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Color Doppler ultrasound evaluation of testicular blood flow in stallions

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Abstract

The objectives of this study were to evaluate the potential use of color Doppler ultrasound to characterize blood flow to the stallion testis, and to establish reference values for Doppler measures of blood flow in the testicular artery of the stallion. Both testes from each of 52 horses were examined using a pulsed-wave color Doppler ultrasound with a sector array 5/7.5 MHz transducer with a 1 mm gate setting. Peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI), and pulsatility index (PI) of the testicular artery were measured in each of two locations, the convoluted aspect (spermatic cord) and the marginal aspect of the artery (on the epididymal edge of testis). We found that: (1) all measures were obtainable; (2) except for EDV, the majority of the measures were higher at the cord location than at the marginal aspect of the artery ($P < 0.05$); and (3) measures for left and right testes were similar ($P > 0.10$). Resulting measures from 41 of these stallions (82 testes) that appeared free of testicular pathology provide useful reference values for clinical evaluation. Evaluation of 11 cases with testicular pathology suggested further investigation of possible effects of these various conditions on testicular blood flow and testicular function.

Keywords: Stallion; Testis; Artery; Color Doppler; Equine; Pathology

1. Introduction

Color Doppler ultrasound has become a method of choice to evaluate vasculature of various organs, including testes. In human medicine, this technique has been employed to evaluate blood flow in the testicular artery and has been applied in diagnosing testicular

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pathologies associated with altered blood flow, such as torsion of spermatic cord, testicular infarction, or varicocele [1–4]. Color Doppler ultrasound appears to be useful in identifying early inflammatory or neoplastic changes of the testes and epididymides, as well as in evaluating other scrotal disorders of men [5,6]. Color-flow imaging of testicular vasculature has been reported to be essential for distinguishing between torsion of spermatic cord (lack of blood flow) and epididymoorchitis (increased blood flow) [7]. The characteristic pulsed Doppler waveforms of the testicular artery at various locations have been described and reference values of the measures of blood flow in this vessel have been reported for men [8].

In recent years, Doppler ultrasonography has become increasingly available for use in veterinary practice. To date, published Doppler work on the peripheral vasculature in horses includes blood flow characteristics of hind and thoracic limb arteries of normal horses, renal arteries, carotid artery, and uterine and ovarian arteries [9–14]. The use of pulsed-wave, gray-scale Doppler ultrasonography to characterize blood flow in the testicular artery of stallions has been described recently [15]. It has been shown that this method can be applied to objectively measure blood flow of the testicular artery in this animal. Furthermore, that work indicated that sedation with xylazine did not significantly affect measures of blood flow, with the exception of end diastolic velocity (EDV) in the convoluted aspect of testicular artery at the level of spermatic cord. This effect did not significantly alter derived indices of resistivity and pulsatility. Gray-scale Doppler ultrasound has its limitations, however. Identification of small vessels is difficult and

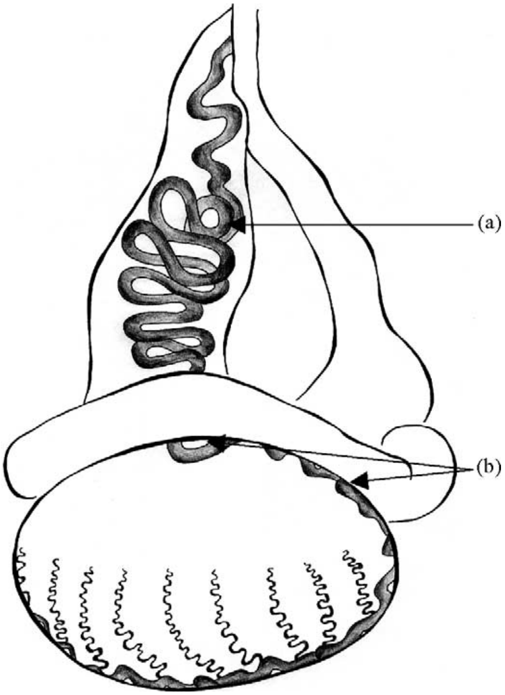

Fig. 1. Testicular artery in the stallion – scheme (a) convoluted aspect and (b) marginal aspect.

evaluation of overall vascularization of the parenchyma of stallion testis is usually not possible with this technology. Also, due to the extremely convoluted course of testicular artery through the testicular cord and the quite tortuous route of this vessel along the testicular edge in stallions (Fig. 1), obtaining images of truly longitudinal sections of its lumen, visible as black thick lines, is technically challenging. Color-coded Doppler ultrasound may help to identify the best locations of elongated fragments of the testicular artery and improve Doppler insonation angle. Furthermore, it may allow visualization of small intratesticular vessels.

