. The reproductive organs of a full-size donkey are similar to that of the full-size horse. Although some seasonal differences in libido are observed, there seems to be little (only for semen pH) or no alteration in seminal parameters during the winter months (Gastal *et al*., 1997).

In donkeys, the accessory genital glands, a series of glands present in between the vas deferens and the root of the penis, are represented with ampulla ductus deference, vesicular, prostate, bulbourethral and urethral glands (Abou-Elhamd *et al*., 2013). They are responsible for the secretion of the seminal plasma, which provides the substrate for conveying the sperm to the female and ensuring final maturation (Morel, 2008).

The accessory genital glands were extensively investigated in different animals, including stallion (Weber and Woods, 1993), bull (El-Khawaga *et al*., 2012), and boar (Clark and Althouse, 2002), with little attention has been paid to those of male donkey (Abou-Elhamd *et al*., 2013). Abou-Elhamd (2005) verified that the accessory genital glands of donkeys are active all over the year, but this activity was more pronounced during spring, and this activity decreases gradually during the summer and autumn seasons to reach its minimal level during winter.

Although ultrasound evaluation of the reproductive tract of stallions was introduced to veterinary practice long ago, this examination is not conducted in a similar way in the donkeys. The anatomical relationships, physical dimensions and ultrasound appearances of the stallion's accessory genital organs were accurately represented by transrectal ultrasonography (Little and Woods, 1987). In order for the veterinarians to identify pathological conditions, the normal architecture of

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the accessory sex glands needs to be described. To the authors' knowledge, there is no ultrasonographic description of the practices and/or characterization of jack accessory genital glands. Therefore, the present investigation was performed to provide a basic knowledge for the purpose of establishing clinical reference values of the different accessory sex glands based on ultrasound topographic and anatomical features in donkeys. In the meantime, verifying the effect of age approaching (young) or surpassing maturity (old) on the accessory sex gland biometry in jacks.

Materials and methods

Animals

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 present study during the period between September to November 2015. Animals were categorized into two aged groups: young peri-mature (≤ 2 years, n=7) and old mature (> 3 years, n=18) groups according to Moustafa *et al*. (2015). Animals´ age was dictated by the appearance of specific dental features (Muylle *et al*., 1999).

All experimental procedures were conducted according to the guidelines of the Ethics for humane treatment of animal use in research and complies with the relevant legislation of Faculty of Veterinary Medicine, Benha University, Egypt.

Technique for trans-rectal ultrasonography

Most examinations were performed per rectum in un-sedated haltered donkeys while they were standing next to a solid wall or tying her to a solid object as described by Pozor and Mcdonnell (2002) using Magic 2200 Vet scanner (Eickemeyer Veterinary Equipment Inc., Germany) equipped with transrectal 6 MHz linear transducer. An ultrasound probe was fixed to a slightly arched fiberglass extension rod of approximately 2 cm diameter and 40 cm in length. This was necessary due to size limitations constrain hindering hand and arm introduction entirely into the rectum as is possible in the full-size equine.

Transrectal examination of donkeys was done by introducing a gloved, lubricated hand to empty the feces with three fingers just inside the anus. Then, 120 ml of water-soluble lubricant was gently infused into the rectum using a catheter tip syringe for safety introduction of the stiffened probe to the appropriate location for the examination, and to provide good contact between the probe and the rectal wall for improved the imaging of the accessory sex glands. The Bulbourethral glands (BUG) appeared at the dorso-lateral aspect of the pelvic urethra. The lobes of prostatic gland (PROS) were located dorsal to the neck of the bladder. The paired vesicular glands (VG) were located lateral to the neck of the urinary bladder and cranial to the prostate gland. The ampulla ductus deferens (ADD) was scanned from the point dorsal to the bladder with excretory ducts on the dorsal wall of the urethral lumen. Colliculus seminalis (Col. Sem.) was viewed on the dorsal wall of the initial part of the pelvic urethra just caudal to the bladder.

The ultrasound settings (focus, gains, brightness, and contrast) were standardized and adopted for all the examinations. All obtained images were transferred to a computer and analyzed with "Image J" (National Institutes of Health, USA) software. To evaluate the echotexture´s homogeneity and the pixel representative area, each zone of selected images was divided in squares with an area of 200 mm^2. The mean pixel value (MPV) of each image of the glandular regions in a pixel scale varying from 0 (anechoic, dark image) to 255 (hyperechoic, white image) as well as the number of pixels (NP) corresponding to the intensity level (PI) that occurs most frequently in the area were recorded. Means for PI and NP from four spots per each gland were calculated, and the mean of the two glands per animal was used in the statistical analysis.

