Uterine blood flow and perfusion in mares with uterine cysts: effect of the size of the cystic area and age

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Abstract

Transrectal color and power Doppler ultrasonography was used to study uterine blood flow and perfusion in mares with and without uterine cysts. Vascular perfusion of the uterus and blood flow velocities, vascular perfusion, diameter, circumference, and area of a cross section of the mesometrial attachment were evaluated. To study the effect of internal cysts, two matched groups (cystic and control, n = 21 mares/group) were used. Uterine vascular perfusion in mares with cysts was less (P < 0.0001) in the cystic than the noncystic region and less (P < 0.0009) than that for controls. Mares with cysts had lower (P < 0.04) pulsatility index (PI) and greater end diastolic velocity (EDV; P < 0.03) and time-averaged maximum velocity (TAMV; P < 0.05) of the mesometrial vessels than the controls. To study the effect of the size of internal uterine cystic area, paired mares were arranged in four groups (n = 8–11/group): small uterine cystic area (≤ 275 mm²) versus controls and large uterine cystic area (> 410 mm²) versus controls. A small uterine cystic area did not affect uterine hemodynamics. Mares with large uterine cystic area had lower PI (P < 0.05) and greater peak systolic velocity (P ≤ 0.05), EDV (P < 0.009), and TAMV (P < 0.005). To study the effect of age, old versus young mares without cysts were compared (n = 11/group). Old mares had greater EDV (P < 0.02) and TAMV (P < 0.01) than young mares. Results demonstrated, for the first time in any species, reduced uterine vascular perfusion in mares with uterine cysts and a positive association between size of the cystic area and disturbed uterine hemodynamics.


Introduction

Uterine cysts have been described in diverse species of animals, including horses, cattle, ewes, pigs, cats, dogs, elephants, and humans (Dow 1959, 1962, Adams 1975, Ricketts 1975, Al-Dahash & David 1977, Dallenbach-Hellweg 1987, Lipetz et al. 1987, Agnew et al. 2004). In mares, uterine cysts are fluid-filled structures commonly found in the three uterine layers or attached to the exterior surface (Ginther & Pierson 1984) of a normal or chronically inflamed uterus (Kenney & Ganjam 1975). The cysts arise from the endometrium (Kenney & Ganjam 1975, Ricketts 1975, Kenney 1978) or myometrium (reviewed by Stanton et al. 2004). Although the etiopathogenesis of uterine cysts is uncertain, it is likely that abnormal uterine blood flow can contribute to cyst formation as a result of inadequate drainage due to poor venous return and angiosis of arterioles (Schoon et al. 1999). Two additional origins for uterine cysts have been suggested: (1) lymphatic cysts from obstruction of lymphatic channels (Kenney & Ganjam 1975) or from a gravitational effect in the enlarged, gravid, or post partum uterus, resulting in an impediment to fluid drainage and ventral collection of lymph (reviewed by Stanton et al. 2004) and (2) glandular cysts from endometrial glandular distension resulting from periglandular fibrosis (Kenney & Ganjam 1975, Ricketts 1975, Kenney 1978). Glandular cysts range from a few millimeters to 1 cm in diameter and are frequently found in pregnant mares. Lymphatic cysts start as small microscopic structures that enlarge from several millimeters to several centimeters (Kenney & Ganjam 1975, Kenney 1978) and have reported mean diameters ranging from 2 to 48 mm (Tannus & Thun 1995). Cysts are usually located at or near the base of both horns (Bracher et al. 1992, Eilts et al. 1995, Tannus & Thun 1995) in the ventral-most portion of the suspended uterus (Ginther & Pierson 1984). The reported incidence in horses ranges from 13 to 55% (Wilson 1985, Adams et al. 1987, Kaspar et al. 1987, Leidl et al. 1987, Bracher et al. 1992, Tannus & Thun 1995). Uterine cysts appear to have clinical importance (Adams et al. 1987, Leidl et al. 1987, Tannus & Thun 1995, reviewed by Stanton et al. 2004, Yang & Cho 2007).

A higher frequency of uterine cysts in older mares (Adams et al. 1987, Leidl et al. 1987, Bracher et al. 1992) and a progressive incidence of cysts as age advances (Tannus & Thun 1995) have been described. Increased age in mares has also been accompanied by increased severity of endometrial fibrosis, increased uterine vascular dysfunction, and reduced fertility (Doig et al. 1981, Oikawa et al. 1993, Nambo et al. 1995, Grüninger et al. 1998).
Schoon et al. 1999). Considering the reports of a high incidence of cysts and degenerative changes in the uterine artery walls in older mares, there may be a relationship among the presence of uterine cysts, decreased uterine vascular perfusion, and pregnancy loss. However, no study has been reported on the direct effect of age on vascular perfusion of the uterine layers in mares.