The objectives of the present study were to: (1) determine the feasibility of Color Doppler ultrasound for evaluating the vasculature of the stallion testis and (2) to obtain reference values for measures of blood flow in the testicular artery of stallions.

2. Materials and methods

Fifty-two stallions of 10 various horse breeds (Silesian, Arabian, Anglo-arab, Thoroughbred, Oldenburg, Malopolski, Wielkopolski, Half-blood, Hanovarian, Ardennes) aged 3–22 years, were used in this study. All stallions were owned by one of three studs in Poland where they were engaged in active breeding programs. Examinations were done during the physiologic breeding season (May and June 2002).

For examination, stallions were simply restrained by a halter and lead without tranquilization. Standard blinkers were used to obstruct the stallion's view of the ultrasound monitor. For each stallion, each of the two testes was evaluated using pulsed-wave Color Doppler ultrasound with a sector 5/7.5 MHz transducer (300S Pandion Vet, Pie Medical Equipment B.V., Maastricht, The Netherlands). The minimum sample gate setting for this unit (1 mm) was used and angle correction was between 30 and 60°.

As shown in Fig. 2, the transducer was initially positioned to enable visualization of the lumen of the convoluted and the marginal aspects of the testicular artery as red and blue

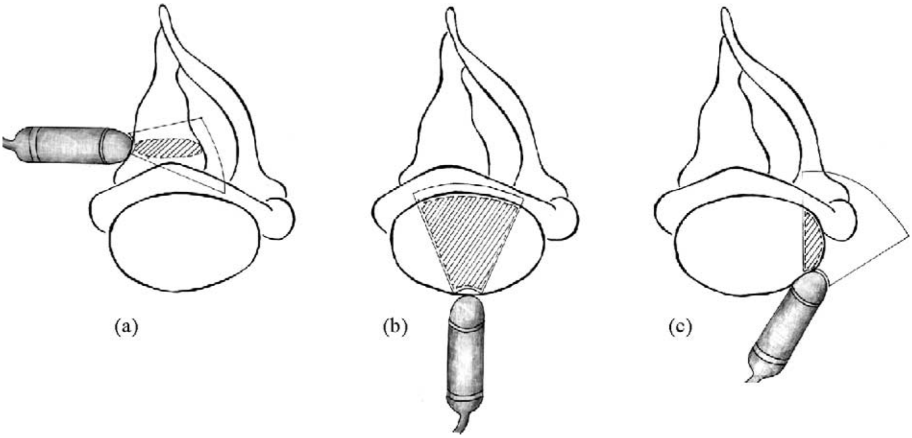

Fig. 2. Transducer orientation: (a) spermatic cord – convoluted aspect; (b) epididymal edge – marginal aspect; and (c) caudal pole – marginal aspect.

areas. After locating the largest and possibly longitudinal or oblique section of the artery in each location (convoluted aspect at the spermatic cord and marginal aspect of the testicular artery on the epididymal edge of testis, close to the caudal pole of the testis), pulsed-wave Doppler analysis was performed. Doppler images and waveforms were stored on videotape for subsequent analysis and calculations.

Using the algorithm package provided with the unit, four measures of blood flow in the testicular artery were calculated for each of the two locations for each of three sweeps per location. The four measures were: peak systolic velocity (PSV); end diastolic velocity (EDV); resistive index (RI) [(PSV − EDV)/PSV]; and pulsatility index (PI) [$\mathrm{PI} = (\text{maximum velocity} - \text{minimum velocity})/\text{mean velocity}$]). For data summary and analysis, the values obtained on the three sweeps 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, measures for the convoluted and marginal aspects of the testicular artery were compared using paired *t*-tests. The relationship of stallion age with each of the four measures was evaluated using Pearson's correlation analysis. Furthermore, differences between four age groups for all the measures were analyzed, using one-way ANOVA (age 3–5 years, $n = 26$; age 6–10 years, $n = 24$; age 11–15 years, $n = 18$; age 16–22 years, $n = 14$).