Anatomical study

Four male donkeys were used for the gross anatomical descriptive study of the accessory genital gland. Xylazine (0.4 mg/kg IV) and propofol (2.0 mg/kg IV) were used for animals´ anesthesia (Matthews and Taylor, 2002), thoroughly bled to death from the common carotid artery and were studied fresh at Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Benha University, Egypt. The gross anatomy of accessory genital glands was studied in-situ and separately. Important anatomic structures of the accessory glands were distinguished and photographed. The nomenclature used in this work was adapted to according to Schaller and Constantinescu (2007).

Statistical analysis

Data of each accessory sex gland's length, width as well echo-pattern (MPV, PI, PN) from the four spots from the two glands are presented as mean (± SEM) and used in the statistical analysis using SPSS statistical package version 16 (SPSS Chicago, Illinois, USA). Student's t-test was to compare between the mean values of premature and mature groups. The association between age, biometry and echo-pattern of the accessory sex organs was computed with Pearson correlation Coefficient. P value was set at < 0.05 to define the significant differences.

Results

The accessory genital glands of the donkeys consisted of four glandes located around the pelvic urethra, these included the paired bulbourethral glands, prostate gland, paired vesicular glands and paired ampullae of ductus deference (Fig.1A – B).

Bulbourethral glands

Bulbourethral glands are paired, oval, and depressed dorso-ventrally in donkeys. It was located on the dorso-lateral aspect of the pelvic urethra at about 4 cm from the anus and 3 cm from the prostate gland (Fig. 2A). It appeared as an oval hypoechoic structure with multiple anechoic spaces throughout the parenchyma (Fig. 2B).

The dimensions (width and length) of right and left BUG were not significantly different between young premature and aged mature donkey groups; though the numerical differences were clear (Table 1). Also, the echo-pattern analysis of BUG did not reveal significant differences between different age groups except for the pixel intensity of the right BUG (38.02± 6.38 vs. 46.61±2.93, respectively) (Table 2).

There was a clear positive correlation between age and BUG length and width ($r=0.407$; $p<0.01$ and $r=0.334$; $p<0.05$), length and width (0.652, $p<0.01$) and MPV and PI (0.968, $p<0.01$). However, there was a negative correlation ($r= – 0.210$, $p<0.05$) between PI and PN (Table 3).

Prostate gland

The prostate gland composed of central isthmus and two lateral lobes; each was rather triangular with a concave dorsal

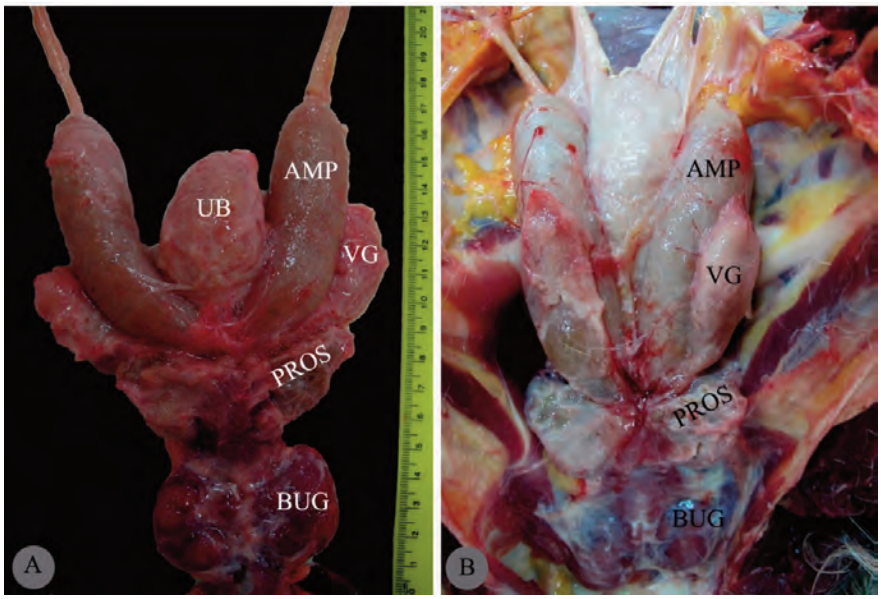

Fig.1 Photographs showing the dorsal view of the isolated (A) and in-situ (B) donkey´s bulbourethral glands (BUG), prostate gland (PROS), vesicular glands (VG), ampullae deferent duct (AMP) and urinary bladder (UB).