Uterine cysts are readily imaged by ultrasound, and transrectal ultrasonography has been an important advancement in the study of uterine cysts, providing detailed research and clinical information (Ginther & Pierson 1984). Ultrasonically, internal cysts are characterized by an anechoic area (fluid) with an irregular echoic wall involving uterine tissue or a prominent bulge in the uterine lumen. Individual, compartmentalized, or multilobulated sacs, pedunculated or not, can be imaged. External cysts usually project outward from the outer surface of the endometrium (Ginther & Pierson 1984, Ginther 1995). In mares, uterine cysts also have been studied by necropsy, histology, transrectal palpation, hysteroscopy, and fiber-optic techniques (Kenney & Ganjam 1975, Wilson 1985, Kaspar et al. 1987, Leidl et al. 1987).

Color and power Doppler ultrasonography is a non-invasive pulse wave technique that has been used recently for transrectal study of blood flow in the reproductive tract of large domestic animals (Ginther 2007). In addition to the uterine artery (Bollwein et al. 1998) and its branches, the mesometrial attachment and vessels located in the endometrium and myometrium can be evaluated for the study of uterine hemodynamics (Silva et al. 2005, Silva & Ginther 2006). Doppler ultrasonography is a potential technique for the study of the pathogenesis and effects of treatment or preventive approaches for uterine cysts. However, no report was found in any species on the use of color Doppler ultrasonography to investigate the relationships between uterine blood flow and uterine cysts.

The purposes of the present study using B-mode and color Doppler ultrasonography (Fig. 1) in mares were to study the incidence of internal and external uterine cysts and the relationship with blood flow. Specific goals were to (1) examine in detail the prevalence of uterine cysts in young and old mares and determine the number, size, and longitudinal and cross-sectional uterine locations of individual cysts (Part 1); (2) test the hypothesis that uterine regions with cysts have low uterine vascular perfusion and determine the effects of cysts on spectral blood flow velocity in the vessels of the mesometrial attachment (Part 2); (3) test the hypothesis that a larger uterine cystic area is associated with changes in uterine vascular perfusion and uterine hemodynamics of the mesometrium than a smaller cystic area (Part 3); and (4) test the hypothesis that older nulliparous mares...
without cysts have lower uterine vascular perfusion than younger mares and determine the effects of age on spectral blood flow velocity in the vessels of the mesometrial attachment (Part 4).

Results

Part 1: prevalence and distribution of uterine cysts

The prevalence of ultrasonographically detected uterine cysts in 76 mares in the three age groups is shown in Table 1. Mean age of mares with uterine cysts was greater than that in mares without detected cysts. The frequency of mares with cysts increased \( P<0.05 \) progressively with age, and mares \( >14 \) years had the highest frequency. By contrast, the majority \( P<0.008 \) of mares without cysts were \( 7–14 \) years. Although the size of the external cystic area \((152 \pm 39 \text{ mm}^2)\) did not differ with the age, the size of the internal cystic area was greater \( P<0.02 \) in mares \( >14 \) years \((523 \pm 165 \text{ mm}^2)\) than in mares \( 7–14 \) years \((121 \pm 47 \text{ mm}^2)\). The distribution of internal and external uterine cysts is shown in Table 2. The mean number of cysts in mares with cysts ranged from 2 to 59 and the mean cystic area ranged from 28 to 3087 \text{ mm}^2\). The number of internal cysts was greater \( P<0.04 \) than that for external cysts, and the mean diameter did not differ between internal \((5.7 \pm 0.7 \text{ mm})\) and external cysts \((5.5 \pm 0.3 \text{ mm})\). In regard to longitudinal locations, the frequency of both internal and external cysts was lower \( P<0.004 \) and \( P<0.006 \) respectively) in the extremities left, right horn anterior and uterine body posterior (LA, RA and BP) than in the central segments left, right horn middle and uterine body middle (LM, RM and BM) and cornual-body junction left, right horn posterior and uterine body anterior (LP, RP and BA). The number of cysts did not differ between the internal and external cysts in the extremities and central segments, and internal cysts were more \( P<0.005 \) predominant than external cysts in the cornual-body junction. There were more \( P<0.0001 \) cysts (total) comprising a larger area \( P<0.0001 \) in the central segments and cornual-body junction than in the extremities. Combinations of internal and external cysts were detected in the majority \((21\ mares, 62\%)\) of the 34 cystic mares. The frequency of mares with only internal cysts \((23.5\%)\) did not differ from that with only external cysts \((14.7\%)\). The incidence of internal cysts in the 21 mares increased \( P<0.0001 \) from the extremities to the cornual-body junction, whereas the external cysts were more \( P<0.02 \) frequently observed in the central segments. The mean age of the 21 mares with both internal and external cysts \((15.9 \pm 1.0 \text{ years})\) was greater \( P<0.007 \) than that for mares with only internal \((10.3 \pm 1.2 \text{ years})\) and only external \((12.3 \pm 2.9 \text{ years})\) cysts. The number of cysts was greater \( P<0.0001 \) in the 21 mares \((17.7 \pm 2.8 \text{ cysts})\) than that in mares with only internal cysts \((8.0 \pm 1.2)\) and mares with only external cysts \((3.2 \pm 1.2)\). The uterine cystic area \((\text{ mm}^2)\) of mares with only internal \((65 \pm 11 \text{ mm}^2)\) and only external \((81 \pm 42 \text{ mm}^2)\) cysts did not differ, but the area was smaller \( P<0.0002 \) than that in mares with both internal and external cysts \((759 \pm 168 \text{ mm}^2)\).