3. Results

Color-coded Doppler ultrasound images of multiple cross-, oblique-, and short-longitudinal-sections of the convoluted aspect of testicular artery in the spermatic cord were easily visualized (Fig. 3a). Arterial pulsation of blood in this vessel was clearly visible during real-time scanning. Visualization of the marginal aspect of the testicular artery was technically more difficult than for the convoluted aspect. However, short longitudinal sections of the lumen of this fragment of the testicular artery were always readily visualized (Fig. 4a). Values of PSV and PI decreased as the artery coursed from the convoluted (spermatic cord) to the marginal (epididymal edge) aspect of the testicular

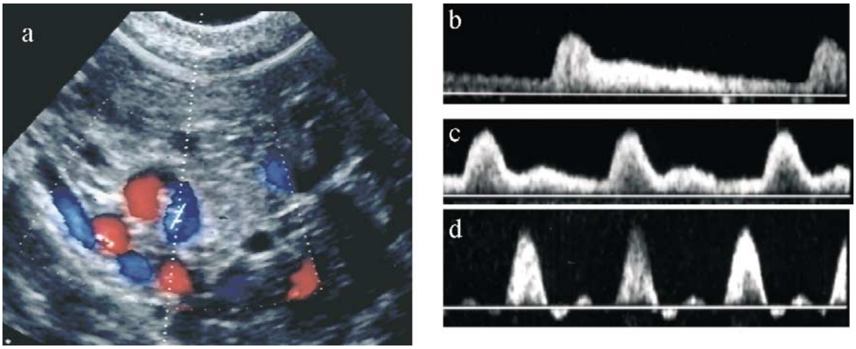

Fig. 3. Convoluted aspect of testicular artery: (a) Color Doppler ultrasound image; (b) monophasic non-resistive waveform; (c) biphasic, resistive waveform; and (d) biphasic, highly resistive waveform with retrograde flow.

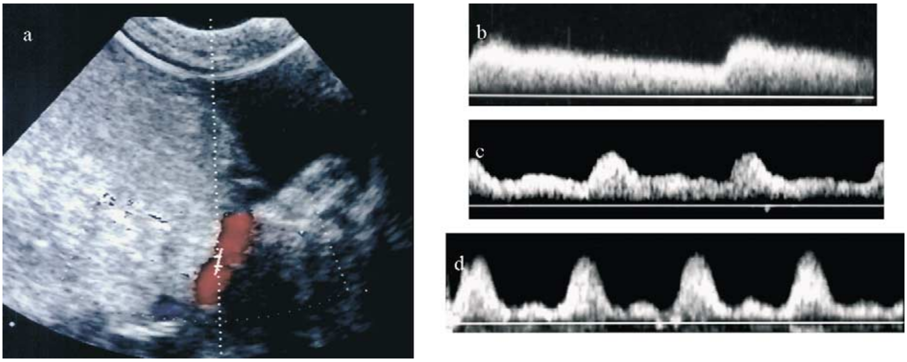

Fig. 4. Marginal aspect of testicular artery: (a) Color Doppler ultrasound image (b) monophasic non-resistive waveform (c) biphasic, non-resistive waveform; and (d) biphasic, resistive waveform.

artery ($P < 0.05$; Table 1). Values of RI showed a similar trend, however, these differences were not significant ($P > 0.10$). Only EDV increased significantly in the marginal aspect compared to the convoluted aspect of the testicular artery.

Table 1 summarizes results for 82 testes of 41 stallions. The remaining 11 stallions had various types of testicular pathology that were visualized with B-mode ultrasonography; therefore, these stallions were excluded from analysis of normal reference ranges. Measures for the left and right testicular artery were similar ($P > 0.10$, paired t-test). Intratesticular arteries were visible as small red or blue dots scattered throughout the parenchyma. These were too small to obtain pulsed Doppler measures of blood flow, probably due to the limitations of the equipment used.