Table 1. Ultrasonographic biometry of donkey accessory sex glands

Accessory sex organs		Animal groups	Width (mm) Mean	SE	P value	Length (mm) Mean	SE	P value
Bulbo-urethral gland	Right	Premature	15.80	0.59	ns	26.34	1.27	ns
		Mature	21.69	1.46		32.12	2.20	
	Left	Premature	17.36	2.18	ns	27.53	1.77	ns
		Mature	21.02	0.94		33.65	1.31	
Prostate gland	Right	Premature	21.16	0.81	*	38.39	3.04	ns
		Mature	25.15	1.24		43.32	1.48	
	Left	Premature	20.69	0.62	*	37.74	2.95	*
		Mature	25.09	1.23		45.32	1.16	
Vesicular gland	Right	Premature	18.97	1.61	ns	27.57	3.94	ns
		Mature	28.08	2.77		45.55	3.08	
	Left	Premature	18.20	1.62	ns	28.74	3.97	ns
		Mature	28.81	2.71		45.00	3.14	
Ampulla ductus deferens	Right	Premature	17.11	2.83	*			
		Mature	24.36	1.05				
	Left	Premature	16.38	2.50	ns			
		Mature	24.92	1.37				
Colliculis seminalis		Premature	12.51	1.74	ns	16.80	2.74	ns
		Mature	16.43	1.25		22.83	1.28	

* indicated significant differences at P< 0.05. ns referred to non-significant differences

surface extended along the caudo-lateral border of the vesicular glands. It was viewed above the neck of the urinary bladder at about 10 cm from the anus (Fig. 2C). The prostatic lobes appeared as a hypoechoic elongated structure enclosing an echogenic lumen (Fig. 2D).The width of the right lobe, and width and length of left lobe of prostate gland showed significant ($p<0.05$) differences between young premature and old mature donkeys (Table 1). Analysis of the echo-pattern (MPV, PI and PN) of prostate gland showed numerical differences between young premature and old mature animals. Nevertheless, PN of left lobe was significantly different (Table 2).

Animal age was significantly correlated with prostate gland length ($r=0.336$, $p<0.05$). At the same time, length and width of the lobes were positively correlated ($r=0.596$, $p<0.01$). The correlations between age and echo-pattern characteristics were not significantly affirmed (Table 3).

Table 2. Echopattern features of donkey accessory genital glands

Accessory sex organs		Animal groups	Mean Pixel value		P value	Pixel intensity		P value	Pixel number		P value
			Mean	SE		Mean	SE		Mean	SE	
Bulbo-urethral gland	Right	Premature	41.90	6.22	ns	38.02	6.38	*	14.65	0.81	ns
		Mature	49.31	3.12		46.61	2.93		13.41	0.39	
	Left	Premature	41.81	6.52	ns	37.25	6.26	ns	13.50	0.98	ns
		Mature	48.17	3.76		43.78	3.86		13.45	0.33	
Prostate gland	Right	Premature	43.74	4.77	ns	37.27	6.07	ns	16.28	0.70	ns
		Mature	48.22	3.84		41.67	4.02		15.23	0.48	
	Left	Premature	41.84	7.03	ns	38.02	8.05	ns	16.50	0.44	*
		Mature	45.33	3.13		38.86	3.98		18.01	1.44	
Vesicular gland	Right	Premature	54.42	7.80	*	51.77	7.17	*	16.84	0.91	ns
		Mature	74.66	3.96		71.19	4.23		16.51	0.44	
	Left	Premature	55.39	6.31	*	51.23	6.63	*	17.03	0.41	*
		Mature	71.71	4.14		67.36	3.93		15.99	0.54	
Ampulla ductus deferens	Right	Premature	54.45	6.22	*	49.66	6.03	*	16.28	0.91	*
		Mature	78.85	4.60	*	72.08	4.36		14.08	0.67	
	Left	Premature	52.01	5.50	*	49.98	6.61	ns	17.38	0.79	*
		Mature	75.63	5.21		69.35	5.73		14.62	0.50	
Colliculis seminalis		Premature	75.01	6.30	ns	68.88	6.90	ns	6.25	0.23	ns
		Mature	85.91	5.63		75.16	5.65		6.02	0.22	

