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Original Research Article

Hemodynamic Changes of the Uterine Artery in Mares With Different Uterine Pathologies

Key words

Doppler ultrasonography;
endometritis;
intrauterine cyst;
notch

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Abstract: Cysts, fluid accumulation, inflammatory changes and tumors are common uterine pathologies in mares. It was aimed to investigate the change of uterine hemodynamics in mares according to uterine pathologies (endometritis or intrauterine cysts). The study contained 28 mares and the groups formed as 9 gynaecologically healthy diestrus mares (Group H), 9 mares with endometritis (Group E) and 10 mares with intrauterine cyst (Group C). The mean uterine diameter (UD) in Group H (2.29±0.18 cm) was significantly lower than in Group C (2.97±0.19 cm) (P<0.05) and in Group E (3.47±0.29 cm) (P<0.01). The diameters of the intrauterine cysts ranged between 0.51 cm and 1.83 cm in Group C. The highest diameter of the uterine artery (UA) was observed in Group E. Hemodynamic indices of UA (PI and RI) in Group E were not significantly different from those in Group C (P>0.05). However, PI and RI values in Group H were significantly lower than both in Group E and Group C (P<0.001). The UA waveform in Group E had an early diastolic notch while systolic notches and small diastolic peaks were observed in the UA waveforms of the mares in group C. Doppler USG is considered as a useful tool to analyze the waveform and hemodynamics of the UA related to the different pathologies (cyst or endometritis). It was concluded that not only Doppler indices but also characteristics of the waveforms of the UA should be evaluated to understand the hemodynamic effect of the uterine pathologies.

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Introduction

Doppler ultrasonography is a non-invasive technique that enables the examination of physiological and pathological changes in the blood supply of the female reproductive system (1). Pulsatility index (PI) and resistance index (RI) values, which are angle-independent, are used to identify abnormal waveforms (2). The PI characterizes blood flow of the uterine artery (UA) and exhibits a wave-shaped profile throughout the estrous cycle in mares, which are negatively correlated with plasma estrogen levels during estrus (3). In humans, decreased uterine blood flow and poor uterine perfusion during the luteal phase are associated with reduced chances of conception and infertility (4).

The most common forms of uterine pathology detected by ultrasonography in the non-pregnant mare are accumulations of intrauterine fluid, inflammatory changes (increase in uterine wall thickness, edema of the endometrial folds, presence of luminal content, etc.), uterine cysts (5). Uterine cysts are fluid-filled and they can be glandular or lymphatic in origin. Glandular cysts, which are typically microscopic features, originate from the endometrial glands. These cysts are commonly undetectable by reproductive ultrasonography and are usually <10 mm in diameter (6). The second form of uterine cysts is lymphatic in origin and generally is larger than endometrial cysts. Most uterine cysts detected by ultrasonography that protrude into the uterine lumen are lymphatic. Lymphatic cysts can be measured from as small as a few millimeters to as large as several centimeters and they are pedunculated or sessile. The establishment of pregnancy may be impaired in mares with multiple large (> 3 cm) cystic areas (single cyst or cluster of multiloculated cysts) (7).

Rectal palpation and ultrasound examinations identify the uterine fluids that may be indicative of endometritis. An endometrial swab or endometrial biopsy provides the

microscopic analysis of the uterine pathology (8). Although different methods have also been used to diagnose endometrial diseases, the most effective and practical technique used to collect endometrial material has been the commercial cytobrush/swab collector (9). Rahawy and colleagues (10) have recommended ultrasound and cytological techniques for the diagnosis of endometritis in mares. Doppler ultrasound improved the diagnosis of endometritis as it showed alterations in uterine vascularization (11). Da Silva-Álvarez and colleagues (12) recommended power Doppler ultrasound in combination with computerized image analysis which has the potential to be a very useful tool in the diagnosis of endometritis in mares. In addition, real-time characterization of uterine blood flow and changes in uterine vascular perfusion and mesometrial PI in response to semen infusion in mares were evaluated (13).

Considering all the above, we hypothesize that pulsed Doppler ultrasound could detect uterine hemodynamic alterations associated with uterine pathologies (endometritis or intrauterine cysts) in equines. It was aimed to determine if the indices of UA are a good marker of uterine pathologies in mares.

