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Feasibility of Pulsed Wave Doppler for Evaluation of Testicular Hemodynamics during pre and Post-pubertal Periods in Egyptian Male Donkeys

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ABSTRACT

The objective of this work was to study the feasibility of pulsed wave Doppler ultrasonography for evaluation of testicular hemodynamics and to establish its normal values during pre- and post-pubertal in clinically healthy male Egyptian donkeys. Both testes from each of 14 mature (6-10 years) and 10 imматures (1-1.5 years) male donkeys were examined using caliper (testicular length, width and depth). Then, a portable color Doppler ultrasound was used to measure the pulsatility index (PI), resistive index (RI), Systolic velocity peak (SVP) and end diastolic velocity (EDV) of the testicular arteries in each of two locations. There were no significant differences between the caliper measurements and blood flow indices of the left and right testes. All indices measurements at the spermatic cord (in the middle of pampiniform plexus) were achievable in pre- and post-pubertal animals. All values were higher at the convoluted location than at the marginal aspect of the artery ($P < 0.05$) except EDV. The wave forms at convoluted segment were mainly biphasic (resistive) and that at marginal part were mainly nonphasic (non-resisrtive). There were non-significant correlations between each of the blood flow measurements and the age (Pearson, for PI, $r = -0.065$; RI, $r = -0.07$; SVP, $r = 0.16$; and EDV, $r = -0.25$; $P > 0.05$). In conclusion pulsed wave Doppler ultrasound characterization of blood flow of the donkeys' testis is possible, and will likely become a useful tool for quantitative evaluation of the testicular vasculature/hemodynamic in male donkey. Further studies are needed on both normal and abnormal testes to clarify the usefulness of this technology in diagnosis of infertility problems of Egyptian male donkey.

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Introduction

Donkeys (*Equus asinus*) have been considered the most important working livestock in Egypt since ancient centuries. They used to pull small cars, to hold cereals, and other field services. In Asia, Africa and Latin America, the species is under new pressure as a source of meat and hide for the expanding Chinese market (Gacem *et al.*, 2020). Despite that, few studies on that genus identify the physiology and functional anatomy of the male reproductive system. The reproductive organs of a full-size, libido and seminal parameter in male donkeys (jackasses) have many resemblances to that in horses (Gastal *et al.*, 1997; Kandiel and El Shafey, 2017).

Adequate flow of blood to the testis is essential to its function. The testicular artery passes through the inguinal canal in the spermatic cord and emerges near the anterior pole of the testis (the main part of adult donkey testicles have a light craneo/caudal inclination) and continues along the dorsal surface of the testes (epididymal edge of the testis); it penetrates the tunica albuginea and branches through the testis in the tunica vasculosa, which is located below the tunica albuginea and extends to the epydidimal margin (Gacem *et al.*, 2020). Every vascular insult substantially affects spermatogenesis, testicular length and can permanently affect the production of sperm from both the affected and contralateral testis. Testicular vascularization plays an important role in testicular function and spermatogenesis, decreasing the blood supply can cause ischemic damage (Al-Naffakh, 2012). The testicular artery showed prominent tortuosity, which is necessary to maintain a testicular temperature of about 4–6°C below the body temperature, given the countercurrent heat exchange mechanism (Kastelic *et al.*, 1997). The broad extension of the testicular artery in contact with the pampiniform venous plexus facilitate the movement of heat from arterial blood to venous blood and helps to minimize the speed of blood flow (Brito *et al.*, 2004; Gacem *et al.*, 2020). Therefore, the study of testicular vasculature in equines may be particularly relevant for early detection and treatment of certain pathological conditions (Pozor, 2007; Gacem, 2020).