Figs. 3 and 4 illustrate waveforms characterizing blood flow in the testicular artery of stallions at each location evaluated. Characteristics of the waveforms obtained from the convoluted aspect of the testicular artery appeared to vary among stallions from "resistive" to "non-resistive." "Resistive" waveforms were associated with a great difference between systolic and diastolic velocity of blood flow and with high values of RI. They were usually biphasic with clearly distinguishable systolic and diastolic peaks. "Non-resistive" waveforms were monophasic with only one, systolic peak. Diastolic velocity

Table 1
Blood flow measures of testicular arteries of 41 stallions ($n = 82$ testes)

Measures	Convoluted (spermatic cord)			Marginal (marginal artery)		
	Mean	Range	S.E.M.	Mean	Range	S.E.M.
PSV (cm/s)	26.1^{a}	12–51	0.91	22.2^{b}	8–59	1.22
EDV (cm/s)	5.4^{a}	0.3–14.7	0.31	7.9^{b}	3.5–20.0	0.42
RI	0.78	0.56–0.99	0.010	0.63	0.39–0.85	0.012
PI	1.99^{A}	0.96–3.96	0.076	1.15^{B}	0.55–2.29	0.045

S.E.M., standard error of the mean; PSV, peak systolic velocity; EDV, end diastolic velocity; RI, resistive index [$\mathrm{RI} = (\mathrm{PS} - \mathrm{ED})/\mathrm{PS}$]; PI, – pulsatility index [$\mathrm{PI} = (\text{maximum velocity} - \text{minimum velocity})/\text{mean velocity}$]. Values with different superscripts differ (a, b: $P < 0.05$; A, B: $P < 0.001$).

decreased gradually during the cardiac cycle; the difference between PSV and EDV, as well as the derived RI values was small. There were only a few examples of such a pattern in this location. Waveforms obtained from the marginal aspect of the testicular artery were mostly "non- resistive" and monophasic, however, "resistive" waveforms were also obtained in a few cases in this location. This pattern was observed in stallions with highly "resistive" waveforms at the convoluted aspect of the artery (spermatic cord).

Among the various blood flow measures, the greatest variation was observed in EDV in the convoluted aspect of the artery. In a few instances, retrograde diastolic flow was observed in this location, especially in the testes of four aged stallions > 18 years old.

Across the range of ages, the associations of age with each of the four blood flow measures were not significant (Pearson R: 0.004–0.29). Also, differences between age groups were not significant ($P > 0.10$) for any of the measures, except EDV and RI for the convoluted aspect of testicular artery. The EDV was significantly greater and RI was significantly lower for the 11–15 years age group than for the 16–22 years age group ($P < 0.05$; Figs. 5–7).

The 11 cases of pathology identified included four unilateral hydroceles, three varicoceles, two atrophic testes, one 180° torsion of spermatic cord, and one testicular tumor.

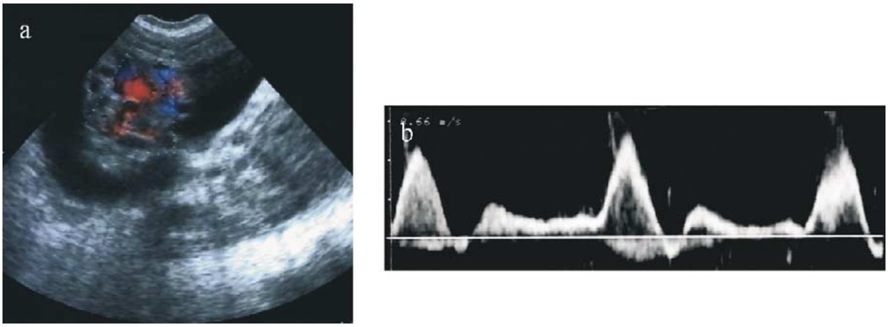

Fig. 5. Hydrocele (a) Color Doppler ultrasound image of the area of spermatic cord and (b) blood flow waveform of the convoluted aspect of testicular artery.

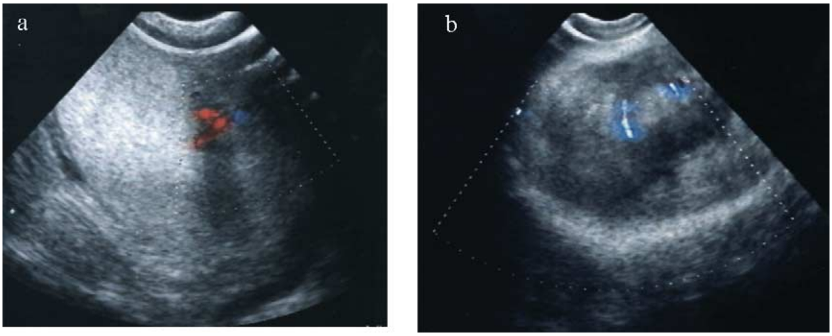

Fig. 6. Color Doppler ultrasound images of testicular pathologies: (a) vascular malformation within testicular parenchyma and (b) testicular tumor.