Materials and methods

General design

In total, 50 mares were examined to get the number of 28 mares in this study. The study groups were formed as 9 gynaecologically healthy mares (group H), 9 mares with endometritis (group E) and 10 mares with intrauterine cysts (group C). The mares both with cysts and endometritis were not included in the study. The mares were not mated or inseminated during the study. The cytological examinations were performed luteal phase of the estrous cycle. All ultrasound evaluations were performed in mid-diestrus, 10 to 12 days after ovulation.

Animals and experimental groups

Twenty eight Arabian mares (7-14 years old; 350-435 kg) were enrolled for the study between March and June 2021 in the Northern Hemisphere (latitude 41° N). The body condition scores of the mares were between 5 and 7 (14). All mares were from the same herd. Mares were kept in an open shed and outdoor grass paddock under natural light with access to water and mineral salt (ad libitum). They received 2 kg of concentrated balanced grains daily.

Eight of 10 mares in group C and all mares in group H have foaled in the last year. However, only 2 of 9 mares in group E have foaled in the last year. At the end of the study, the mares in Group C were naturally mated at the next estrus and their pregnancy status was recorded. Also, the routine breeding protocol was continued for healthy mares, and they did not have any problems in conceiving. The first examination for pregnancy diagnosis of mares mated in both group H and group C was performed on the 14th day after mating. Also, the vitality of the mare and the fetus were followed by ultrasonography from the diagnosis of pregnancy until delivery.

B-mode and Doppler USG

The internal genital tract (ovaries and uterus) was examined transrectally by the same operator in all groups with B-mode ultrasonography (Esaote MyLab One Vet, Esaote Pie Medical, Genova, Italy) equipped with linear veterinary transducer (SV 3513 Vet; Esaote Pie Medical, Genova, Italy). Follicles larger than 35mm were visualized daily by ultrasonography until ovulation occurs. The ovulation (day 0) was confirmed by the disappearance of the largest follicle and visualization of the newly forming corpus luteum (CL) in ultrasonography examination. The date of ovulation was noted for each mare. Ovaries in all groups were examined for the presence of CL and small follicles (< 20 mm). The widest cross-sectional uterine diameter (UD), edema of the endometrial folds, presence of luminal content, number, localization and diameter of uterine cysts were visualized on left and right uterine horns. Related diameters were calculated automatically by the USG equipment software. Grading of the uterine inflammatory fluid echo pattern was performed by grayscale echogenicity (15). The researchers (15) have defined this classification as: Grade 1: The intrauterine fluid is very echogenic (almost white) and represents large amounts of suspended debris. On occasion, it may be difficult to see the junction of the fluid and the uterine wall. Grade 2: The intrauterine fluid is echo dense (light gray) but less so than grade 1. Grade 3: The intrauterine fluid is dark gray due to a few hypoechoic foci suspended in an anechoic medium. Grade 4: The intrauterine fluid is black or anechoic. Subjective evaluation of the uterine edema was classified according to Samper and Pycock (16). The related classification was defined as follows: 0: No edema, 1: Minimal detectable uterine edema, 2: Moderate uterine edema, 3: Significant edema, 4: The highest level of normal edema, little free fluid can be detected in the lumen, 5: Abnormal uterine edema characterized by an irregular and disorganized appearance.

The UA was found the way Bollwein and colleagues (1) described. Uterine blood supply was studied by the investigation of the left and right uterine arteries. The UA was examined as published previously (1). The color Doppler was used to visualize UA by using a 5 MHz transducer. Three UA diameters were determined at the same location using a linear array ultrasound transducer. The hemodynamic changes of the UA were visualized by using pulsed wave Doppler USG using a 5 MHz transducer. The angle of insonation was approximately 60° during spectral measurements. At least three consecutive systolic peaks with similar amplitude and velocity were included for analysis. The PI and RI values of UA were automatically calculated by the Doppler machine software. To minimize the variations, settings were kept constant (B mode frequency 10 MHz, color

flow Doppler frequency 5 MHz, gain 28%, pulse repetition frequency 1 KHz, Pulsed wave Doppler frequency 5 MHz, gain 70%, pulse repetition frequency 2 KHz).