Doppler ultrasonography is commonly used as a diagnostic imaging tool in veterinary practice (Rubens *et al.*, 2006). However, identification of small vessels and assessment of the

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overall vascularization of the organs using gray-scale ultrasound is difficult. Color Doppler ultrasound has become a tool of choice for assessing the vasculature of various abdominal and pelvic organs, including uterus and ovaries in females, and testes in males (Bollwein *et al*., 2002; Pozor and McDonnell, 2004, Pozor *et al*., 2011; Ortega-Ferrusola *et al*., 2014; Dzieciol *et al*., 2014). Doppler analysis was used as an additional tool for male evaluation in various species, such as stallions (Pozor and McDonnell, 2002 and 2004; Ortiz-Rodriguez *et al*., 2017), toms (Brito *et al*., 2015), kids (Samir *et al*., 2015), rams (Batissaco *et al*., 2013), bulls (Gloria *et al*., 2018, Rodrigues *et al*., 2020), dogs (England *et al*., 2017), donkeys (Gacem *et al*., 2020) and men (Pinggera *et al*., 2008). Spectral Doppler ultrasonographic assessments of arterial indices of male genital tract in bulls could give useful information regarding the progression of sexual maturation (Rodrigues *et al*., 2020).

The Duplex Doppler ultrasonography was used to obtain information on the anatomical features of the vasculature and blood flow (existence, direction and velocity) and simultaneously provides two-dimensional ultrasound and pulsed-wave Doppler imaging. Sidhu (1999) used this technique for assessment of the blood flow in men's testicular artery. This tool has been used for the diagnosis of some testicular pathologies in human medicine since a decade (Sidhu, 1999; Pavlica and Barozzi 2001; Sriprasad *et al*., 2001). In addition to the distinction between sperm cord torsion (lack of blood flow) and epidididyo-testicular inflammation (increased blood flow) (Dubinsky *et al*., 1998). It was used also for diagnosis of spermatic cord torsion in stallions (Pozor and McDonnell, 2004; De Bock *et al*., 2007). Using color Doppler, the blood flow characteristics of uterine and ovarian arteries were investigated (Bollwein *et al*., 1998 and 2002). Doppler ultrasonography as a useful tool has been developed in reproductive studies in humans (Middleton *et al*., 1989; Al-Naffakh, 2012), stallions (Pozor and McDonnell 2004), bulls (Gloria *et al*., 2018; Rodrigues *et al*., 2020) and dogs (Günzel Apel *et al*., 2001; Gumbsch *et al*., 2002; Carrillo *et al*., 2012). There are few and scarce studies on the Doppler blood flow parameters of the testicular artery in healthy growing and adult male donkeys (Gacem *et al*., 2020), The testicular artery and accessory genital glands were examined and Doppler indices were recorded for jackass in Spain and its correlation to semen production (Gacem *et al*., 2020). To our knowledge it has not been well established under Egyptian condition. So, the aim of this study was to determine the feasibility of pulsed wave Doppler ultrasound for evaluation of the testicular vasculature. Moreover, to establish normal values for testicular hemodynamic in pre- and post-pubertal clinically healthy male donkeys.

Materials and methods

Animals and Management

In this study fourteen mature (6-10 years) and ten immatures (1-1.5 years) clinically healthy male donkeys of mixed breeds were used. All males were kept under the same environmental and managemental condition in the Veterinary Teaching hospital, Assiut University (Latitude 32 ° N and 27° E), Egypt. The animals were fed total mixed ration ad libitum and accessed water supply freely. These animals were examined during the breeding season (May to August) and to avoid the effect of circadian rhythmicity on blood flow the animals were examined at affixed time between 9.00 and 12.00 a.m. (Zaidi *et al*., 1995; Gacem *et al*., 2020).

All Institutional and National Guidelines for the care and use of animals were followed according to the Egyptian Medical Research Ethics Committee (no. 14 – 126), also in in accordance with the Ethics Committee on Animal Experimentation of Faculty of Veterinary Medicine, Assiut University, Egypt.

Caliper measurements

The testicular measurements of 14 mature male donkeys (both sides) were taken using caliper. The testicular length, breadth and depth as well as condition of the epididymal tail were recorded to exclude the pathological affections and confirm the soundness of the jacks. All testes were found in the scrota of the immature males.

Doppler examination

Before the start of the experiment and for training of the males to be acquainted with the testicular examinations, the testes were slowly palpated and examined three times/week for two to three weeks. During examination, male donkeys were restrained by a halter in stanchion without tranquilizers. The animals were not anxious and/or nervous during inspection. Each of the two testes for each male was evaluated using a portable color Doppler ultrasound device (MyLab30 Vet device; Esaote, Pie Medical, Firenze, Italy), equipped with a 5–7.5-MHz linear veterinary probe, LV513.

