PSM Veterinary Research ISSN: 2518-2714 (Online) – An International Peer-reviewed Journal

Research Article 2018 | Volume 3 | Issue 2 | 45-52

Article Info

Open Access
Citation: Elbaz, H.T., Marey, M.A., Abdel Razek, E.M., Moustafa, I.A., 2018. Ultrasonographic Evaluation of Accessory Sex Glands and Testes of Stallion during Non-breeding Season. PSM Vet. Res., 3(2): 45-52.

Received: October 8, 2018

Accepted: November 26, 2018

Online first: November 27, 2018

Published: December 22, 2018

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Ultrasonographic Evaluation of Accessory Sex Glands and Testes of Stallion during Non-breeding Season

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Abstract
In the current study, the accessory sex glands of 10 stallions were visualized with trans-rectal ultrasonography during non-breeding season using a linear array B-mode veterinary ultrasound high-frequency transducer (5-7.5 MHz). Also, the echogenicity and measurements of all scrotal contents (testes, epididymis, and spermatic cords) were evaluated using transcutaneous ultrasonography. The results revealed no significant differences ($P > 0.05$) in the measurements of ampulla, vesicular gland, prostate gland and bulbourethral gland within right and left lobes in the same stallion and between different examined stallions. Additionally, there were no significant differences ($P > 0.05$) in the measurements of testes, scrotal width and tail of the epididymis. Of note, there are limited variations in echogenic characters within and between stallions as most accessory genital glands have no secretions or fluid. In conclusion, the normal reference ranges defined in this study can be used in the routine clinical evaluation of reproductive organs of stallions in the non-breeding season.
Keywords: Stallion, Ultrasound, Testis, Accessory sex glands, Non-breeding season.

INTRODUCTION

Palpation and ultrasonographic examination of the internal genital tract of the stallion can be done as a part of the routine breeding soundness examination. This technique is an excellent diagnostic tool for investigating various pathological disorders of the scrotum and the internal genitalia of the stallion (Love, 1992; Turner, 1998). However, ultrasound examination of stallions is performed much less frequently, typically than mares except when there is a problem that requires reliable diagnosis (Pozor, 2005). Ultrasonographic examination is mainly performed in the evaluation of stallions presenting for routine breeding soundness examinations, abnormalities of the ejaculate, or ejaculatory dysfunction (Ball, 2008 and Turner, 2011), as well as examination problems in stallion and geldings (Schnobrich *et al.,* 2016). Also, sonographic evaluation may be performed in stallions during breeding and non-breeding season (Najjar *et al.,* 2012). In fact, pathology of the internal reproductive organs of the stallion is relatively uncommon. Therefore, most veterinarians are unfamiliar with examination of these structures and measurements of ultrasonographic imaged accessory sex glands (Pozor and McDonnell, 2002).

Testicular ultrasound is an easy method to determine testicular sperm production using testicular width (Najjar *et al.,* 2012). Stallions showed an evident lack of sexual interest during non-breeding season, which may be associated with the lower testosterone during this season (Leme *et al.,* 2012). Leme et al. (2012) found that the effects of season were not significant either for testicular volume or for any semen parameter. Meanwhile, previous study reported that the total and lumenal diameters of the vesicular glands increased significantly after sexual preparation and decreased significantly after ejaculation (Weber *et al.,* 1990). Therefore, the aim of this study was to characterize the size and sonographic features of the ampullae of the ductus deference, seminal vesicles, prostate, bulbourethral glands with evaluation of the scrotum and its contents in the Egyptian native stallions during non-breeding season.

MATERIALS AND METHODS

All experiments were conducted in accordance with the ethics statement and guiding principles for the care and use of research animals promulgated by the faculty of veterinary medicine at Sadat City, Egypt. In the faculty farm, ten Egyptian native stallions (8-12 years old) were raised and housed together in a pen that was isolated from mares. The study was conducted during non-breeding season (March, 2016). No fertility problems were known or suspected in any of the stallions. All stallions were restrained before examination by halter and cross-ties in standing stocks and fecal balls were manually evacuated from the rectum by lubricated hand.