Cytological examination

Cytobrush (CB) technique was performed using a cytology brush instrument (Equine Cytology Brush, Ref. 17214/2960; Minitube GmbH, Tiefenbach, Germany) as reported before (17). The slides were stained with MGG Diff Quick stain set (ADR Group, Mediko Kimya, Istanbul, Türkiye) according to the manufacturer's instructions. Slides were examined by a light microscope (Olympus CX41, Tokyo, Japan) at x400 magnification. A digital camera (Olympus SC30, Tokyo, Japan) combined with a software package program allowed digital documentation and further analysis. In total, 200 cells were counted to determine the percentage of PMNs in each slide (17). The ratios of PMNs less than 0.5%, between 0.5% and 5%, between 5% and 30%, and higher than 30% were used to determine the classifications as 0, +1, +2 and +3; respectively (18, 19).

Statistical analysis

Statistical analyses were performed with the SPSS 13.0 package program. The normal distribution of the data was checked by using Shapiro-Wilk test. The comparison of the groups about USG measurements were applied with One-way ANOVA and Duncan tests. Values were given as mean ± standard error of the mean (SEM). The significance level was accepted as $P<0.05$.

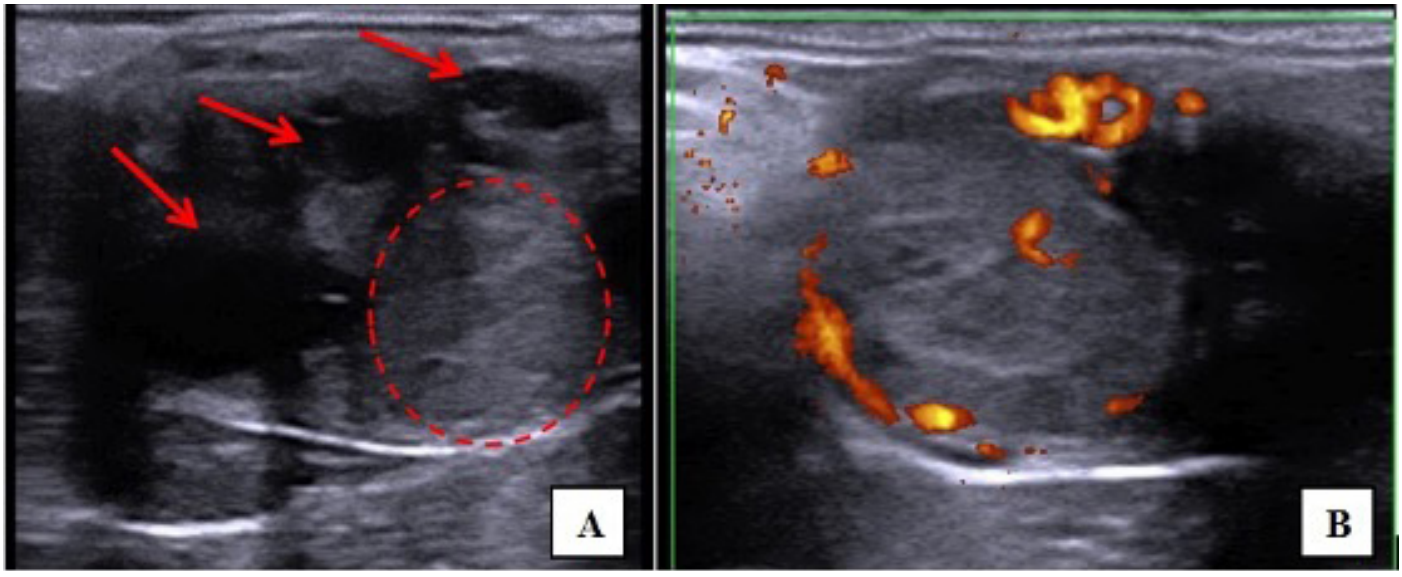

Figure 1: A. The presence of CL and small follicles (< 20 mm). Red circle: CL. Red arrows: Small follicles. B. Power Doppler image of an active CL

