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Uterine blood flow evaluation in bitches suffering from cystic endometrial hyperplasia (CEH) and CEH-pyometra complex

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ABSTRACT

Doppler ultrasound is a useful diagnosis tool to evaluate uterine blood flow in different canine reproductive states. The aim of this study was to describe and compare uterine blood flow in bitches suffering from cystic endometrial hyperplasia (CEH) and CEH-pyometra complex (CEH-P). Ninety diestrous bitches were clinically, hematologically, and ultrasonographically classified into four groups: (1) clinical signs, leukocytosis, CEH, and uterine luminal contents (CEH-P, $n = 31$); (2) asymptomatic bitches with CEH and uterine contents (CEH-C, $n = 15$); (3) asymptomatic bitches with CEH without uterine contents (CEH, $n = 16$); and (4) normal diestrous bitches (ND, $n = 28$). The widest cross-sectional diameter and uterine wall thickness (W) of uterine horns were measured using two-dimensional ultrasound. Peak systolic velocity and end diastolic velocity of uterine arteries were measured by Doppler ultrasound in all the bitches. Peak systolic velocity and end diastolic velocity were higher in CEH-P than in CEH-C, CEH, and ND ($P < 0.01$); although both parameters did not show differences among the latter three groups ($P > 0.1$). Conversely, resistance index (RI) reported lower values in CEH-P than that in the other three groups ($P < 0.01$), being ND higher ($P < 0.01$) than both CEH-C and CEH which did not differ between themselves ($P > 0.1$). When all the bitches were considered, correlation between RI and cross-sectional diameter was $r = -0.69$ ($P < 0.01$) and between RI and uterine wall thickness was $r = -0.02$ ($P > 0.1$). It is concluded that the uterine artery blood flow velocity of bitches suffering from pyometra was higher, not only from normal bitches, but also from females with endometrial hyperplasia. Furthermore, endometrial hyperplasia, accompanied or not by luminal contents, had a higher blood flow velocity than normal uterus. Hemodynamic parameters appear as useful markers to differentiate uterine pathologic conditions.

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1. Introduction

The cystic endometrial hyperplasia-pyometra complex (CEH-P) is one of the most prevalent uterine diseases in the female dog [1]. Middle-aged to old, diestrous bitches are frequently affected [1]. Cystic endometrial hyperplasia is a

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subclinical disease characterized by the proliferation of endometrial glands resulting in the formation of fluid-filled cysts and luminal uterine contents may be present in some cases [2,3]. Pyometra, which is associated with purulent contents, presents severe systemic signs of illness because of the bacterial infection [4]. Conversely, when the uterine contents are serous, bloody, or mucoid, the condition is generally not associated with any clinical sign other than infertility [1,5,6]. Sterile uterine fluids can be considered as the initiating stage that progresses into pyometra after uterine bacterial colonization occurs [1].

In dogs, ultrasonographic evaluation of the uterus provides valuable information to diagnose uterine diseases. The thickness of the uterine walls and the presence of luminal contents can be determined, allowing differentiation between pregnancy and pathologic conditions [7–9]. Although it has been reported that the echogenicity of the uterine contents suggests cellularity, it has also been claimed that ultrasonographic differentiation of the contents is difficult [8–10].

Doppler ultrasonography is a noninvasive technique that provides information about the organs perfusion [11]. This technique has found to be a useful tool to diagnose numerous gynecologic lesions in several species [12–14]. In women and cows, uterine pathologic conditions may cause a blood flow increase that can be detected by Doppler ultrasound.

In dogs, this technique has been used to evaluate uterine arteries blood flow during estrus, normal and abnormal pregnancy, and puerperium [15–17]. Although the characterization of uterine blood flow by Doppler ultrasound will probably contribute to the precocious diagnosis of CEH and pyometra, this technique has not been studied in bitches with uterine diseases so far. Thus, the aim of this study was to describe and compare uterine blood flow in normal diestrous bitches and other suffering from CEH and CEH-P complex.