Methods for locating and visualizing each structure were performed as previously described by (Weber *et al.,* 1990) with minor modifications. Briefly, a trans-rectal palpation of the internal reproductive tract was performed before introducing the transducer to locate the pelvic urethra, ampullae, seminal gland and prostate gland. After palpation, the accessory sex glands of each stallion were examined using a well-lubricated (5-7.5 MHz) linear array B-mode veterinary ultrasound transducer (Sonoscape-A5V, Shenzhen, China). The length and height of each accessory genital gland was measured to the nearest millimeter using the ultrasound built-in measuring ruler. The testes were pushed ventrally into the scrotum with one hand, while the calipers were placed over the widest part of the scrotum with the other hand for taking the total scrotal width of a stallion. The ultrasound probe was placed longitudinally across one testicle to measure the length of the testis. Additionally, the probe was turned to a cross-sectional axis in order to measure the width of the testis. The tail of epididymis was visualized in caudal part of testis and spermatic cord was visualized in dorsal part of testis.

Statistical analysis

Data are presented as mean ± standard error of mean (SEM). Statistical analyses were performed using IBM® SPSS® Statistics 24 (IBM Corp., Armonk, NY, USA). Student's t-test was applied to compare the data between paired structures, while one-way ANOVA followed by LSD post hoc multiple comparison test was used to compare the data obtained from different stallions. The results were considered statistically significant at $P< 0.05$.

RESULTS

Measurements of accessory sex glands, scrotum, and its contents

The ultrsonographic examination of accessory sex glands, scrotum, and its contents revealed, no significant difference ($P >0.05$) in measurements between right and left lobes of ampulla, vesicular gland, prostate gland and bulbourethral gland of stallions during the non-breeding season (Table 1). Moreover, there was no significant difference ($P >0.05$) in measurements of testes, scrotal width, spermatic cord and tail of epididymis in the examined stallions (Table 2).

Echogenicity of testes and accessory sex glands Testes

In the present study, the ultrasonography characteristics of testes, and accessory sex glands were performed*. The obtained findings* demonstrated that the testicular parenchyma was homogenous and uniformly echogenic. The mediastinum testis was white linear structure of greater echogenicity than testicular

parenchyma short or long in certain stallions (Figure 1). The scrotal layer appeared very echogenic structure and varied in its thickness between stallions with presence of clear hypoechoic space or line separating it from the testis in some stallion.

Table 1. Measurements of accessory genital glands (Ampulla, vesicular, prostate, bulbourethral glands) in stallions during non-breeding season.

Accessory genital gland	Measurements (mm)	Mean ± Std. Error	Minimum	maximum	*P* values
Ampulla	Length right	61.15 ± 1.02	52.50	63.50	6.75
	Diameter right	11.74 ± 0.76	7.40	15.00	
	Length left	60.58 ± 1.21	50.60	63.50	
	Diameter left	10.46 ± 0.61	8.00	13.20	
Vesicular gland	Length right	45.35 ± 2.44	35.00	56.90	15.83
	Diameter right	12.57 ± 0.82	9.30	18.70	
	Length left	48.27 ± 2.40	37.80	59.90	
	Diameter left	12.85 ± 0.63	9.40	15.20	
Prostate gland	Length right	52.69 ± 3.15	36.70	66.60	32.35
	Diameter right	21.69 ± 2.55	8.30	36.20	
	Length left	50.47 ± 2.76	32.70	61.50	
	Diameter left	25.45 ± 3.28	10.40	43.40	
Bulbourethral gland	Length right	34.04 ± 2.04	24.50	40.50	22.57
	Diameter right	13.98 ± 0.70	11.30	18.40	
	Length left	36.79 ± 2.35	26.00	47.60	
	Diameter left	14.09 ± 0.93	11.90	20.10	

Table 2. Measurements of the scrotum and its content (Scrotal width, testes, tail of epididymis and spermatic cord) in stallions during non-breeding season.

Scrotal contents	Measurements	Mean ± Std. Error	Minimum	maximum	*P* values
Scrotum	Scrotal width (SW)	11.00	10.00	12.5	1.88
Testes	Length right	59.80 ± 2.74	38.40	69.90	17.01
	Diameter right	41.72 ± 2.51	27.10	51.50	
	Length left	60.52 ± 1.79	49.40	70.50	
	Diameter left	41.24 ± 3.42	23.80	60.70	
Tail of epididymis	Length right	17.59 ± 0.82	12.60	21.40	20.27
	Diameter right	20.40 ± 1.00	14.70	23.90	
	Length left	20.89 ± 1.73	15.40	34.20	
	Diameter left	20.79 ± 1.50	14.90	32.80	
Spermatic cord	Diameter right	27.17 ± 1.39	21.50	35.30	1.96
	Diameter left	24.94 ± 1.40	17.50	32.40	

The tail of the epididymis was easily identified on the caudal end of the testicle and appeared hypoechoic or less echoic than the testicular parenchyma resembling globular or oval appearance and had echogenic particles or spots in the center (Figure 2). Neither head nor body of the epididymis could be imaged clearly in all stallions.