Part 2: effect of cysts

The presence of internal cysts did not affect the vascular perfusion, diameter, circumference, and area of the cross section of the mesometrial attachment (Table 3). The cystic group had a lower PI, lower RI (approached significance), greater end diastolic velocity (EDV), and greater time-averaged maximum velocity (TAMV) than the controls. No difference between groups was found for peak systolic velocity (PSV) values. The percentage of Doppler signal in the cystic region was less than in the middle of the uterine horns of controls (Table 3; Fig. 2). The percentage of uterine tissue with Doppler signals of vascular perfusion in the cystic group was also less \( P<0.0001 \) in the cystic region than in the adjacent region without cysts (Table 3; Fig. 3). No difference in percentage of uterine vascular perfusion was detected between left and right horns in control mares (data not shown), and the average was used in the analyses. The region without cysts in the cystic group did not differ from the average for the two horns in the control group.

Part 3: effect of size of cystic area

Mares with a small uterine cystic area \((\leq 275 \text{ mm}^2)\) had an area of \(180 \pm 24 \text{ mm}^2\) (range \(32–275 \text{ mm}^2)\).
individual cysts were fewer \( (P<0.004) \) in number and less \( (P<0.007) \) in diameter in the small cystic area than in the large cystic area (Table 4). No significant differences between mares with a small cystic area and the paired controls were found at the mesometrial attachment for spectral end points, vascular perfusion, cross-sectional diameter, circumference, or area, except for a lower RI (approached significance) in the group with a small cystic area. Uterine vascular perfusion percentage in the cystic region was less \( (P<0.04) \) than that in the adjacent region without cysts and also less \( (P<0.02) \) than that in the control mares (Table 4; Fig. 3). The vascular perfusion percentage in uterine regions without cysts did not differ from the percentage in controls.

Mares with a large uterine cystic area \( (>410 \text{ mm}^2) \) had an area of \( 1110 \pm 314 \text{ mm}^2 \) (range \( 411–2854 \text{ mm}^2 \)). The large uterine cystic area did not affect vascular perfusion, diameter, circumference, and area \( (\text{mm}^2) \) of the mesometrial attachment compared with the paired controls (Table 4). The PI was lower \( (P<0.05) \) and the RI was not different when compared with the control group. The PSV \( (P\leq0.05) \), EDV \( (P<0.009) \), and TAMV \( (P<0.005) \) were greater at the mesometrial attachment in the cystic group than in the paired controls (Table 4; Fig. 4). Differences in vascular perfusion between regions and groups were similar to those for the small cystic area.

**Part 4: effect of age in mares without cysts**

In mares without detected cysts, the mean ages in the young and old groups were \( 6.7 \pm 0.7 \) and \( 16.6 \pm 0.5 \) years respectively. A gated artery of the mesometrial attachment had a greater EDV \( (P<0.02) \) and TAMV \( (P<0.01) \) in old mares \( (3.40 \pm 0.3 \) and \( 5.81 \pm 0.4 \text{ cm/s} \) respectively) than in young mares \( (2.65 \pm 0.1 \) and \( 4.68 \pm 0.2 \text{ cm/s} \) respectively). A positive correlation \( (r=0.44; P<0.04) \) between age and TAMV was found and EDV tended \( (P<0.07) \) to be positively correlated \( (r=0.38) \) with age. No other significant differences between young and old mares were detected for any of the other end points measured at the mesometrial attachment and uterus (data not shown).

### Discussion

The use of color or power Doppler ultrasonography has become one of the best available and reliable techniques for the diagnoses and studies of uterine hemodynamics *in vivo* (Ginther 2007). The present study demonstrates for the first time in any species the relationships between uterine cysts and uterine hemodynamics, using ultrasonographic Doppler technology. The findings can be helpful for understanding the origin and effects of cysts. New information was obtained with regard to the reduced uterine perfusion in cystic areas and the absence of a

| Table 2 Uterine distribution of cysts in mares*.
<table>
<thead>
<tr>
<th>Cross-sectional location†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitudinal location‡</td>
</tr>
<tr>
<td>Overall</td>
</tr>
<tr>
<td>Extremities</td>
</tr>
<tr>
<td>Central segments</td>
</tr>
<tr>
<td>Cornual-body junction</td>
</tr>
</tbody>
</table>

*abc Frequencies or means with different letters within a column are different \( (P<0.02) \). *b Means within a row (internal versus external) with different letters are different \( (P<0.005 \text{ to } P<0.04) \).

‡ Internal (no. cysts/mare) External (no. cysts/mare) Total (no. cysts/mare)§ Average for left and right horns of mares without cysts was used.