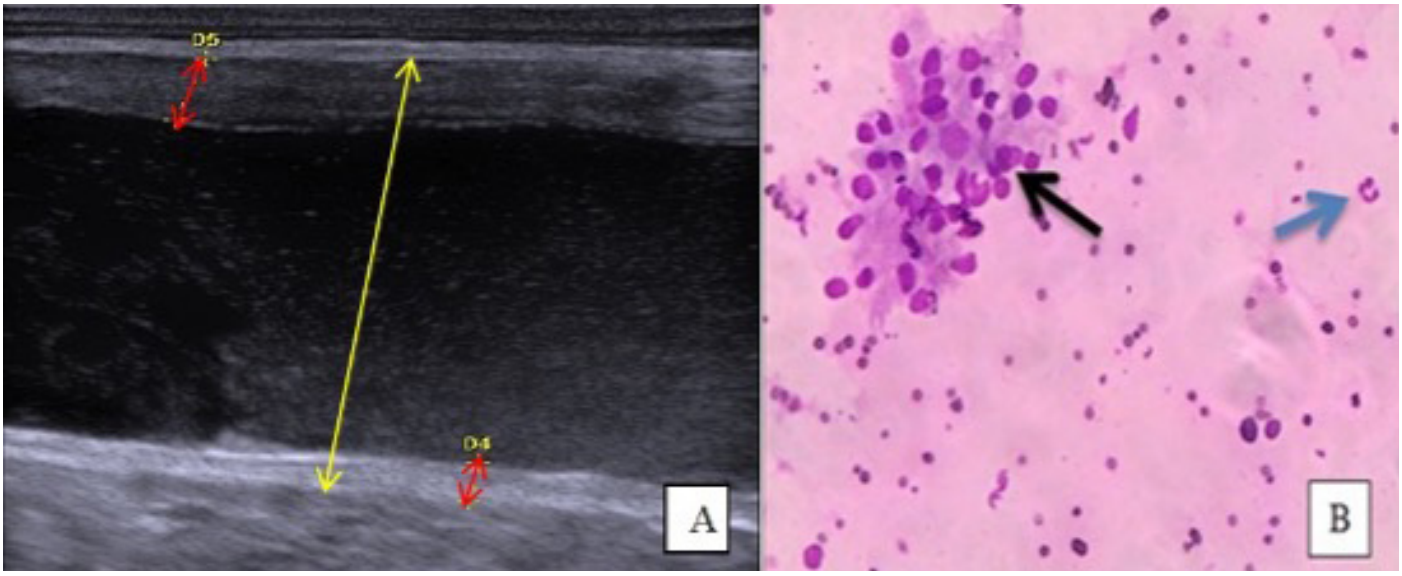

Figure 2: A. Transrectal USG image of the uterus in a mare with endometritis and presence of the intrauterine fluid. Red arrows: Excessive edema of the endometrial folds (D_4: 3.69 mm and D_5: 6.08 mm). Yellow arrow: Increased uterine diameter (4.02 cm). B. Cytology smear obtained by CB in a mare with +2 endometritis. Black arrow: PMNs. Blue arrow: Neutrophil