As shown in Fig. 1, the B-mode ultrasound (7.5 MHz probe) was used for visualization of the testicular artery and testicular evaluation then the color Doppler mode (5 MHz probe) was used to confirm the artery. Pulsed-wave Doppler analysis was performed after locating the largest segment of the artery (convoluted part, middle of pampiniform plexus, Fig. 1A) at the spermatic cord, where the probe was located horizontally in a lateromedial plane, perpendicular to the spermatic cord and close to the abdomen (Gacem *et al*., 2020). For the marginal part of the testicular artery, the probe placed in oblique plane (dorso-ventral) with about 70 inclination angle at edge of the testes close to the tail of the epididymis (Fig. 1B, Gacem *et al*., 2020). When at least three similar and successive arterial Doppler waveforms with a maximum Doppler shift were displayed, the image was frozen and saved for analysis. The four indices recorded for each of the two locations were: pulsatility index (PI) [PI = maximum velocity-minimum velocity); resistive index (RI) [(peak systolic velocity – end diastolic velocity) / peak systolic velocity]; systolic velocity peak (SVP) and end diastolic velocity (EDV). Moreover, the Systolic velocity/diastolic velocity ratio (SV/DV ratio) was also recorded. All measurements were obtained with an angle of insonation between 30 and 60 and with a gate setting of 1 mm (Ginther and Utt, 2004). All examinations were performed by one operator to avoid the individual variations and minimized the gaps in interoperation techniques.

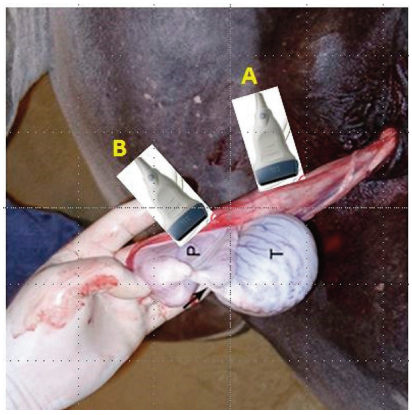

Fig. 1. Transducer orientation: (A) spermatic cord – convoluted part; (B) epididymal edge – marginal aspect (T, testis; P, tunica vaginalis paraitalis).

Statistical analysis

The normality of the data was examined using the Shapiro–Wilk test. For data summary and analysis, the values obtained on the three spectral waves were averaged to obtain a single mean value for each measure at each location. Mean values for the left and right testes were compared using paired t-tests. Similarly, parameters measured for the convoluted and marginal aspects of the testicular artery were compared using paired t-tests. The relationship of male donkey age with each of the four parameters was evaluated using Pearson's correlation analysis. Furthermore, differences between the two age groups for all the measures were analyzed, using paired t-tests. Significance was set at P < .05. All collected data were statistically analysed using SAS (SAS, 2008).

Results

The testicular measurements of 14 mature male donkeys are presented in Table 1. All testes were descended into the scrota of the immature donkeys (cryptorchidism was not recorded). The testicular dimensions did not show any difference between the two sides (right and left, Table 1). Two-dimensional B-mode ultrasonography images allowed for the establishment of homogeneous echogenicity of the testis. The mediastinum was well visualized as a centrally positioned hyperechoic line in mature males, but was tedious to be seen in immature individuals. Using color Doppler, the visualization of the testicular artery at the level of spermatic cord was easy and was more difficult at the marginal segment (epididymal edge). All measurements of the 28 testes in mature males were presented in Table 2. Values of PI at the convoluted segment (spermatic cord) were significantly higher (1.90±0.1, P< 0.05) than that at marginal aspect (1.39±0.06, epididymal edge). The difference between values of RI at the convoluted segment (spermatic cord, 0.84±0.01) and the marginal aspect (epididymal edge, 0.67±0.01) was not significant. Values of SVP and SV/DV ratio showed similar trends (41.37±2.5, 5.78±0.28 vs. 28.46±1.71, 3.20±0.06 respectively, P< 0.05, Table 2). In the opposite of that, values of EDV at the marginal aspect were significantly higher (9.49±0.45,) than that at the convoluted segment (5.89±0.29, P< 0.05) (Table 2).