### Table 3 Mean ± S.E.M. extent of mesometrial vascular perfusion and blood flow velocities and uterine vascular perfusion in mares with internal uterine cysts versus control mares without detected cysts.

<table>
<thead>
<tr>
<th>End points</th>
<th>Group</th>
<th>Internal cysts*</th>
<th>Control*</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of mares</td>
<td>21</td>
<td>21</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Mesometrial attachment†</td>
<td>Cross-sectional view</td>
<td>Vascular perfusion (scores 1–4)</td>
<td>2.9 ± 0.2</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>17.1 ± 0.6</td>
<td>17.1 ± 0.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Circumference (mm)</td>
<td>66.2 ± 1.8</td>
<td>65.7 ± 1.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>240.2 ± 15.3</td>
<td>218.6 ± 12.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PI (index)</td>
<td>1.60 ± 0.08</td>
<td>1.80 ± 0.08</td>
<td>( P&lt;0.04 )</td>
<td></td>
</tr>
<tr>
<td>RI (index)</td>
<td>0.71 ± 0.02</td>
<td>0.75 ± 0.02</td>
<td>( P&lt;0.09 )</td>
<td></td>
</tr>
<tr>
<td>PSV (cm/s)</td>
<td>13.6 ± 0.7</td>
<td>12.7 ± 0.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>EDV (cm/s)</td>
<td>3.7 ± 0.3</td>
<td>3.1 ± 0.2</td>
<td>( P&lt;0.03 )</td>
<td></td>
</tr>
<tr>
<td>TAMV (cm/s)</td>
<td>6.3 ± 0.4</td>
<td>5.4 ± 0.3</td>
<td>( P&lt;0.05 )</td>
<td></td>
</tr>
<tr>
<td>Uterine vascular perfusion (%)</td>
<td>Cystic region</td>
<td>7.6 ± 1.2a</td>
<td>14.1 ± 1.6d</td>
<td>( P&lt;0.0009 )</td>
</tr>
<tr>
<td>Adjacent to cystic region</td>
<td>12.7 ± 1.6b</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant. PI, pulsatility index; RI, resistance index; PSV, peak systolic velocity; EDV, end diastolic velocity; TAMV, time-averaged maximum velocity. *b Uterine vascular perfusion means with different letters within a column are different \( (P<0.0001) \).

*The presence or absence of external cysts was not considered in the cystic group. Controls had neither internal nor external cysts and were paired with mares with cysts of similar age. Vessels of the mesometrial attachment averaged for the two uterine horns. \( \% \)Percentage of the endometrium and myometrium with color Doppler signals of blood flow. \( \% \)Average for left and right horns of mares without cysts was used in the analyses.
detected effect of age on uterine vascular perfusion in mares without cysts.

The frequency of mares with cysts in this study was greater than that reported previously (Adams et al. 1987, Kaspar et al. 1987, Leidl et al. 1987, Eilts et al. 1995, Tannus & Thun 1995). A progressive increase in the frequency of mares with cysts with the advancing of age (Tannus & Thun 1995) and a lower mean age of mares without cysts (Adams et al. 1987, Leidl et al. 1987, Bracher et al. 1992, Eilts et al. 1995) agrees with our findings. Although the overall number of cysts and the size of the cystic area increased with age, our findings indicated that old age was associated with increase in the internal cystic area, whereas the size of the external cystic area did not differ between mares of intermediary versus advanced age.

To our knowledge, this is the first ultrasonographic study to consider internal and external cysts separately. The majority of the mares with cysts had both internal and external cysts. The size of cysts was not affected by their location (internal or external). However, external cysts were present in lower number/mare and were smaller in area than internal cysts. The extremities of the uterus were least affected by internal and external cysts. The cornual-body junction was more affected by internal than external cysts. Considering only mares with both internal and external cysts, a progressive increase in the frequency of internal cysts from the extremities to the cornual-body junction of the uterus was found and agrees with previous reports (Bracher et al. 1992, Eilts et al. 1995, Tannus & Thun 1995) that suggested a higher prevalence of endometrial cysts in the caudal portion of the uterine horns. During the embryo mobility phase (11–15 days after ovulation; Ginther 1983a, Leith & Ginther 1984), the embryo has been observed to move through cystic areas (Ginther & Pierson 1984). However, it is unknown whether extensive cysts could interfere with embryo mobility and thereby with the requisite exposure of the entire uterus to the mobile embryo in the prevention of luteolysis (McDowell et al. 1988). Fixation of the embryo occurs almost always in the caudal portion of one of the uterine horns (Ginther 1983b), but it is unknown whether cysts in
endometrium and myometrium with color Doppler signals of blood flow. The average for left and right horns of mares without cysts were used in end points between mares with uterine cystic area.

**Table 4** Mean ± S.E.M. extent of mesometrial vascular perfusion and blood-flow velocities and uterine vascular perfusion in mares with a small (≤275 mm²) or large (>410 mm²) internal uterine cystic area compared to controls.