* indicated significant differences at P< 0.05. ns referred to non-significant differences

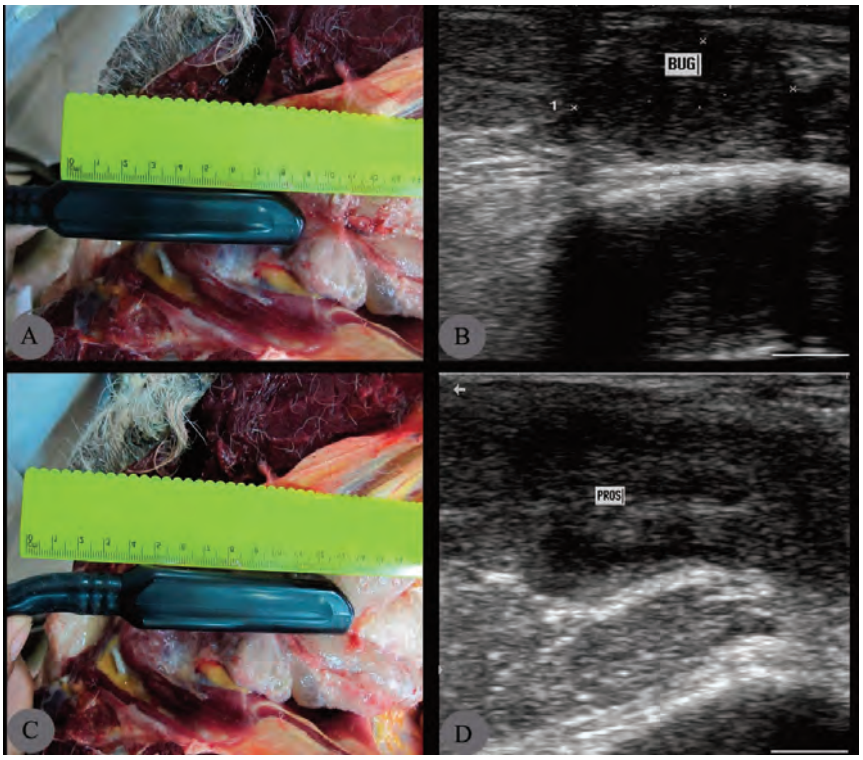

Fig.2. Photographs showing the in-situ probe orientation (A and C) during ultrasound examination of bulbourethral (BUG) and prostate (PROS) glands and its echo-graphic appearance (B and D) in donkeys. BUG appeared as an oval hypoechoic structure with multiple anechoic spaces throughout the parenchyma. PROS appeared as hypoechoic elongated structure enclosing an echogenic lumen.

Table 3. Correlation between biometry and echo-pattern of donkey accessory sex organs

		Length	Width	MPV	PI	PN
1. Bulbourethral gland	Age	0.407**	0.334*	0.025	0.026	0.152-
	Length		0.652**	0.152-	0.149-	0.076-
	Width			0.014-	0.016	0.137-
	Mean pixel value (MPV)				0.968**	0.281-
	Pixel intensity (PI)					0.210-*
2. Prostate gland	Age	0.336*	0.215	-.004-	0.011-	0.063
	Length		0.596**	0.189-	0.251-	0.087-
	Width			0.096	0.043	0.188-
	Mean pixel value (MPV)				0.956**	0.385**
	Pixel intensity (PI)					0.344*
3. Vesicular gland	Age	0.340*	0.350*	0.367*	0.316*	0.358-*
	Length		0.794**	0.331*	0.246	0.339-*
	Width			0.216	0.166	0.318-*
	Mean pixel value (MPV)				0.964**	0.518**
	Pixel intensity (PI)					0.407-**
4. Ampulla ductus deferens	Age		0.451**	0.419**	0.396**	0.255-
	Width			0.395**	0.376**	0.132-
	Mean pixel value (MPV)				0.979**	0.218-
	Pixel intensity (PI)					0.124-
5. Colliculus seminalis	Age	0.324	0.27	0.111	-.020-	0.09
	Length		0.821**	0.001	0.137-	0.392-
	Width			0.016	0.088-	0.219-
	Mean pixel value (MPV)				0.922**	0.321-
	Pixel intensity (PI)					0.192-

*, **. Correlation is significant at the 0.05 and 0.01 levels, respectively (2-tailed).