Table 1. Testicular measurements of 14 mature male donkeys

	Left (cm)	Right (cm)
Testicular length	9.63±0.21	9.69±0.13
Testicular width	6.36±0.14	6.28±0.1
Testicular depth	6.51±0.14	6.58±0.11

The values of all measured parameters RI, PI, SVP, EDV and SV/DV at the convoluted segment in mature animals were higher than that in immature donkeys (Figs. 2 and 3). All previous parameters were very difficult and tedious to be measured at marginal aspect during pre-pubertal period in male donkeys.

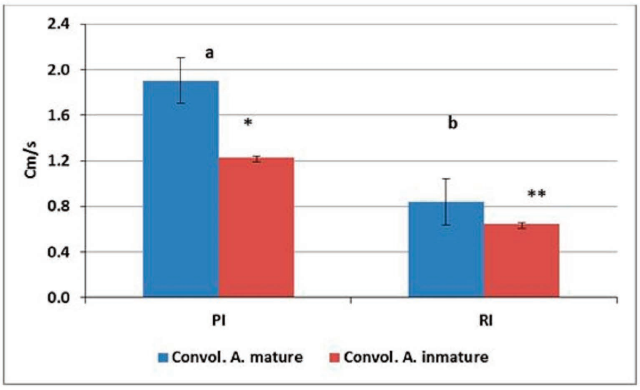

Fig. 2. Mean values and SEM of pulsatility index (PI, cm/s), resistance index (RI, cm/s) in the testicular artery at convoluted aspect in mature (n = 14) and immature (n= 10) male donkeys. (different letters and astricks mean significant difference)

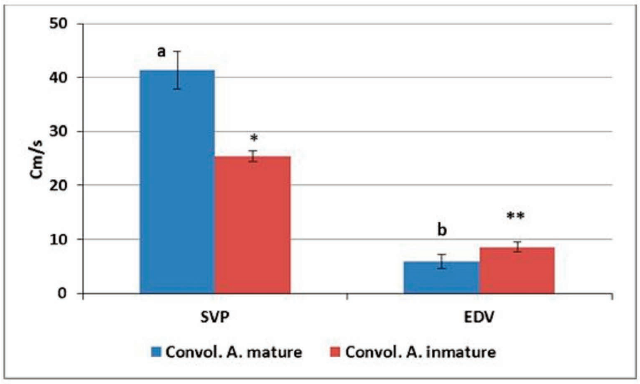

Fig. 3. Mean values and SEM of Systolic velocity peak (SVP, cm/s) and End diastolic velocity (EDV, cm/s) in the testicular artery at convoluted aspect immature (n.= 14) and immature (n.= 10) male donkeys (different letters and astricks mean significant difference)

There were non-significant correlations between each of the four blood flow measurements and the age (PI, r = –0.065; RI, r = –0.07; SVP, r = 0.16 and EDV, r = –0.25, P> 0.05, Fig. 4).

The waveforms of blood flow obtained in the donkeys' testicular artery were reported at the two sites of interrogations. The waveforms at the convoluted segment were predominantly biphasic and characterized by a major difference between the systolic and diastolic velocity of blood flow and high RI (resistive) values (Fig. 5a). With only one, systolic peak (non-resistive) and diastolic velocity slowly decreased during the cardiac process, only two individuals displayed monophasic

Table 2. Blood flow measures of testicular of 14 mature male donkeys (n. = 28 testes)

	Convoluted testicular artery (Spermatic cord)		Marginal testicular artery (near Epididymis tail)	
	Mean ± SEM	Range	Mean ± SEM	Range
PI (cm/s)	1.90±0.1[a]	1.41-2.47	1.39±0.06[b]	1.32-1.76
RI (cm/s)	0.84±0.01	0.76-0.91	0.67±0.01	0.61-0.70
SVP (cm/s)	41.37±2.5[a]	30.2-53.0	28.46±1.71[b]	21.9-42.3
EDV (cm/s)	5.89±0.29[a]	4.5-8.1	9.49±0.45[b]	7.9-11.2
SV/DV	5.78±0.28[a]	4.16-7.77	3.20±0.06[b]	3.14-3.38

The Doppler indices including; pulsatility index (PI), resistance index (RI), Systolic velocity peak (SVP), end diastolic velocity (EDV) and Systolic velocity/Diastolic velocity ration (SV/DV).
Values with different superscripts are significantly differ (P< 0.05)

waveforms in the convoluted aspect; the discrepancy between SVP and EDV and the derived RI values was small (Fig. 5b). Waveforms obtained from the testicular artery's marginal aspect (epididymal edge) were "non-resistive" and monophasic (Fig. 6a), also in only two cases the reported waveforms were resistive (biphasic) at the marginal aspect of the testicular artery (Fig. 6b).