<table>
<thead>
<tr>
<th>End points</th>
<th>Small cystic area</th>
<th>Large cystic area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cystic</td>
<td>Control</td>
</tr>
<tr>
<td>Number of mares</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Individual cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>4.5 ± 0.9ᵃ</td>
<td>–</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>5.6 ± 0.5ᵃ</td>
<td>–</td>
</tr>
</tbody>
</table>
| Mesometrial attachment†
| Cross-sectional results |
| Vascular perfusion (score 1 to 4) | 3.1 ± 0.3         | 3.2 ± 0.2         | 2.8 ± 0.3         | 2.8 ± 0.3         |
| Diameter (mm)    | 18.3 ± 0.6         | 18.1 ± 0.8        | 16.1 ± 0.8        | 16.1 ± 0.8        |
| Circumference (mm) | 70.1 ± 2.1     | 68.5 ± 2.7        | 63.9 ± 2.7        | 62.7 ± 1.9        |
| Area (mm²)       | 270.5 ± 16.9      | 244.7 ± 21.3      | 220.8 ± 25.7      | 191.4 ± 8.4       |
| Spectral results |
| PI (index)       | 1.57 ± 0.11        | 1.76 ± 0.11       | 1.49 ± 0.08ᵇ      | 1.81 ± 0.15ᵇ      |
| RI (index)       | 0.70 ± 0.02ᵃ       | 0.75 ± 0.02ᵇ      | 0.71 ± 0.02ᵇ      | 0.75 ± 0.03ᵇ      |
| PSV (cm/s)       | 12.7 ± 1.0         | 13.6 ± 0.8        | 14.8 ± 1.3ᵇ       | 11.9 ± 1.1ᵇ       |
| EDV (cm/s)       | 3.6 ± 0.3          | 3.4 ± 0.3         | 4.2 ± 0.5ᵇ        | 2.8 ± 0.2ᵇ        |
| TAMV (cm/s)      | 5.8 ± 0.5          | 5.9 ± 0.5         | 7.2 ± 0.6ᵇ        | 5.0 ± 0.4ᵇ        |
| Uterine vascular perfusion (%)‡ |
| Cystic region    | 8.9 ± 2.1ᵇ         | 15.6 ± 2.4ᵇ      | 6.9 ± 1.3ᵇ        | 12.3 ± 2.0ᵇ       |
| Adjacent to cystic region | 12.3 ± 2.4ᵇ     | 12.8 ± 1.9ᵇ      |

PI, pulsatility index; RI, resistance index; PSV, peak systolic velocity; EDV, end diastolic velocity; TAMV, time-averaged maximum velocity.ᵃᵇMeans with different letters between the two cystic groups are different (P<0.007).ᵃᵇᶜUterine vascular perfusion means with different letters within a column are different (P<0.04).ᵃᵇᶜᵈMeans within a pair of columns (cystic versus control) with different letters are different (P<0.05), except that the difference approaches significance (P<0.07) for the comparison of the RI index between the small cystic area and its control.

*The presence or absence of external cysts was not considered in the cystic group. Controls had neither internal nor external cysts and were paired with mares of similar age with internal cysts. †Vessels of the mesometrial attachment averaged for the two uterine horns. ‡Percentage of endometrium and myometrium with color Doppler signals of blood flow. §Average for left and right horns of mares without cysts were used in the analyses.

The capability of color and power mode Doppler ultrasonography as an effective method for detecting the differences in uterine vascular supply and perfusion between mares with and without cysts was demonstrated for the first time in any species. To our knowledge, this is the first study on uterine vascular perfusion based on power mode Doppler ultrasonography in farm animals; previous studies used the color flow mode (Silva et al. 2005, Silva & Ginther 2006). Power Doppler increases the sensitivity for displaying blood flow within the soft tissue three- to fivefold, compared with conventional color display for Doppler ultrasonography (Ginther 2007). The greater sensitivity allows the evaluation of vessels with small diameter or slow blood flow that do not appear on a conventional color flow image because of incompatible velocity ranges and Doppler angles (Ginther 2007). The earlier study using color Doppler imaging and scoring from 1 to 4 found constant low endometrial vascular perfusion from 1 to 16 days after ovulation in nonpregnant mares; however, in pregnant mares, an increased uterine vascularity was observed after 11 days (Silva et al. 2005). Therefore, to evaluate potential weak vascular perfusion with minimum artifacts in cystic regions, the percentage of color signals was evaluated in the power mode for the study of uterine vascular perfusion. In addition, color Doppler signals

[Figure 4 Spectral color Doppler graphs from a gate (indicated by an arrow) placed in a color signal from a vessel of the mesometrial attachment of a mare (A) without and (B) with cysts. Spectral results are given in the boxes. Mares with uterine cystic area > 410 mm² (B) had a lower PI and greater velocities for PSV, EDV, and TAMV at the mesometrial attachment when compared with their paired controls (A). There was no difference for spectral end points between mares with uterine cystic area ≤275 mm² and their controls.]

within the endometrium are commonly inadequate for the production of spectral waveforms (Silva et al. 2005). Therefore, blood flow indices and velocities collected from the arteries of the mesometrial attachment have been used as an indirect method for the evaluation of uterine blood flow and perfusion (Silva et al. 2005, Ginther 2007).