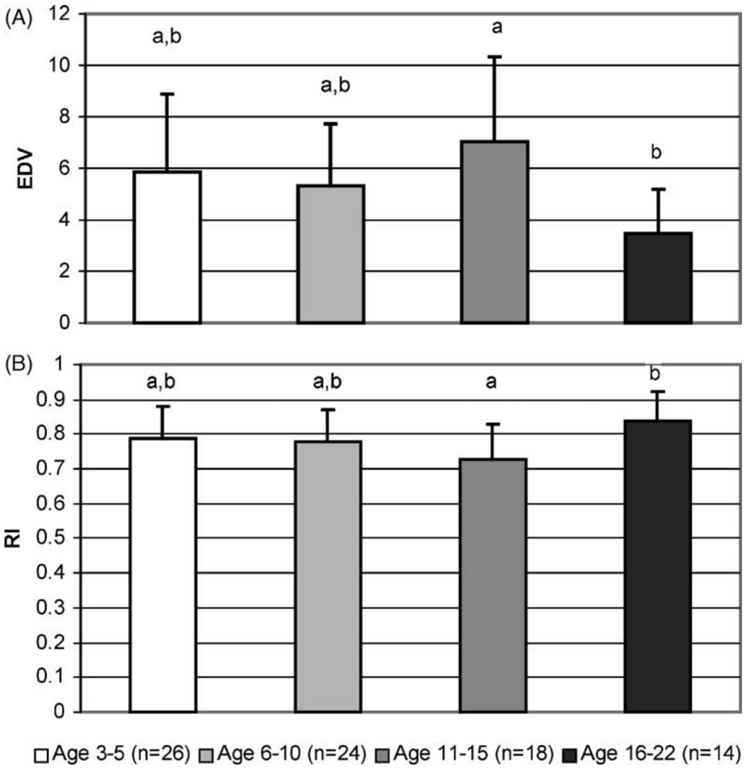

Fig. 7. Mean values and standard deviations of EDV: (A) and RI and (B) from convoluted aspect of testicular artery, for four age groups. (A and B) Values with different letters (a, b) differ significantly $P < 0.05$.

For all of these stallions, except for the case with testicular tumor for which blood flow measures were not obtained, all measures of blood flow in the testicular artery of the affected side fell within normal limits (Table 2). However, a trend for high values of PSV, RI and PI, especially in cases with varicocele, was observed (Fig. 8). Also, in a few instances, ultrasound images, as well as the blood flow waveforms, were unusual.

In four instances of mild–moderate hydrocele, testicular blood flow did not appear affected. In one severe case, with a presence of thick ‘‘layer’’ of fluid within the vaginal cavity surrounding the entire testis as well as the spermatic cord, turbulent blood flow in testicular artery at the level of the spermatic cord was evident. The contour of the waveforms of blood flow obtained in this case suggests the presence of a substantial amount of blood moving slowly forward or even backwards during systolic phase of the cardiac cycle, while the majority of blood was moving forward forming the characteristic systolic peak (Fig. 5). Early diastolic retrograde flow of blood cells in the testicular artery was also observed in the case of 180° torsion of the spermatic cord.

In all three instances of varicocele, the Color-coded image of arterial blood flow enabled ready distinction of the distended lumen of the venous network (pampiniform plexus) or

Table 2
Blood flow measures of testicular arteries of 10 stallions with testicular pathologies

Measures	Mean (range)			
	Testes with hydrocele ($n = 4$)	Testes with varicocele ($n = 3$)	Atrophic testes ($n = 2$)	180° Torsion of spermatic cord ($n = 1$)
Convoluted				
PSV (cm/s)	32.6 (24.3–43.3)	31.0 (26.3–37.3)	36.3 (27.0; 45.5)	28.7
EDV (cm/s)	4.35 (2.70–5.30)	5.67 (4.30–7.00)	7.85 (8.00; 9.70)	2.70
RI	0.84 (0.79–0.90)	0.81 (0.77–0.84)	0.76 (0.65; 0.87)	0.90
PI	2.57 (2.16–3.12)	2.05 (1.65–2.34)	1.79 (1.22; 2.35)	2.58
Marginal				
PSV (cm/s)	23.4 (13–32.3)	19.8 (19.0–20.5)	27.2 (23.3; 31.0)	13.0
EDV (cm/s)	5.93 (4.70–8.00)	7.65 (7.00–8.30)	11.35 (9.70; 13.00)	7.00
RI	0.71 (0.57–0.87)	0.62 (0.57–0.67)	0.58 (0.57; 0.59)	0.46
PI	1.53 (0.97–2.30)	1.16 (0.89–1.43)	0.97 (0.96; 0.97)	0.60