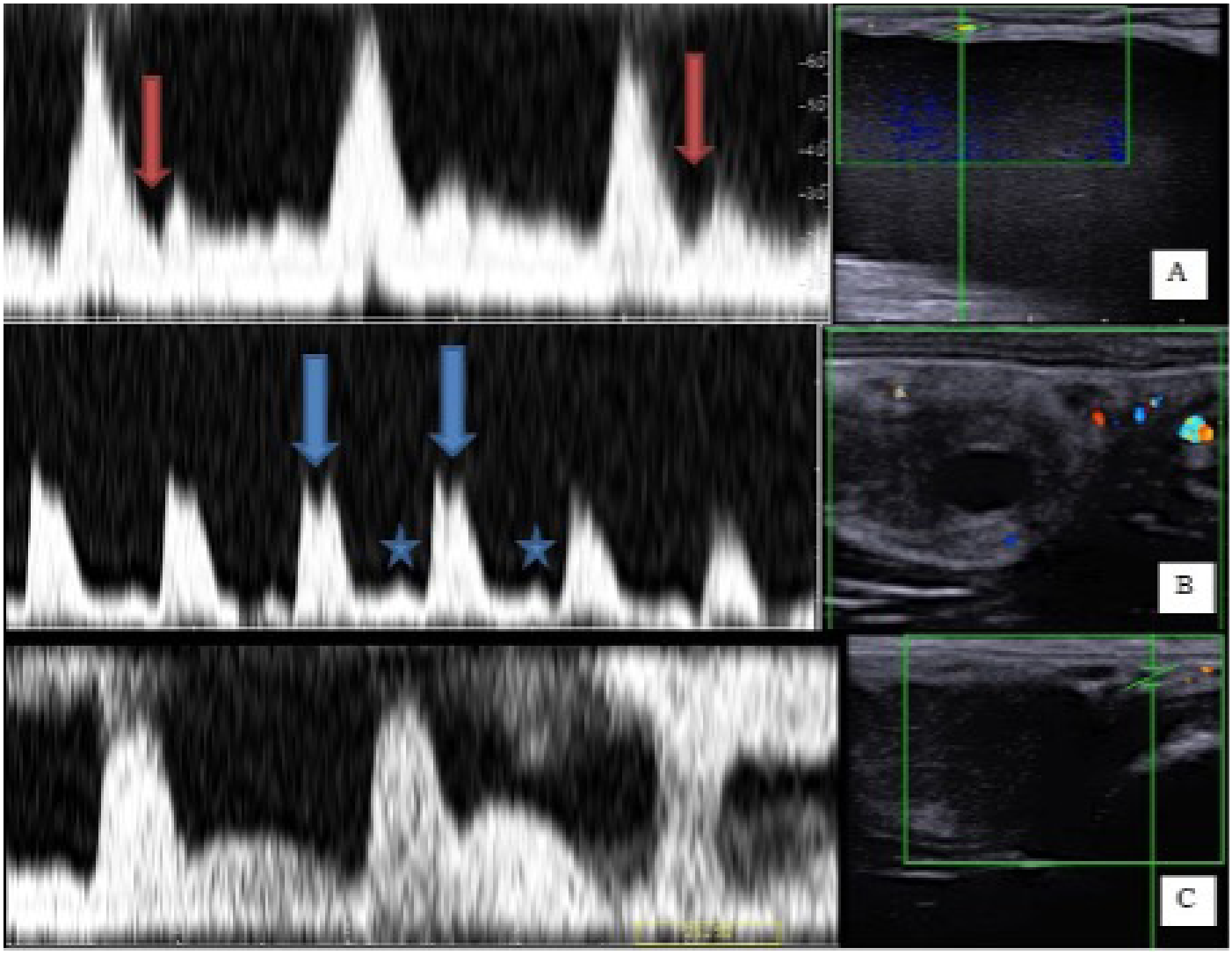

Figure 3: Waveforms and color mapped of UA in all groups. A. Early diastolic notches (red arrows). Color mapped of the UA and the presence of the intrauterine fluid accumulation. B. Waveform evident with systolic notches (blue arrows) followed by a small diastolic peak (blue stars). Color mapped of the UA and the presence of an intrauterine cyst. C. Spectral Doppler graph and typical pattern of UA waveform. Color mapped of the UA and transversal section of the healthy uterus in the luteal phase

Results

The mean ages of the mares related to groups were 8.4±0.50 years in Group H, 12.25±1.03 years in Group E, and 11.2±0.37 years in Group C. Significant rises were determined in the mean ages of the mares both in Group E and Group C than in Group H ($P<0.01$) while the ages between Group E and Group C were not significantly different ($P>0.05$). In B-mode ultrasonography, all mares which were not mated/inseminated, had follicles less than 20mm and corpora lutea on the ovaries (Figure 1).

In nine mares in Group E, endometritis was diagnosed by transrectal palpation, CB, and transrectal USG (Figure 2). All mares in Group E had chronic endometritis. In each mare of group E, an excessively toned, partially pendulous, and enlarged uterus was detected by rectal palpation. Cytology smears of Group E were classified as PMN +2 (Table 1). However, none or less than 0.5% PMNs was observed in Group H and Group C.