The lower vascular perfusion in cystic regions when compared with adjacent noncystic regions and with the middle segments of both horns in mares without cysts supported the hypothesis that cysts are spatially associated with reduced uterine vascularity. The association between cysts and reduced uterine vascular perfusion was local; the reduced perfusion did not extend to other regions of the uterus. The reduced uterine vascular perfusion for both small (≤275 mm²) and large (>410 mm²) cystic areas when compared with the controls of similar age, and reproductive status did not support the hypothesis that local reduced vascular perfusion is more pronounced when the cystic area is large. Speculatively, reduced uterine vascular perfusion in the vicinity of cysts as well as interference with embryo dynamics and implantation could contribute to infertility and loss of impartment of growth of the conceptus. In this regard, endometrial receptivity, conceptus implantation, and pregnancy development are dependent on an adequate uterine circulation in humans (Sterzik et al. 1989, Chien et al. 2004). In addition, impairment of fetal growth due to restricted blood supply has been described in sheep (Harding et al. 1985).

Mares with small uterine cystic area versus paired controls of similar age and reproductive status were not different in the mesometrial blood flow velocities (PSV, EDV, and TAMV) and PI or in the vascular perfusion, diameter, circumference, and area of a cross section of the mesometrial attachment. However, mares with a large uterine cystic area had increased mesometrial PSV, EDV, and TAMV and decreased PI when compared with the paired controls. These findings support the hypothesis that a large uterine cystic area has a greater effect on uterine hemodynamics than a small area. The increased mesometrial blood flow velocities (PSV, EDV, and TAMV) may have been a response to the increased resistance to the uterine blood flow (lower perfusion) in mares with large uterine cystic area. Oxidative stress is a critical factor in processes involving ischemia/reperfusion (Ferrari et al. 1990) and has been correlated with endothelial dysfunction and increase in vascular tone of peripheral vessels (Yang et al. 1998, Zhou et al. 2005). Furthermore, greater blood flow velocities (EDV and PSV) have been found in arterial segments with narrowed lumen (Zwibel & Perrellito 2005). The elevated mesometrial PSV might also reflect a compensatory increase in cardiac systole from the increased uterine vascular resistance (decreased perfusion) as well as resistance at the mesometrial attachment. The myocardium has the ability to increase its force of contraction in response to a severe increased arterial resistance (reviewed by Frohlich 1983). The increased blood velocities detected in the mesometrial attachment and the decreased blood flow perfusion in uterine cystic regions in the present study indicates that vascular defects may play a role in the development of cysts. This is supported by histological studies that suggested that angiosis of arterioles in the endometrium and lymphangiectasia are possible precursors of lymphatic cysts (reviewed by Schoon et al. 1999). Therefore, it is likely that the cysts were a response rather than a cause of the vascular dysfunction. In addition to the vascular dysfunction (Schoon et al. 1999), the presence of lymphatic (Kenney & Ganjam 1975, reviewed by Stanton et al. 2004) and glandular (Kenney & Ganjam 1975, Ricketts 1975, Kenney 1978) distention may also contribute to continued development of uterine cysts.

The hypothesis that old mares without cysts have lower uterine vascular perfusion than noncystic young mares was not supported. The mares did not have a history of pregnancy or parturition during the past 10 years, unlike mares of similar age on breeding farms, and this may account for the lack of difference in vascularity. It has been suggested that age may influence the incidence of uterine angiopathies in mares (Oikawa et al. 1993, Nambo et al. 1995, Schoon et al. 1999). Although mild uterine angiopathies may occur independent of age, the severity of uterine angiosis has been associated with the number of pregnancies and parturitions in addition to the age in mares (Grüninger et al. 1998). A higher uterine resistance in multiparous mares than uniparous mares has been reported (Bollwein et al. 1998); however, the relationship between uterine vascular perfusion and age was not studied. Although the uterus has the ability for restoration post partum, elastosis of the arterial wall is a frequent degenerative change of uterine vessels in multiparous aged mares (Grüninger et al. 1998). Aging induces a series of structural, architectural, and compositional modifications in the vasculature even in healthy individuals (rats: Guyton et al. 1983; humans: reviewed by Lakatta 2002), and these modifications are associated with a decrease in vascular elasticity (Tao et al. 2004) and an increase in thickness of the arterial wall (Vaitkevicius et al. 1993). Studies in humans have demonstrated that both arterial stiffness and speed of the blood flow increase progressively with the advancing of age in healthy individuals (Avolio et al. 1985, Vaitkevicius et al. 1993, Mitchell et al. 2004). Therefore, an age-related increased stiffness or decreased distensibility of the arterial walls may have contributed to the greater mesometrial blood flow velocities (EDV and TAMV) in older mares in the present study.