Vesicular glands

Vesicular glands were paired, pear-shaped with rounded blind end and a constricted neck. They were located at about 14 - 15 cm from the anus (Fig. 3A). Ultrasonographically, each gland was seen with a thin echogenic wall surrounds anechoic lumen (Fig. 3B).

The dimensions of right and left VG showed numerical non-significant differences between young premature and aged mature donkeys, and between the right and left glands (Table 1).

The echogenicity of right VG was slightly higher than left one. The analysis of echo-texture of VG revealed a clear significant ($p<0.05$) influence of sexual maturity on MPV and PI of the gland (Table 2).

A clear significant ($p<0.05$) correlation was noticed between donkey's age and VG dimensions and echo-pattern. Likewise, there was a positive correlation between length and width ($r=0.794$, $p<0.01$) and MPV ($r=0.331$, $p<0.05$). Pixel number was negatively correlated with VG length ($r= -0.339$, $p<0.05$) and width ($r= -0.318$, $p<0.05$) (Table 3).

Ampulla Ductus Deferens (ADD)

Ampulla of the ductus deferens constituted the terminal glandular widening part the ductus deferens. ADD seemed cylindrical in shape dorso-lateral to the urinary bladder at about 20 cm from the anus (Fig. 3C). Ultrasonographically, ADD of donkeys appeared as a hypoechoic elongated mass with a very scarce slightly anechoic lumen (Fig. 3D).

The ADD appeared longer than the ultrasound probe in most of the cases, therefore the width was the only measurable aspect. The width of right ADD was noticeably ($p<0.05$) larger in old mature (24.36±1.05 mm) than that of young premature (17.11±2.83 mm) donkeys. Such difference was numerically feasible for left ADD (Table 1).

The analysis ADD echogenicity in terms of MPV, PI and PN showed a substantial ($p<0.05$) dissimilarities between old mature and young premature donkey groups. Interestingly, PN was higher in premature than mature animals (Table 2).

The correlation between age and ADD width ($r=0.451$), MPV ($r=0.419$) and PI ($r=0.396$) was statistically ($p<0.01$) verified. Beside the positive correlation between ADD width and MPV ($r=0.395$) and PI ($r=0.376$) was clearly ($p<0.01$) noticed (Table 3).

Colliculus seminalis

Colliculus seminalis was viewed as a widening in the initial part of the pelvic urethra just caudal to the prostate gland. Ultrasonographically, it appeared as an echogenic mass with highly branched echogenic septa enclosing hypoechoic areas due to highly vascularization and sponge like depiction (Fig. 4). The dimensions of Col. Sem. did not reveal statistical differences between young premature and old mature donkeys. Echo-pattern analysis of Col. Sem. did not show statistical significant changes between aged mature and young premature animals (Table 2). Also, the correlation between age, and Col. Sem. dimensions and echo-pattern was not significantly proven (Table 3).

Discussion

In the course of recent twenty years, various diagnostic approaches have become accessible to enable reproductive evaluation of the stallion. An assessment of breeding stallion´s infertility has depended on physical examination of the reproductive tract, as well as spermiogram (sperm number, motility, and morphology) evaluation. Ultrasonography is an easily applicable noninvasive method, provides information related to the clinical fertility evaluation of the jack. Transrectal ultrasound could be used in a number of pathological conditions of prostate, vesicular gland ... etc. In horses, transrectal ultrasonography has proved to be a useful adjunct to palpation in evaluating the anatomic relationship, physical dimensions and the acoustic characteristics of the stallion accessory sex glands