The mean examination time of 15-20 minutes was considered adequate for visualizing both sides of the artery's two locations (convoluted and marginal). The longest time was needed to evaluate the marginal region.

Discussion

The testicular measurements did not differ between sides (right and left testes); a possible explanation for these results may be related to the sample of this study being composed of one breed of male donkeys (Egyptian) as well as a relative few number of the examined animals.

Several authors investigated the correlation between testicular hemodynamics and semen quality in both humans and certain animal species (Pinggera *et al*., 2008; Zelli *et al*., 2013; Ortiz-Rodriguez *et al*., 2017; Gloria *et al*., 2018). However, in this study we did not tackle issues of testicular vasculature association with semen properties because the aim primarily was focused on the feasibility of pulsed wave Doppler for evaluation of testicular hemodynamics during pre and post-pubertal periods in Egyptian male donkeys. On the other hand, Gacem *et al*. (2020) found that color pulsed-wave Doppler ultrasound imaging showed an important reduction in testicular artery blood flow at the level of the pampiniform plexus compared to that of the capsular artery, which would result in a reduced testis temperature and tissue oxygen pressure, and influence sperm quality. Similar results were recorded in the current study that concerned to Doppler-measured testicular artery blood flow variables in the jackass. Since the use of

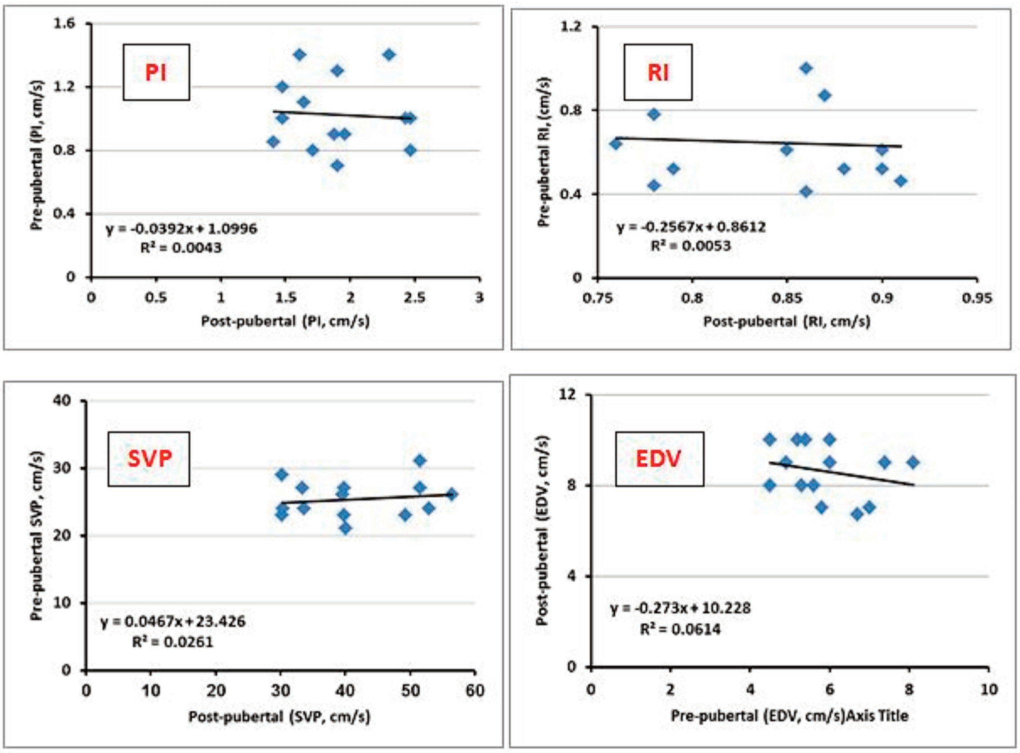

Fig. 4. Mean values of pulsatility index (PI, cm/s), resistance index (RI, cm/s), Systolic velocity peak (SVP, cm/s) and End diastolic velocity (EDV, cm/s) in the testicular artery at covoluted parts in Post-pubertal (n.= 14) and Pre-pubertal (n.= 10) male donkeys (scatter plot showing non-significant correlations between age and recorded indices).