In conclusion, power mode Doppler ultrasonography was effective for the evaluation of the uterine vascular perfusion in mares with and without cysts. Uterine cystic regions had lower uterine vascular perfusion than
noncystic regions. A large uterine cystic area (mm$^2$) was associated with lower PI and higher blood flow velocities in the mesometrial attachment. In mares without cysts, aging increased the pulse wave velocities (EDV and TAMV) in the mesometrial attachment but did not alter uterine blood flow resistance and vascular perfusion of the uterine layers. The results indicated that uterine vascular disturbances should be considered as an additional potential cause of uterine cysts.

### Materials and Methods

#### Animals

Animals were handled in accordance with the United States Department of Agriculture Guide for Care and Use of Agricultural Animals in Research. The study was done during November in the Northern Hemisphere (latitude 43 °N). The mares ($n = 76$) were mixed breeds of large ponies and apparent pony–horse crosses weighing 270–410 kg (mean 300 ± 6 kg) and were 3–25 years of age (mean 11.8 ± 0.6 years). The score for body condition for all mares was high throughout the experiment (score ≥ 7; Henneke et al. 1983). Mares were kept under natural light in an open shelter and outdoor paddock and were maintained on alfalfa/grass hay with access to water and trace-mineralized salt. Mares had docile temperament and no macroscopic abnormalities of the reproductive tract as determined by ultrasound examination (Ginther 1995a), except that some had uterine cysts. Only one examination per mare was used. Reproductive status was based on previous records obtained every day or alternate day by ultrasound scanning of the ovaries and uterus (Ginther 1995b). The age of mares was estimated from dental characteristics (American Association of Equine Practitioners Manual 2002).

#### Ultrasonography

Mares were scanned using a duplex B-mode (gray scale) and pulsed-wave color Doppler ultrasound instrument (Aloka SSD-3500; Aloka America, Wallingford, CT, USA) equipped with a finger-mounted 7.5 MHz convex-array transducer (UST-995-7.5). The principles, techniques, and interpretation of B-mode examination of the mare reproductive tract have been reviewed (Ginther 1995b). The brightness and contrast controls of the monitor and the gain controls of the scanner were standardized to constant settings (Gasatal et al. 1998).

The color and power flow modes were utilized to display signals for blood flow in the vessels of the endometrium, myometrium, perimetrium, and mesometrial attachment (Fig. 1). In the power Doppler mode, the degree of uterine vascular perfusion was based on the estimated proportion of tissue with color spots, as described previously and validated using the color Doppler mode in mares (Silva et al. 2005). The color gain setting was kept constant. Real-time B-mode and color and power flow mode images of continuous scans were captured with an online digital videotaping system and stored for selection of images for illustrative purposes.

The B-mode and color and power Doppler uterine end points were evaluated while the entire uterus and mesometrial attachment were being scanned in a slow continuous motion several times (Ginther 2007). The uterus was divided into nine imaginary segments of similar length (Ginther 1995a) as follows: LA, LM, LP, RA, RM, RP, BA, BM, and BP (Fig. 1). The nine segments were utilized to identify three distinctive uterine portions as follows: extremities (LA, RA, and BP), central segments (LM, RM, and BM), and cornual-body junction (LP, RP, and BA). Scanning started at the caudal portion of the uterus (close to the cervix), and the transducer was directed toward each horn as described (Ginther 1995a). Longitudinal and cross-sectional planes of the uterine body, horns, and mesometrial attachment were acquired. To obtain a cross section of the mesometrial attachment and its accompanying vessels, the transducer was positioned dorsocaudally within 1 cm of the surface of each horn, directed cranially, and rotated on its vertical axis (Silva et al. 2005, Silva & Ginther 2006). Mares were slightly sedated during Doppler scans with detomidine hydrochloride (1 mg per mare, i.v., Dormosedan; Pfizer Animal Health, Philadelphia, PA, USA) to optimize ultrasound color images and minimize the presence of artifacts due to animal movement.

For the spectral Doppler mode, the velocity range limit was set at 19.9 cm/s and the Doppler filter at 100 Hz (Silva et al. 2005). However, the setting for the range of flow velocity detection was adjusted, when indicated, to obtain the optimal spectral graph (Ginther 2007). The sample cursor or gate was set at a width of 1.5 mm. The angle of insonation was unknown. The gate was placed within the image of a lumen of a vessel in the mesometrial attachment of each uterine horn, using the most prominent color signal and pulse repetition frequency as a guide. A Doppler spectrum with three cardiac cycles was generated, and the optimal cycle was used for spectral measurements (Fig. 1). This procedure was done two more times, and the mean of the three measurements for each spectral end point was used in the statistical analyses.