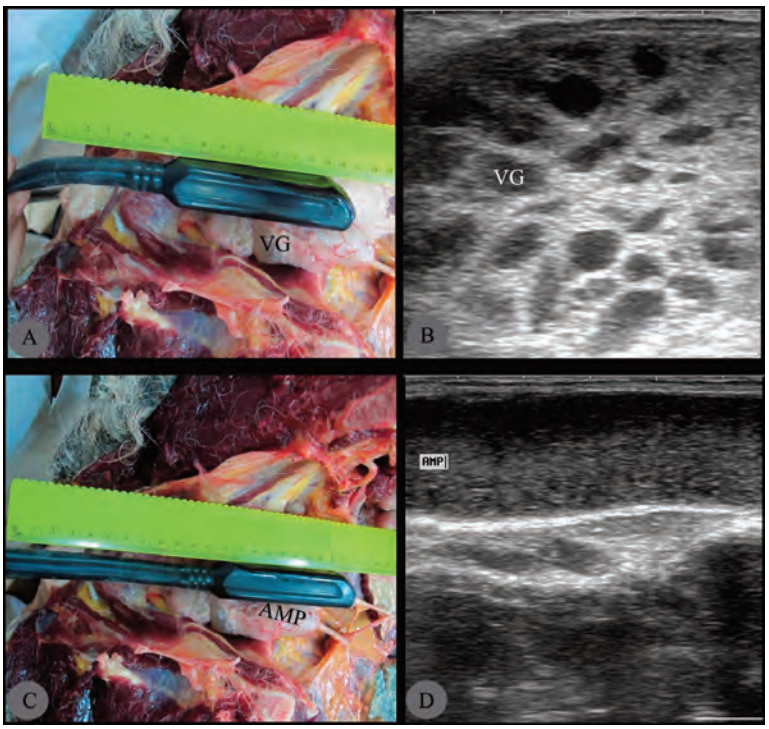

Fig.3. Photographs showing the in-situ probe orientation (A and C) during ultrasound examination of vesicular glands (VG) and ampulla deferent duct (AMP) and its echo-graphic appearance (B and D) in donkeys. VG appeared as a thin echogenic wall surrounds anechoic lumen. AMP appeared as a hypoechoic elongated mass with a very scarce slightly anechoic lumen.

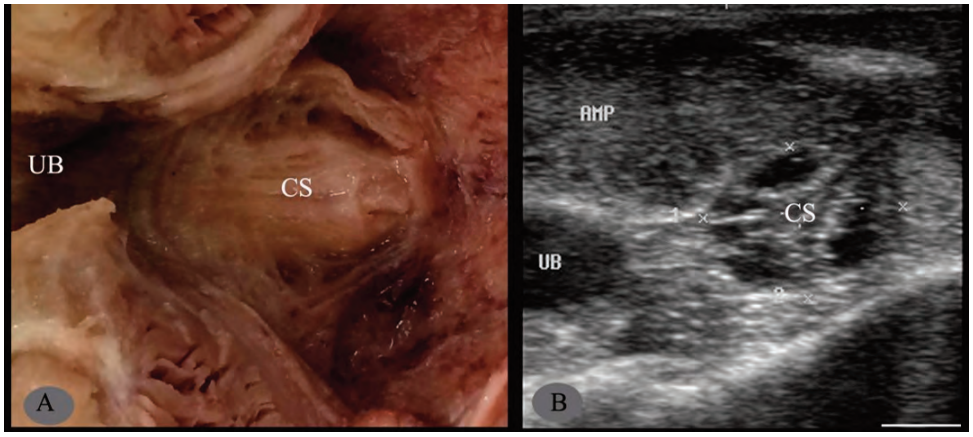

Fig.4. Photographs showing the in-situ anatomic position (A) and echo-graphic appearance (B) of colliculus seminalis (CS) in donkey. Colliculus seminalis appeared as an echogenic mass caudal to urinary bladder (UB) and caudo-ventral to ampulla deferent duct (AMP) with highly branched echogenic septa enclosing hypoechoic areas due to its high vascularization, ascribed as sponge like depiction.

and related structures (Little and Woods, 1987). Unfortunately, the palpation of the donkey sex accessories was not feasible due to the small size rectum as well as the smooth texture of glands accused to its detection difficulty. These add an importance to the ultrasound as a non-invasive diagnostic technique in this species.

In the current study, BUG appeared as an oval hypoechoic structure with multiple anechoic spaces throughout the parenchyma. This shape and features come in accordance with the gross anatomical description made by Mai (2014). Also, the measured dimensions were close to that reported in horses (19.7±4.6 mm in width and 32.4±6.7 mm in length) formerly (Little and Woods, 1987). Age of animals referenced with maturity onset did not influence statistically on BUG dimensions or echo-pattern, though the numerical distinctions were clear. This is perhaps due to the close age of animals or small size sample under study, regarding that these donkeys already post-pubertal and perhaps some of them approached the age of maturity. Nevertheless, there was a clear positive correlation between age and BUG dimensions, and also between length, and width, MPV and PI. In stallions, the dimensions of BUG is correlated with its functional activity where the length and width of the bulbourethral glands increased significantly after sexual preparation and decreased significantly after ejaculation (Weber *et al*., 1990). Contri *et al*. (2008) found that the dorso-ventral dimension of the BUG was never significantly different between sexual rest and after ejaculation, but it did at the erection in jackass.