The ultrasonography examination of spermatic cord revealed that the heterogeneous appearance of the vascular pampiniform plexus with anechoic black circular areas surrounded by more hyperechoic regions (Figure 3). For ampulla, it was appeared as echogenic tube with small hypoechogenic central lumen surrounded by a uniformly echogenic wall. The parenchyma was appeared as a mottled appearance and echogenicity was the same in seven stallions (7/10) and the glands were empty from the secretion (Figure 4). Mean lengths of right and left lobes of ampulla were (61.15 ±1.02 & 60.58±1.21 mm) and diameters were (11.74±.76 &10.46±.61 mm), respectively (Table 1).

Indeed, the ultrasonography examination of the vesicular glands was difficult to be palpated or visualized in some stallion. The obtained findings revealed that the size

and echogenicity of the glands were variable within stallions from right to left and also between different examined stallions (Figure 5). The empty seminal vesicles appeared as elongated, flattened, and somewhat irregular soft tissue structures with a thin echogenic wall and echoic or heteroechoic lumen in seven stallions (7/10). Notably, non-echoic lumen was detected in the gland of one stallion. The maximum vesicular gland length and diameter were (59.90 &18.70 mm), respectively (Table 1).

For prostate gland, it was appeared as large heterogeneous gray-white and contain multiple small anechoic fluid-filled pockets or bands in eight stallions (8/10) (Figure 6). Only two stallions had small gland with very low anechoic pockets surrounded by highly echogenic wall. The right and left lobes of the prostate were easily identified in all stallions. The maximum prostate gland length and diameter were (66.60 & 43.40mm), respectively (Table 1).

The bulbourethral glands were easily identified in all stallions just lateral to pelvic urethra. The maximum bulbourethral gland length and diameter were (47.60 & 20.10 mm), respectively. The bulbourethral glands appeared as oval structures, mottled with multiple small hypoechogenic spaces throughout the parenchyma in all stallions (10/10) with well-defined border (Figure 7).

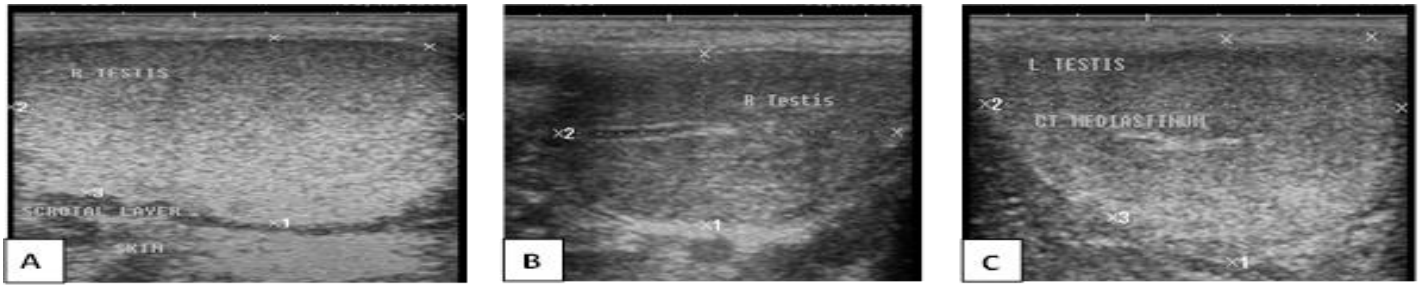

Fig. 1. Ultrasound image of testes: (A) more echogenic testicular parenchyma (B) hypoechogenic testicular parenchyma with central more echogenic line (mediastinum testis) (C) echogenic testicular parenchyma with central more echogenic line (mediastinum testis).