2. Materials and methods

2.1. Animals and experimental groups

Ninety, 1 to 14-year-old, 3 to 40 kg, cross, and pure-bred diestrous (15–60 days after the end of the previous estrus) bitches that were admitted in Teaching Hospital of the Faculty of Veterinary Sciences of the National University of La Plata, Argentina from 2009 to 2014 were included in this study. The females were evaluated for presence of clinical signs of systemic illness (fever, inappetance, depression; [18]), and leukocytosis (White blood cell count (WBC) >17,000 cells/mL; [19]). Finally, their uteri were ultrasonographically examined as described in the following (ultrasonographic measurements) and the animals were classified into the following groups:

- pyometra group: the presence of clinical signs, leukocytosis, CEH, and uterine luminal content (CEH-P, n = 31). Twenty-two of these bitches had purulent vulvar discharge, whereas nine bitches showed no

discharge. These animals were classified as open- and closed-cervix pyometra, respectively.

- cystic endometrial hyperplasia with luminal contents: asymptomatic bitches with normal WBC, CEH, and uterine contents (CEH-C, n = 15)
- cystic endometrial hyperplasia without luminal contents: asymptomatic bitches, with normal WBC and CEH without uterine content (CEH, n = 16)
- normal diestrus: bitches having no clinical, hematologic, or ecographic signs (ND, n = 28 [4]).

2.2. Ultrasonographic measurements

Two-dimensional ultrasonographic evaluations of the uterus were carried out with a 6-MHz convex transducer (Toshiba Core Vision Pro, Japan). The dogs were positioned in lateral recumbency and gently restrained. No sedation was used. Acoustic gel was applied to the transducer and coupled directly to the clipped skin. The widest cross-sectional diameter (D) and uterine wall thickness (W) of uterine horns were measured immediately after body bifurcation [9].

Color Doppler was conducted to localize uterine arteries at both sides of the uterine body, and pulsed-wave Doppler was performed to obtain the waveforms [20]. The angle of insonation was manually corrected according to previous reports [21]. Measurements with an angle less than 20° were only considered for analysis. Three consecutive waveforms with maximum Doppler shift were included in the study [21]. Peak systolic velocity (PSV; m/s) and end diastolic velocity (EDV; m/s) were measured, whereas resistance index [RI = (PSV–EDV)/PSV] was automatically calculated.

2.3. Statistical analysis

Peak systolic velocity, EDV, and RI of the left and right uterine arteries were compared using Student's *t* tests. Also in CEH-P, PSV, EDV, and RI of bitches with open- and closed-cervix pyometra were compared using the same test. Values of PSV, EDV, and RI were analyzed by one way ANOVA followed by Tukey's test, using age and body weight as covariates (SPSS 19.0; SPSS, Chicago, IL, USA). To further interpret the findings, RI was correlated with D and W using Pearson's correlation test. All data were presented as mean ± SEM and *P* < 0.05 was considered significant.

3. Results

No differences were observed for PSV (*P* > 0.1), EDV (*P* > 0.5), or RI (*P* > 0.5) between left and right uterine arteries in any group. Thus, results were calculated including both arteries. In addition, no differences were found in PSV, EDV, and RI between bitches with open- and closed-cervix pyometra (*P* > 0.1); therefore, all of these bitches were analyzed together. In CEH-P, the waveforms were characterized by a diastolic flow extending to the next cardiac cycle. In CEH-C and CEH, the EDV was recorded in

14 of 15 cases and 12 of 16 cases, respectively. In ND, 10 of 28 bitches did not show an end diastolic flow.

Peak systolic velocity ($P < 0.01$; Fig. 1) and EDV ($P < 0.01$; Fig. 2) were higher in CEH-P than that in CEH-C, CEH and ND, although both parameters did not show differences among the latter three groups ($P > 0.1$). Conversely, RI reported lower values in CEH-P than that in the other three groups ($P < 0.01$; Fig. 3), being ND higher ($P < 0.01$) than both CEH-C and CEH which did not differ between themselves ($P > 0.1$). No influence of age and body weight was found in PSV, EDV, and RI ($P > 0.1$). When all the bitches were considered, correlation between RI and D was $r = -0.69$ ($P < 0.01$) and between RI and W was $r = -0.02$ ($P > 0.1$).