PSV, peak systolic velocity; EDV, end diastolic velocity; RI, resistive index $[\mathrm{RI} = (\mathrm{PS} - \mathrm{ED})/\mathrm{PS}]$; PI, pulsatility index $[\mathrm{PI} = (\text{maximum velocity} - \text{minimum velocity})/\text{mean velocity}]$.

lymphatic system from the lumen of the artery. The velocity of blood flow in the venous system was too low to be detected by our equipment, so the flow was not visible in Color on the screen.

One atrophied testis had an enlarged arterial vessel within the testicular parenchyma (vascular malformation) in the region of possible testicular trauma (Fig. 6a). All measures of blood flow in this vessel were within the limits presented here for normal stallions for the marginal aspect of testicular artery.

The testis with a tumor was enlarged, with a denser texture and heterogeneous echostructure. Numerous arterial vessels, with characteristic pulsation and Color-coded blood flow, were visible within the capsule surrounding this pathology as well as inside the

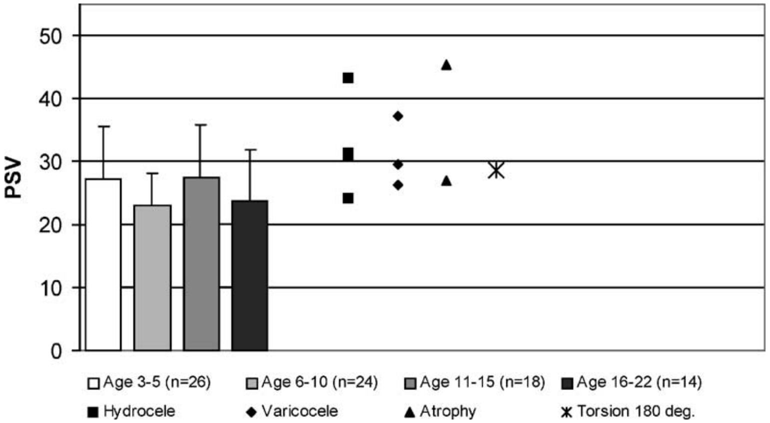

Fig. 8. Mean values and standard deviations of peak systolic velocity (PSV) from convoluted aspect of testicular artery, for four age groups (bars) and PSV values for testes with pathologies (scatter plot).

tumor itself (Fig. 6b). However, measures of blood flow in these vessels were not obtained, possibly due to low sensitivity of equipment used in this study.

4. Discussion

Color Doppler ultrasound was used in the stallion to objectively evaluate blood flow to the testes. This technique appeared to be more reliable in visualizing short fragments of the longitudinal sections of the lumen of the testicular artery than gray-scale ultrasound. Due to better insonation, Color Doppler seemed to improve the accuracy of the measurements of blood flow velocities and calculation of indices. However, even though Color Doppler ultrasound greatly facilitated measure of blood flow in the testicular artery of stallions, due to the tortuous route of the testicular artery in the spermatic cord and on the epididymal edge of stallion testis, visualizing longitudinal luminal sections remains time-consuming and tedious.

Waveforms of blood flow to human and canine testes appear to have "non-resistive" and monophasic character [8,16,17]. We have obtained mostly "resistive", biphasic waveforms of blood flow to stallion testes at the level of spermatic cord and mostly "non-resistive", monophasic waveforms from the marginal aspect of testicular artery. Middleton et al. [8] suggested that high-resistance waveforms, occasionally obtained in men, might have originated from sampling arteries other than testicular arteries in the area of spermatic cord, such as the cremasteric and deferential arteries. However, these vessels are located on the periphery of the spermatic cord in the stallion, and it is quite unlikely that they would be visualized in the central area of spermatic cord. "Resistive" character of the waveforms from the convoluted part of the stallion testicular artery may be due to the horizontal orientation of the long axis of testes, located close to the body wall, and relatively short spermatic cord with highly convoluted artery.