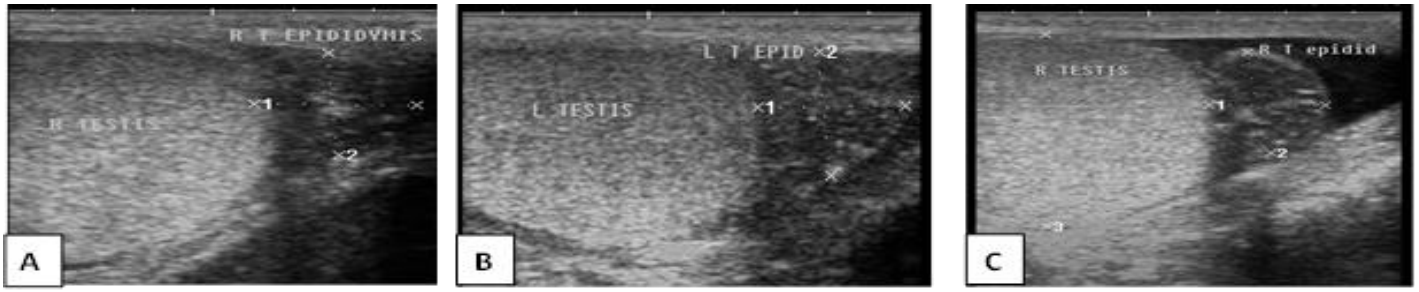

Fig. 2. Ultrasound image of testis and tail of epididymis :(A), (B), (C) hypoechogenic tail with white (hyper-echogenic) lines in center. Note the decreased echogenicity of the epididymis relative to the adjacent testicle.

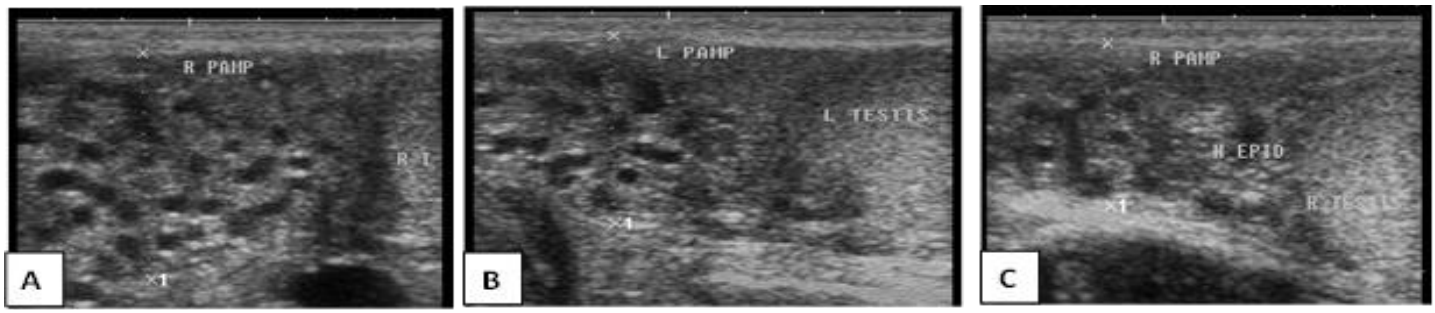

Fig. 3. Ultrasound image of pampiniform plexus :(A) anechoic black circular areas surrounded by more hyperechoic regions (B), (C) note the body of epididymis more hardly to visualized.