#### Uterine vascular perfusion

Vascular perfusion of the uterus (all layers combined) was estimated subjectively by one operator from the cross sections of a horn using the power flow function. The following ultrasound settings were used: velocity range limit, 6.22 cm/s; flow filter, 3; and frame rate, 6 Hz. Only the color signals that appeared to be within the limits of the uterus were considered. The uterine vascular perfusion was estimated subjectively using the percentage of uterine tissue with color signals of blood flow during real-time cross-sectioning of the uterus in a continuous span of 1 min. Multiple cross sections were viewed because of animal and uterine movements and the variation resulting from angles of insonation. In mares without cysts (controls), regions of the middle segments of left and right horns were used. In mares with cysts, uterine vascular perfusion was estimated at a cyst location with the most prominent internal cysts versus the adjacent uterine region without internal uterine cysts.

#### Evaluation of the mesometrial attachment

Each entire mesometrial attachment was scanned in a slow continuous motion for 1 min. The dimensions of a cross section...
of the mesometrial attachment of each side were obtained. The selected cross section of the mesometrium was oblong and contained sections of vessels in various planes. No attempt was made to determine if a cross section represented a single vessel or multiple vessels. Measurements of the mesometrium were performed from one still image in B-mode, using the split-screen function with the color mode image on one side to facilitate location and delineation of the cross section (Ginther 2007). The diameter (mm) was obtained from an average of height and width. The area (mm²) and circumference (mm) of each cross section of the mesometrium was determined, using the scanner’s tracing function. The vascular perfusion of the attachment in the cross section was estimated subjectively by one operator during scanning in the color flow Doppler mode, using a pulse repetition frequency of 8 Hz and constant color gain settings and scoring from 1 to 4 (minimal to maximal, including fractional scores).

Spectral assessment of the blood flow velocity in an artery at the mesometrial attachment was performed as described (Silva et al. 2005, Silva & Ginther 2006). The end points for the gated intramesometrial color signal were resistance index (RI), pulsatility index (PI), PSV, EDV, and TAMV. The meaning and formulas for these spectral end points are well established (Zwiebel & Pellerito 2005, Ginther 2007).

Part 1: prevalence and distribution of uterine cysts

The prevalence of uterine cysts was determined in the pool of 76 mares. The mares were assigned to one of the three age groups as follows: <7, 7–14, and >14 years (15–25 years). Uterine cysts were classified, based on their cross-sectional locations, as internal when located within the endometrium and/or myometrium, and external when located outside and apparently attached to the perimetrium. For longitudinal location of cysts, internal and external uterine cysts were assigned to each of the nine segments and three portions of the uterus within each mare.

Part 2: effect of cysts

From the pool of 76 mares, 21 pairs were used to study the association between uterine cysts and uterine and mesometrial vascular perfusion and mesometrial blood flow velocities (PSV, EDV, and TAMV). The age and number of days post-ovulation were balanced within pairs of control mares (uterine cysts not detected) and mares with cysts.

Part 3: effect of size of cystic area

Out of the 21 pairs from part 2, 19 were used to evaluate the association between the size of the cystic area (mm²) and the uterine and mesometrial vascular perfusion and mesometrial blood flow velocities (PSV, EDV, and TAMV). The mares were assigned to a group in which the cystic area of the cystic member of the pair was ≤275 mm² (11 pairs) versus >410 mm² (8 pairs). Two pairs in which the cystic member had an intermediary cystic area (312 and 360 mm²) were not considered. Therefore, mares were arranged in four groups: small uterine cystic area (≤275 mm², n=11) paired with controls (n=11) and large uterine cystic area (>410 mm², n=8) paired with controls (n=8). The diameter (mm) of each cyst was obtained from an average of height and width. To determine the area (mm²) of a cyst, the cyst was considered circular in shape, and the formula for area of a circle (πr²) from mean diameter was utilized. The sum of the area for all internal cysts was used to represent the total or combined internal uterine cystic area. The same approach was used to determine the external uterine cystic area per animal.

Part 4: effect of age in mares without cysts

From the pool of 76 mares, 22 mares without cysts (uterine cysts not detected) were used to study the effect of age on uterine and mesometrial vascular perfusion in the absence of cysts. The mares were assigned into two age groups: young (3–10 years, 6.7 ± 0.7 years; n=11) and old (15–21 years, 16.5 ± 0.5 years; n=11). Mares of the intermediate age were not used in order to increase the age difference between the two age groups. None of the mares had foaled during the last 10 years, and 10 out of 11 young mares (<10 years) had never been bred. The remaining young mare had an unknown reproductive history.

Statistical analyses

Paired and unpaired Student’s t-tests were used to locate differences within and between two groups respectively. The area (mm²) of internal and external cysts in different uterine portions and the three age classes were analyzed by one-way ANOVA and Duncan’s multiple range tests. Chi-square tests of independence were used to examine the differences in frequency data. Pearson’s correlation analyses were performed between age and Doppler end points in part 4. A probability of P≤0.05 indicated that a difference was significant, and probabilities between P>0.05 and ≤0.1 indicated that a difference approached significance. Data are presented as mean±S.E.M., unless otherwise indicated.

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