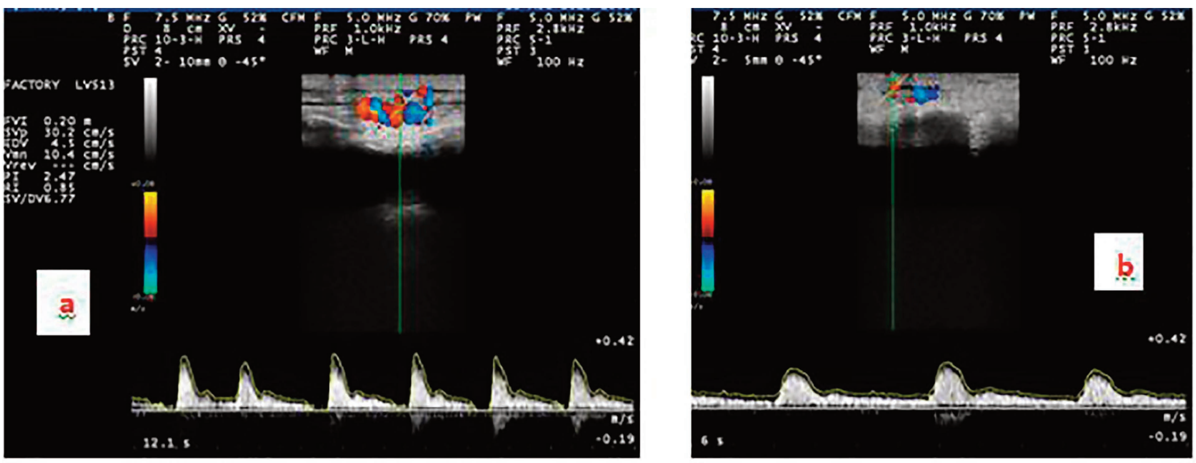

Fig. 5. Convoluted aspect of testicular artery: Spectral wave-forms of testicular blood flow in male donkey. (a) Biphasic wave-form. (b) Monophasic waveform.

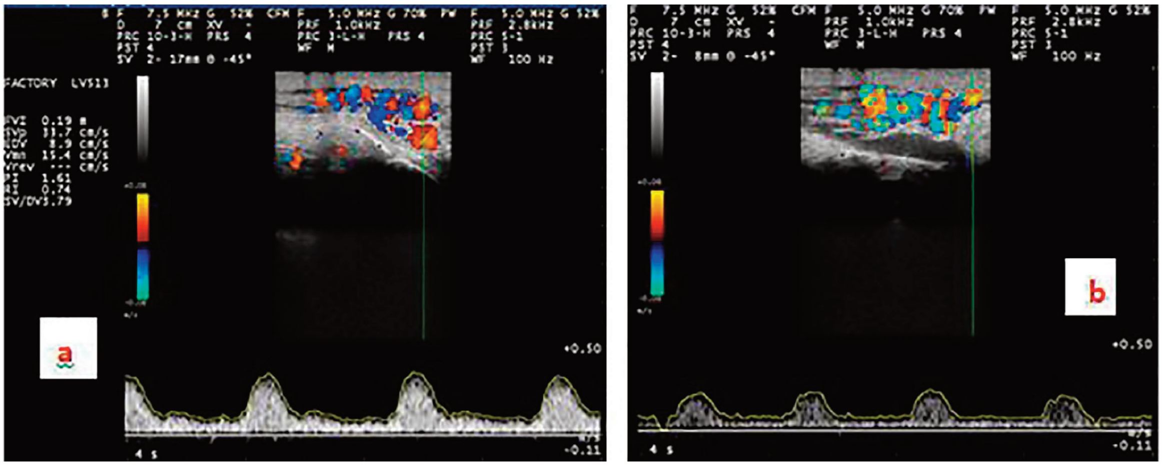

Fig. 6. Marginal aspect of testicular artery: Spectral wave-forms of testicular blood flow in male donkey. (a) Biphasic wave-form. (b) Monophasic waveform.