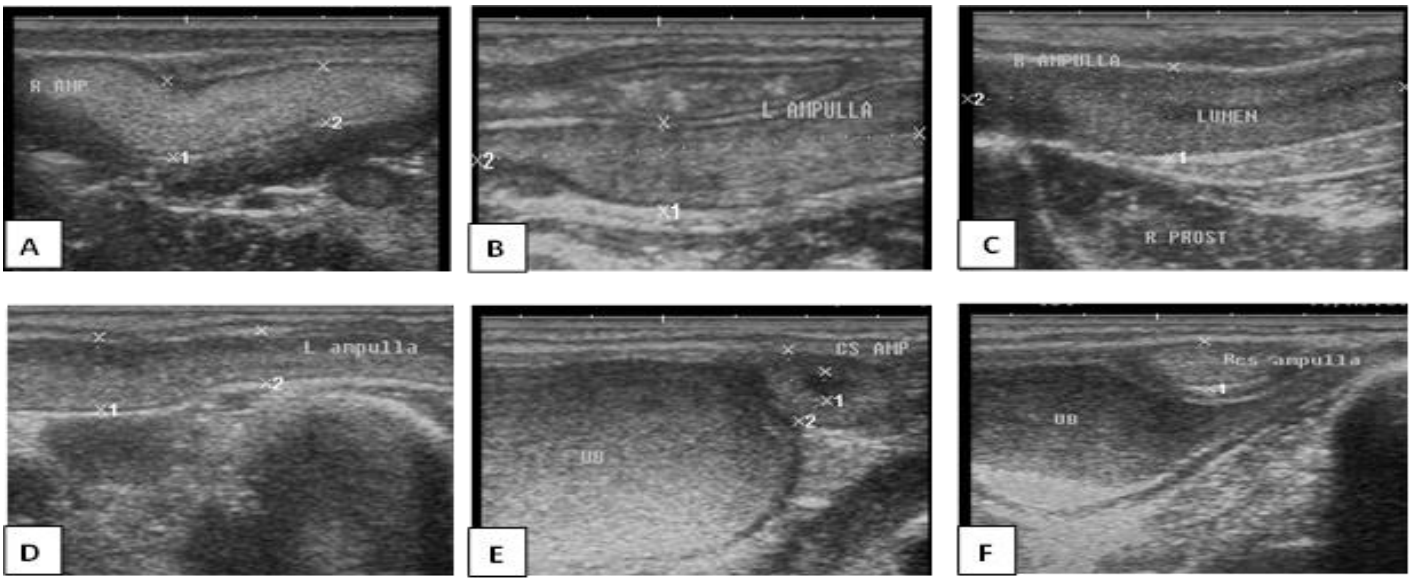

Fig. 4. Ultrasound image of ampulla: (A) more echogenic lumen (B) echogenic lumen (c) hypoechogenic lumen with dark line in the center (D) hypoechogenic lumen with dark line in dorsal part (E) C.S ampulla with non-echogenic lumen (F) C.S ampulla with echogenic lumen.

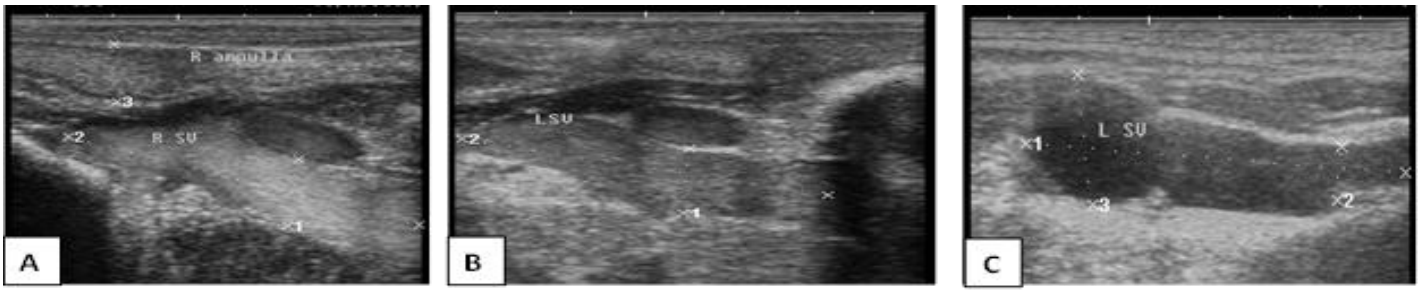

Fig. 5. Ultrasound image of vesicular glands: (A) small more echogenic lumen (B) hypoechogenic lumen (C) non-echogenic lumen.