Middleton et al. [8] described Doppler waveforms of the arteries of testes of normal men and established reference values for measures of testicular blood flow. Similar to our results, they reported that the velocity waveforms of supratesticular arteries were quite variable, in contrast to capsular and intratesticular arteries. The RI for capsular and intratesticular arteries of human testes were smaller than in supratesticular arteries, while in stallions, values of mean RI in the marginal aspect of the artery were only slightly lower than in the convoluted aspect. This difference was statistically significant. Due to limitations of the instrumentation, we were not able to evaluate blood flow in intratesticular arteries.

Of the four measurements that were obtained in this study, two directly reflect velocities of blood flow in arterial vessels during the cardiac cycle (PSV and EDV). However, values of EDV are known to be considerably variable and not consistent between measurements [9]. Calculated indices (RI and PI) seemed to be more sensitive indicators of arterial blood flow than PSV and EDV, since they provided information on vascular impedance, not only velocity. Of these two indices, RI was more sensitive in differentiating abnormal waveforms, especially for the assessment of transplanted kidney [18]. Furthermore, the RI of the testicular artery seemed to be the most useful clinical measure of blood flow to and within the testis and epididymis. It is usually altered by inflammatory processes [19] and aging [20]. Epididymoorchitis was consistently associated with decreased values of RI, due to hyperemia, while it was increased in aging males, most likely due to degenerative changes and increased resistance of testicular tissue. Also, RI in humans was lower in undescended than in descended testes [21]; the RI values were inversely proportional to testicular histology score. Recently, Biogiotti et al. have suggested that RI, as well as PSV values, are reliable indicators of sperm production, since these parameters were highly correlated with ‘‘sperm production rate score’’ [22]. Surprisingly, in the dog [23], varying degrees of spermatic cord torsion had no measurable effect on RI of the capsular of parenchymal artery, while in rats a high degree of testicular torsion decreased RI, culminating in non-pulsatile Doppler waveforms, obtained at the level of spermatic cord [24]. The difference in results may be due to different locations of the evaluated vessel, different species, and technique.

In the present group of stallions, for the majority blood flow measures of the testicular artery, age was not a significant factor. However, old stallions had lower values of EDV and greater values for RI than ‘‘middle-age stallions’’ (11–15 years) in the convoluted aspect of testicular artery. It was shown that RI values were lower in prepubertal than in pubertal boys and were increased in aging men [20,25]. Furthermore, it was postulated that larger volume of testes are associated with lower RI [25].

Retrograde diastolic blood flow seen in a few old stallions suggested some age-related vascular changes. Similarly, retrograde blood flow in the case of 180° torsion of spermatic cord suggested an effect of this condition on testicular function, even in the absence of clinical signs of substantial vascular compromise. We have seen a few other cases of 180° torsion of spermatic cord in stallions that were also associated with retrograde blood flow (Pozor and McDonnell, unpublished data). Inversion of diastolic blood flow, as well as increased RI were also observed in cases with incomplete torsion of the spermatic cord in men [1]. Therefore, a larger number of such cases should be studied in order to determine the clinical significance of this condition.

Turbulent blood flow was seen in one severe case of hydrocele. It would be useful to study blood flow in the testicular artery in cases of hydrocele of varying severity.

Color Doppler ultrasound proved to be helpful in visualizing certain pathological conditions of the scrotum in men due to characteristic distribution of blood vessels in normal testicular parenchyma versus inflammatory or neoplastic changes [7]. We have seen characteristic vascularization of testicular tumor in the stallion and abnormal course of large blood vessels within the testicular parenchyma after trauma (vascular malformation).

Obtaining repeatable values of Doppler measures of arterial blood flow was technically challenging. Therefore, the majority of authors use average values obtained from two to seven sweeps [11–13]. In this study, we did not address issues of repeatability and reproducibility of measures. Of particular interest would be the effect of technician experience on measures for the various parameters, and the number of measurements needed per each parameter to achieve efficient accuracy. These have been reported to be important factors in Doppler evaluation of various blood vessels [26–29]. Further work designed specifically to address questions of reliability of Doppler measures of stallion testicular arteries should be done.

In conclusion, Color Doppler ultrasonographic characterization of blood flow of the stallion testis is possible, and will likely become a useful tool for objective evaluation of the stallion testis, particularly in cases of various scrotal disorders. These data, representing 52 stallions of various ages, breeds and size types, provide reasonable reference values as a starting point for clinical evaluation. Further data, both for normal and abnormal testes, are needed to improve the usefulness of this technology.

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