In Group E, the mean UD and uterine wall thickness were 3.47±0.29 cm and 4.30±0.28 mm; respectively. The amount and echogenicity of the intrauterine fluid in Group E was Grade 2 (Table 1). Subjective evaluation of uterine edema, 2 of the 9 mares in Group E exhibited Grade 3, while the remainder presented with Grade 2 (Table 1). Edema of the endometrial folds, presence of intrauterine fluid, and rise in UD was visualized in Group E by transrectal USG whereas absence of edema of the endometrial folds, absence of the intrauterine fluid, and UD were 2.29±0.18 cm in Group H. The mean UD of all groups is presented in Table 2. The mean UD in Group H was significantly lower than in Group C ($P<0.05$) and in Group E ($P<0.01$). According to trans-rectal ultrasonography, single (n=6 mares) and double intrauterine cysts (n=4 mares) were observed in Group C. The cysts were located on the right uterine horn (n=5 mares) and on the left

Table 1: Uterine edema, echogenicity of intrauterine fluid, PMN grades in mares with endometritis

Mares in Group E	Uterine edema						Echogenicity of intrauterine fluid				PMN (%)			
	0	1	2	3	4	5	Grade 1	Grade 2	Grade 3	Grade 4	(0) <0.5 %	(+1) 0.5-5%	(+2) 5-30%	(+3) >30%
No.1			+					+					+	
No.2			+					+					+	
No.3			+					+					+	
No.4				+				+					+	
No.5			+					+					+	
No.6			+					+					+	
No.7			+					+					+	
No.8				+				+					+	
No.9			+					+					+	

uterine horn (n=5 mares). The diameters of the intrauterine cysts ranged between 0.51 cm and 1.83 cm (1.27±0.10 cm). The diameter of UA (DUA) was measured on the color image of UA in all groups. Accordingly, the mean DUA in Group C (3.85±0.04 mm) was significantly lower than in Group E (3.94±0.02 mm) (P<0.05). In Group H, DUA (3.79±0.03 mm) was significantly lower than in Group E (P<0.01) while significant differences in DUAs were not observed between Group H and Group C (P>0.05) (Table 2).

Blood flow in UA was analyzed by calculation of PI and RI. The PI and RI values of all groups are presented in Table 3. Both PI and RI values in Group E were not significantly different from those in Group C (P>0.05). However, PI and RI values in Group H were significantly lower than both in Group E and Group C (P<0.001).

The waveform of the UA belong to the groups were presented at Figure 3. In Group E, the waveform was characterized by a systolic peak followed by diastolic flow, extending throughout the cardiac cycle with prominent early diastolic notch. Additionally, systolic notches and small diastolic peaks were observed in the UA waveforms of the mares in Group C. In Group H mares, the biphasic waveform was characterized by a short fall in peak systolic flow and was continuous with diastolic flow. The diastolic component continued with the previous systolic component and presented throughout the cardiac cycle which indicated good uterine perfusion in Group H.

When the measurements of the study were completed, 10 mares in Group C were naturally mated at the next estrus. One mare had pregnancy loss on Day 45 due to the localization of the embryonic vesicle next to the intrauterine cyst. All but 1 of the rest of 8 mares became pregnant and gave birth to a healthy foal. Also, all mares in Group H had healthy foals in the next breeding.

Discussion

Infertility problems increase in parallel with the mare's age. A decrease in fertility is seen in mares older than 15 years (20). Leidl and colleagues (21) reported that there was no evidence of cysts in mares under 10 years of age. Consistent with the previous report, the mean age of the mares in group C was greater than 10 years. Carnevale and

Table 2: Mean and standard error of the UD and DUA in all groups

	Group E (n=9)	Group C (n=10)	Group H (n=9)
UD (cm)	3.47±0.29[a]	2.97±0.19[a]	2.29±0.18[b]
DUA (mm)	3.94±0.02[a]	3.85±0.04[b]	3.79±0.03[b]

[a,b] Different letters in the same line indicate the significant difference as P<0.05

Table 3: Mean and standard error of the UA resistance index (RI), and pulsatility index (PI) of the mares

	Group E (n=9)	Group C (n=10)	Group H (n=9)
PI	2.34±0.09[a]	2.22±0.10[a]	1.65±0.06[b]
RI	0.85±0.00[a]	0.84±0.01[a]	0.76±0.01[b]

[a,b] Different letters in the same line indicate the significant difference as P<0.001

Ginther (22) indicated that old age was associated with increased endometrial inflammation, reduced pregnancy rate, increased embryo-loss rate, increased severity of endometrial fibrosis, increased uterine vascular dysfunction, and reduced fertility in mares. Also, a progressive increase in the frequency of mares with cysts with the advancing of age has been reported (23). Although the mean ages of the mares in Group E and Group C were not older than 15 years in this study, they were significantly higher than in Group H. The similar results emphasized that uterine problems become common when the age progresses.