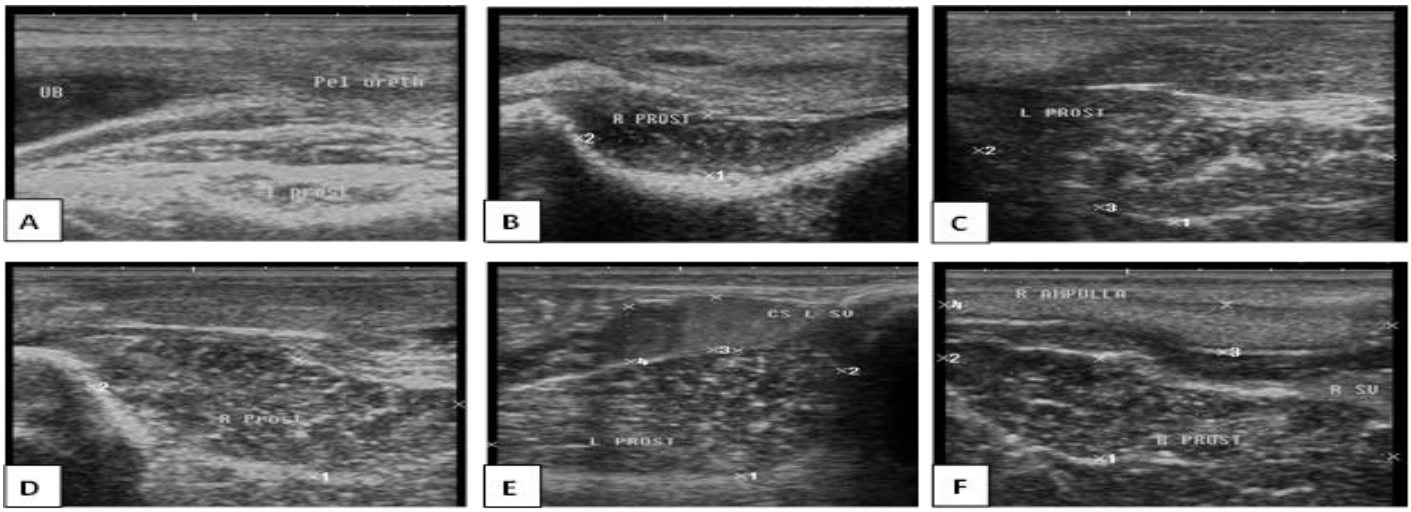

Fig. 6. Ultrasound image of prostate glands :(A) Small echogenic gland with more echogenic outline (b) hypoechogenic gland with more echogenic outline (C), (D) large gland with more white streaks in the lumen (E), (F) large gland with hypoechogenic lumen with small white streaks in the lumen.

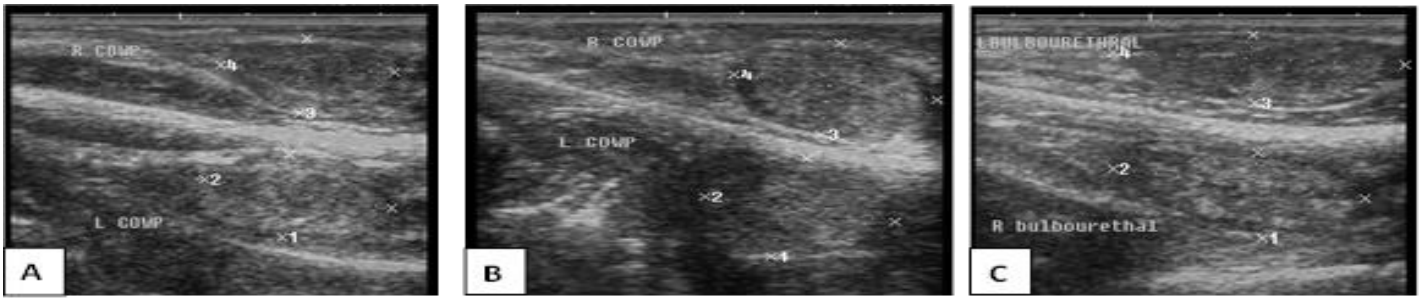

Fig. 7. Ultrasound image of bulbourethral glands: (A), (B), (C) oval structures, mottled with multiple small hypoechogenic spaces with well-defined echogenic border.

DISCUSSION

The ability to evaluate the internal reproductive tract of the stallion is a skill that is required for any veterinarian performing routine breeding soundness evaluations (Turner, 2014). The data of the current study provides guidelines for ultrasonography and size measures of accessory sex glands and testes in stallions in native Egyptian breed during non-breeding season.

Our results showed that there was no significant difference (P >0.05) in the measurements of testes, scrotal width and tail of epididymis between stallions in the non-breeding season. It has been shown that stallions showed an evident lack of sexual interest which may be associated with the lower testosterone during this season (Leme *et al.,* 2012). Concurrently, Palmer et al. (1998) reported that testicular measurements of the stallion were the same in the spring and autumn seasons meanwhile, testicular weight increased during the periods coinciding with the photoperiod variations. On the other hand, it has been shown that the volume, total sperm count and motility in fresh semen were significantly higher in summer than in winter, while sperm concentration was significantly lower in summer compared to the other seasons (Janett *et al.,* 2003).