Current data revealed significant changes in prostate gland dimensions in mature as compared with premature groups, though its echo-pattern except for PN was not different in donkeys. Moreover, animal age was significantly correlated with prostate gland length. These findings indicated the effect of sexual activity, controlled by testosterone hormone production, after age of maturity on prostate gland measures, but not on its secretory contents. Thompson *et al*. (1980) presented that the stallion accessory sex glands depend on the presence of androgens for their development and maintenance. The prostate gland increases in size and decrease in echogenicity with teasing (England, 2005). The length and width of prostate gland lobes in indigenous Nigerian male donkey was 4.1±0.27 and 1.9±0.2 cm, respectively (Mai, 2014). The mean dorso-ventral diameter (±SD) of the prostate gland of horses more than 3 years old was 32.9±6.4 mm, while that of geldings was 12.8±3.1 mm (Rantanen and Mckinnon, 1998). Contri *et al*. (2008) showed that the dorso-ventral dimensions of the right and left prostate gland lobes were never significantly different between sexual rest (2.99±0.32 and 3.00±0.3cm) and after ejaculation (3.03±0.33&2.99±0.31cm), but it did at erection (3.42±0.25 and 3.46±0.32cm) in jackass.

An outstanding characteristic of the male accessory apparatus in Equidae (stallion, jackass and zebra) is the presence of very large ampullae and seminal vesicles (Mann and Lutwak-Mann, 1981). In the current results, the vesicular gland dimensions of right and left VG showed numerical differences between premature and mature donkeys. The echogenicity of right VG was slightly higher than left one. The analysis of echo-texture of VG revealed a substantial impact of sexual maturity on MPV and PI of the gland. Beside there was a significant correlation between donkey's age and VG dimensions and echo-pattern. Pixel number was negatively correlated with VG length and width (r= – 0.339 and – 0.318, respectively). Pozor and McDonnell (2002) showed that the vesicular gland measures were greater for heavier horses than for those of other groups, though it was similar for small size breeds of horses (Miniature and ponies). The echogenic characteristics of vesicular glands obviously differed, perhaps due to divergence in recent sexual activity. Contri *et al*. (2008) showed that the dorso-ventral dimensions of the VG significantly decrease after ejaculation.

In the current study, characterization of donkeys ADD by means of ultrasound revealed a considerable ($p<0.05$) divergences between mature and premature aged animals in terms of MPV, PI and PN. Besides, there was a clear ($p<0.01$) positive correlation between age and ADD width (r=0.451), MPV (r=0.419) and PI (r=0.396). Contri *et al*. (2008) showed that the dorso-ventral measures of different duct ampulla in jackass were significantly bigger at the time of erection as compared with sexual rest and after ejaculation. In stallions, most of ampullar duct engorgements (93%) happened preceding to the begin of ejaculation (Weber and Woods, 1993). This indicated the significance of sexual stimulation on the gland activity and consequently its dimensions.

Ultrasonography can be approved to evaluate the cavernous tissues´ physical status and to identify urethral lesions, such as calculi or stenosing scars (Schumacher, 2007). Many stallions with ejaculatory problems were found to suffer from large cysts at the Col. Sem. (Pozor *et al*., 2011). In this study, ultrasonographic investigation of Col. Sem. did not proven statistical differences between premature and mature donkeys in terms of the dimensions and echo-pattern. This phenomenon might be due to testosterone production in sufficient amounts to enhance the development and functional activity of the cavernous tissue of Col. Sem., as the animal approaching maturity. So far to the authors' knowledge, there is no publications were available to discuss the present findings.

This might be due to fact that the Col. Sem. is less frequently screened during routine examinations, possibly because this area is screened less carefully for the pathologies than the more proximal portion of the internal reproductive tract of stallions (Pozor *et al*., 2011).

Conclusion

Ultrasonography is a valuable tool to screen the accessory sex gland of donkeys. Such tool helped herein to determine the dimensions of the glands along the course of the pelvic urethra in healthy intact donkeys reared in Egypt. It was successful to ascertain the presence of some relations between age, measures and the echo-pattern which could be used as a base sire selection as well as further andrological research with special emphasis to sexual activity and pathological conditions.

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