Uterine cysts had two additional origins: Lymphatic cysts from obstruction of lymphatic channels and pooling of lymph in multiparous mares, glandular cysts from endometrial glandular distension resulting from periglandular fibrosis (24, 6). Glandular cysts range from a few millimeters to 1 cm in diameter and are frequently seen in pregnant mares but mean diameter of lymphatic cysts varying from 2 to 48 mm (23, 24). Most uterine cysts detected by ultrasonography that protrude into the uterine lumen are lymphatic (7). In line with the previous reports, the mean diameter of the uterine cysts in our study was 1.27±0.10 cm and the mares were multiparous but not pregnant, which supports the lymphatic nature of the intrauterine cysts in Group C. Tannus and Tunn (23) observed that uterine cysts were most commonly located at the junction of both uterine horns and the body of the uterus, which is also the site of vesicle fixation. Additionally, the pregnancy rates were found lower at Days 14 and 40 in mares with uterine cysts (23). Consistent with the previous report, one mare in our study had an embryonic loss at Day 45 due to the localization of the vesicle next to the intrauterine cyst. Early embryonic losses could be explained by inadequate blood flow and nutrient provision for the conceptus due to improper implantation adjoining a cyst (6). However, Leidl and colleagues (21) reported that endometrial cysts can be recognized together with embryonic vesicles and pregnancies can continue without any problems. Similar to Leidl and colleagues (21), 8 of 10 mares in Group C became pregnant and had healthy foals in this study.

Transrectal palpation, ultrasonography and uterine cytology are the non-invasive, easy, and rapid methods for the diagnosis of endometritis (25). Similarly, three diagnostic methods were used to identify endometritis in mares in this study. The researchers reported that mares with endometritis classified as +2 and +3 had a mean uterine diameter of 4.16 ± 0.28 cm (26). Although the mean uterine diameter in Group E (3.47± 0.29 cm) was significantly higher than in Group H (2.29 ±0.18 cm), it was lower than previously reported. Different results may have arisen due to differences in the cytological stage of endometritis (only +2 PMN) in this study. Rahawy and colleagues (10) stated that the mean uterine fluid echogenicity in cases of endometritis is proportional to the increased endometrial wall thickness. Grading of the uterine inflammatory fluid echo pattern, performed by gray scale echogenicity as the researchers reported (15), identified a moderate amount of slightly echogenic, light gray echodense intrauterine fluid in Group E. This group also demonstrated a marked increase in uterine wall thickness, consistent with previous reports (10,15).

The researchers reported that DUA ranges between 2 to 6 mm in cyclic mares (1). Two maiden mares had a uterine artery diameter of 0.29 and 0.25 cm which was significantly smaller compared with the mean uterine artery diameter of the multiparous mares (0.4±0.07 cm) in a study by Klewitz and colleagues (27). In line with the previous reports, DUA was ranged between 3.79 to 3.94 mm in this study. Similar results were thought to be obtained because all mares were multiparous in this study. Lüttgenau and colleagues (28) observed greater uterine perfusion in mares with endometritis. In addition, it had been reported that the DUA before treatment in mares with acute endometritis was significantly larger than that at the end of treatment (29). Abdelnaby and colleagues (11) stated that uterine artery showed an increase in circumference and area in mares with endometritis. Similarly, in the presented report, the highest DUA was detected in Group E but no difference was observed in DUA between Group C and Group H. This result could be explained by the increase of uterine perfusion in endometritis as Lüttgenau and colleagues (28) indicated.

Uterine artery notches are another sign of the blood flow spectrum. An early diastolic notch is considered to be present when there is a visibly defined upturn of the flow velocity waveform at the beginning of diastole. The presence of an early diastolic notch in uterine artery flow velocity waveforms has been reported to be a good predictor of poor pregnancy outcome. Early diastolic notch depth, double notches and reversed diastolic flow may be accepted as different uterine artery waveforms related to the severity of adverse effects and outcomes (30). Although poor pregnancy outcome was not presented in this study, early diastolic notches were observed in mares with endometritis which also had the highest DUA. The presence of early diastolic notches in Group E can be explained by the researchers (31) who reported that larger uterine vessels and myometrial resistance are highly associated with UA notching. A systolic notch is considered to be present when there is a defined upturn or horizontal flow velocity waveform in the systolic wave (30). If Doppler waves have significant magnitude and are delayed concerning the incident waveform, notching occurs (32). Thaler and colleagues (33) observed that fetal growth retardation rates were significantly higher in pregnant patients with systolic or diastolic notches. Lau and colleagues (34) hypothesized that reverse diastolic flow or double notches in the uterine artery may be the result of progressive deterioration in the underlying vasculopathy. Though pregnant mares were not included in this study, systolic notches were observed in waveforms of the mares with intrauterine cysts. It is thought to occur due to the deterioration in the vasculopathy and the delay concerning the waveform (32,34).