Our data clearly demonstrated that there was no significant difference (P >0.05) in the measurements of right and left ampulla, vesicular gland, prostate gland and bulbourethral gland of stallions in the non-breeding season. Recently Schnobrich et al. (2016) mentioned that there were no statistically significant differences in measurements between the left and right sides of paired structures of accessory genital glands of gelding horses. Also, Weber et al. (1990) showed that the dimension of accessory sex glands in stallion except wall diameter of ampullae and vesicular glands was increased after sexual preparation and decreased immediately after ejaculation. The small size of accessory genital glands is expected as the size of the accessory sex glands increases under the influence of testosterone (Thompson *et al.,* 1980; Holyoak *et al.,* 1994). This may be due to the fact that the hypothalamic-pituitary-testicular axis of normal stallions has a lower activity in the non-breeding season and it is much more responsive to a GnRH challenge (Roser and Hughes, 1992). While, Pozor and McDonnell (2002) reported that there were no significant differences between measures taken before sexual preparation and immediately after ejaculation. Additionally, Leme et al. (2012) reported that stallions expressed very few differences in the reproductive characteristics as semen parameters, percentage of germ cells, and LH and FSH concentrations between the seasons. In the same direction, Vidament et al. (2006) demonstrated that housing stallions next to or together with mares did not increase testosterone concentrations, quantity and quality of spermatozoa, nor did any benefit on semen collection behavior. Ultrasonography evaluation and measures of the accessory sex glands of heavy type stallions (Behn *et al.,* 1997) are slightly greater than those we found for our native light horses. This difference might be related to the differences in the size, types, age, reproductive history, photoperiod of stallions.

Our results showed that the testicular parenchyma was homogenous and uniformly echogenic and the mediastinum testis was white linear structure of greater echogenicity than testicular parenchyma. The tail of epididymis appeared hypoechoic or less echoic than the testicular parenchyma and had echogenic particles or spot in the center. The full length of the head of the epididymis could not be always imaged clearly because the pampiniform plexus masked its upper part. Similar results were obtained by (Pozor, 2005) who performed a breeding soundness evaluation of 113 stallions including ultrasonography of external and internal genitalia. Moreover, (Clay and Clay, 1992) reported a seasonal increase in the testicular parenchyma mass which in turn leads to upregulation of the efficiency of spermatogenesis and sperm production per gram of testicular parenchyma.

In the current study, ampulla appeared as an echogenic tube with small hypoechogenic central lumen surrounded by a uniformly echogenic wall. Vesicular glands were difficult to be palpated and hard to visualize in some stallion. The size and echogenicity of the glands is variable within stallions from right to left and also between stallions. The empty seminal vesicles appear as elongated, flattened, and somewhat irregular soft tissue structures and appeared as a thin, echogenic wall with echoic and heteroechoic lumen in seven stallions (7/10). While (Pozor and Mcdonnell, 2002) reported that echogenicity of accessory genital glands varied widely and dependent upon recent sexual activity of stallions. The size and character of lumen contents varied between paired glands. Previously (Malmgren, 1992) suggested that highly echogenic character of the lumen of vesicular glands, particularly with a difference between paired glands indicate possible inflammation. Increased echogenicity of vesicular gland fluid is associated with the highly viscous gel fraction produced by some stallions.

The prostate gland was appeared large heterogeneous gray-white and contains multiple small or lacking anechoic fluid-filled pockets or bands in eight stallions. Similar results obtained by (Schnobrich *et al.,* 2016). The size of the prostate and number of the fluid-filled spaces increase following sexual stimulation (Weber and Woods, 1992). The bulbourethral glands were appeared as oval structures, mottled with multiple small hypoechogenic spaces throughout the parenchyma in all stallions (10/10) with well-defined border. Similar results obtained by (Schnobrich *et al.,* 2016). It has been found that the accessory sex gland size and content varied with the sociosexual environment, where stallion with harem status had larger glands than those with bachelor stallion status (McDonnell and Pozor, 1995).

CONCLUSION

Taken together, our results clearly demonstrated that there were no significant differences in the measures of testes, epididymis and accessory genital glands within and between stallions in the non-breeding season with ultrasound. Moreover, there were some limited variations in echogenic characters of accessory genital glands and testes. Whereas, the present study provides basic guidelines for ultrasonography and measures of accessory sex glands and testes that can be used during the routine breeding soundness examination of stallion, we cannot exclude the possibility of seasonal variations. Therefore, further investigations are essential to explore the possible seasonal changes in the echogenicity and measures of accessory sex glands and testes in native Egyptian breeds of horses.

CONFLICT OF INTEREST

The authors declare that no competing interests exist.

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