Doppler in human medicine is already well established for the evaluation of testicular perfusion (Middleton *et al*., 1989; Dubinsky *et al*., 1998), further studies are recommended to clarify the relationship between testicular hemodynamics, semen quality and resulted fertility in Egyptian donkeys. This modality seemed to be more effective than gray-scale ultrasound in visualizing the lumen of the testicular artery. Color Doppler appeared to improve the precision of blood flow measurements and the estimation of indices due to improved insonation (Pozor and McDonnell, 2004). Doppler indices are used to provide information on blood flow and vascular impedance that cannot be derived from information about the velocity alone (Pozor, 2007). However, although Color Doppler ultrasound greatly facilitated the measurement of blood flow in the stallion's testicular artery, the tortuous route of the testicular artery in the sperm cord and the epididdymal edge of the stallion testis make the visualization of longitudinal luminal sections time-consuming and tedious (Pozor and McDonnell, 2004). Similarly, in the current study it was noticed also that the monitoring of the convoluted aspect of the testicular artery was easier than that at marginal segment (epididymal edge).

Waveforms of blood flow to human and canine testes seem non-resistive and monophasic in nature (Middleton *et al*., 1989; Günzel Apel *et al*., 2001; Gumbsch *et al*., 2002). The obtained results consistent with that recorded before (Pozor and McDonnell, 2004), where the blood flow to donkey testes at the level of spermatic cord was mostly resistive, biphasic waveforms but was mostly non-resistive, monophasic waveforms at the marginal aspect of testicular artery. We agree with the explanation given earlier that the resistive character of the waveforms of the convoluted part of the stallion testicular artery may be due to the light oblique (craneo/caudal inclination) orientation of the long axis of testes, located near the body wall, and the relatively short sperm cord with a highly convoluted artery. In this study, it was not possible to identify the testicular artery at the marginal aspect in pre-pubertal male donkeys due to small testicular volume. The blood flow in intra-testicular arteries in the stallion could not be assessed due to instrumentation limitations (Pozor and McDonnell, 2004), similarly in the current work; the Doppler-measured testicular artery blood flow variables in the jackass could not be assessed. It was found also in dogs that Doppler evaluation of the testicular artery was easy to perform, except in the intra-testicular region due to low blood flow velocity and the small size of the vessel, which require the use of low PRF and high Doppler gain, thus raising the probability of aliasing artifacts (Günzel Apel *et al*., 2001; Gumbsch *et al*., 2002; Zelli *et al*., 2013). In previous studies, up to 41.5% of examined male dogs did not identify the testicular artery (Gumbsch *et al*., 2002). On the other hand, it was reported that the biometric and arterial indices measured during ultrasonography of the testes and male genital tract in bulls are affected by animal breed and age (Rodrigues *et al*., 2020).

During the cardiac cycle, two of the measures (SVP and EDV) represent blood flow velocities in the arterial vessels. However, the EDV values are considered to be substantially variable and inconsistent between measurements (Cochard *et al*., 2000). Calculated indices (RI and PI) appeared more sensitive to arterial blood flow measures than SVP and EDV, as they offered knowledge not just about velocity but also about vascular impedance. In addition, testicular artery RI appeared to be the most useful clinical measure of blood flow to and within the testis and epididymis. Inflammatory pathways normally change this (Jee *et al*., 1997) and aging (Wielgos *et al*., 1998). In case of epididymo-orchitis and due to hyperemia, there were reduced RI values, although it rose in aging males, most likely due to degenerative changes and high testicular tissue resistance. In human begins, RI was also smaller as well as was inversely proportional to the histology ranking in undescended testes (Atilla *et al*., 1997). In the present group of donkeys, for the majority of blood flow measures of the testicular artery affected significantly with the donkey's age. This could be related to the large testicles in mature donkeys requiring more blood than in immature groups. However, a greater number of such cases should be studied to determine the clinical significance of this condition.

Conclusion

Color Doppler ultrasonographic characterization of the blood flow of the donkeys' testis is possible, and it is likely to become a useful tool for objective evaluation of the testes. The current data may provide appropriate normal values for testicular perfusion in healthy donkeys. Further experiments on both normal and abnormal conditions are required to explain the usefulness of this technology in diagnosis of infertility problems in male donkeys.

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Conflict of interest

The authors declare that they do not have any conflict of interest.

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