A significant increase was reported in Doppler velocities and blood flow rate accompanied by a significant decrease in Doppler indices in mares with endometritis (11). Significantly higher PI and RI were reported in cows diagnosed with mild degree in comparison with cows diagnosed with moderate and severe degrees of clinical endometritis (35). In contrast with Abdelnaby and colleagues (11), significantly higher PI and RI were determined in Group E and Group C than in Group H in this study. Ferreira and colleagues (36) reported that mares with cysts had lower PI and RI of the mesometrial vessels than the controls. Also, they demonstrated reduced uterine vascular perfusion in mares with uterine cysts and a positive association between the size of the cystic area and disturbed uterine hemodynamics. Contradictory to the previous report (36), the rise in PI and RI in Group C than in healthy controls was thought that less disturbed uterine hemodynamics due to smaller sizes of the intrauterine cysts in the present study. Thaler and colleagues (33) reported that notches had significantly higher rates of fetal growth retardation and the presence of both in systolic or diastolic notch, the RI in the UAs on both sides of the uterus were significantly higher than in subjects without a notch. Clark and colleagues (32) indicated that high RI in UA, combined with notching, reflects an abnormal remodeling of the entire uterine vasculature. In line with the findings of the researchers (32, 33), the second reason for the rise in Doppler indices (PI and RI) in Group E and Group C could be the occurrence of the notches (diastolic or systolic) on the spectral waveforms which reflect the abnormal uterine vasculature.

Conclusions

Doppler USG is considered a useful tool to analyze the waveform and hemodynamics of the UA related to the different pathologies (cyst or endometritis). It was concluded that not only Doppler indices but also characteristics of the waveforms of the UA should be evaluated to understand the hemodynamic effect of the uterine pathologies.

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Hemodinamične spremembe maternične arterije pri kobilah z različnimi patologijami maternice

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Izvleček: Ciste, nabiranje tekočine, vnetne spremembe in tumorji so pogoste patologije maternice pri kobilah. Naš namen je bil preučiti hemodinamične spremembe maternice glede na patologijo maternice (endometritis ali intrauterine ciste). V študijo je bilo vključenih 28 kobil, razdeljenih v skupine: 9 ginekološko zdravih kobil v diestrusu (skupina H), 9 kobil z endometritisom (skupina E) in 10 kobil z intrauterino cisto (skupina C). Povprečni premer maternice (UD) v skupini H (2.29±0.18) je bil bistveno manjši kot v skupini C (2.97±0.19 cm) (P<0.05) in skupini E (3.47±0.29 cm) (P<0.01). Premeri inrauterinih cist v skupini C so se gibali med 0.51 cm in 1.83 cm. Največji premer maternične arterije (UA) je bil opažen v skupini E. Hemodinamični indeksi UA (PI in RI) v skupini E se niso bistveno razlikovali od tistih v skupini C (P>0.05). Vrednosti PI in RI v skupini H pa so bile bistveno nižje kot v skupini E in C (P<0.001). Pri skupini E je bil v krivulji UA prisoten zgodnji diastolični vrez, medtem ko so bile v krivulji UA kobil iz skupine C opazne sistolične zareze in majhni diastolični vrhovi. Dopplerjev USG velja za uporabno orodje za analizo valovanja in hemodinamike UA, povezanih z različnimi patologijami (cista ali endometritis). Ugotovljeno je bilo, da je za razumevanje hemodinamskega učinka patologij maternice poleg dopplerskih indeksov potrebno vrednotiti tudi značilnosti valovnih oblik UA.

Ključne besede: Doplerjeva ultrazvočna preiskava; endometritis; intrauterina cista; zareza