4. Discussion

This study reported that Doppler ultrasonography can accurately reflect hemodynamic changes that are present in bitch patients with different uterine pathologic conditions. Although normal bitches presented a significantly higher resistance Doppler waveform of the uterine arteries, the CEH-P Doppler waveforms reported a low resistance pattern. In CEH and CEH-C groups, uterine blood flow was higher than in normal animals but lower than in CEH-P.

These findings are concomitant with previous reports in women, where uterine infection was associated with high uterine blood flow [12,22]. In addition, the high RI of ND versus CEH and CEH-C is in line with a previous canine report [23] but is not in agreement with another one [24], in which RI of normal diestrous bitches was lower than that in bitches suffering from CEH. Moreover, in the present study, RI values found in ND and CEH groups are higher than those reported by England et al. (2012) for 12 bitches but similar to our previous report in normal diestrous female dogs [16].

During an inflammatory response, increased blood flow, vasodilatation, and angiogenesis are present [12]. In

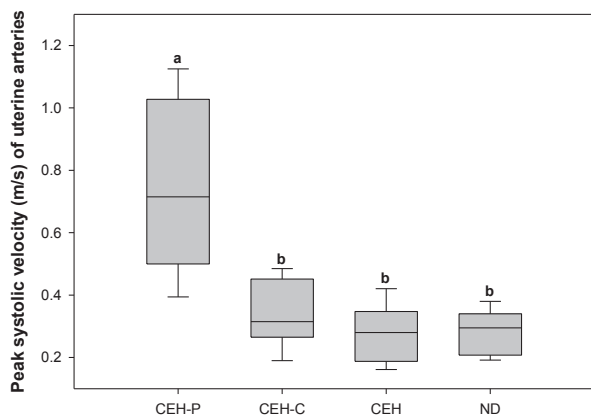


Fig. 1. Peak systolic velocity (mean \pm SEM) of 90 diestrous female dogs with clinical signs, leukocytosis, CEH, and uterine luminal content (CEH-P, $n = 31$), asymptomatic bitches with a normal white blood cell count and CEH with (CEH-C, $n = 15$) and without (CEH, $n = 16$) uterine luminal content and normal diestrous bitches (ND, $n = 28$). Different letters indicate differences ($P < 0.01$) among groups. CEH, cystic endometrial hyperplasia; CEH-P, CEH-pyometra complex.

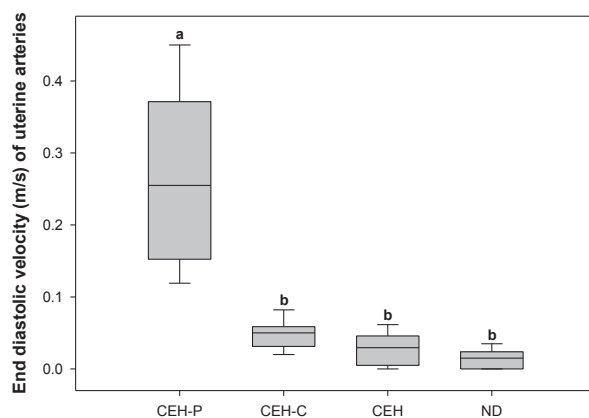


Fig. 2. End diastolic velocity (mean \pm SEM) of the dogs of Figure 1. Different letters indicate differences ($P < 0.01$) among groups.

women, uterine inflammation associated with an infectious process may lead to a diminution of RI values of uterine arteries [12]. Furthermore, a previous study in bitches with pyometra reported an increase in the intra-uterine concentration of prostaglandin E, which may also lead to an increase in uterine perfusion [25,26]. In addition to prostaglandins, nitric oxide, a potent vasodilator, is also released locally in several mammalian species during endometrial inflammation [27,28].

The low vascular resistance detected by Doppler ultrasonography in CEH-P versus CEH-C can be used to support the diagnosis of canine pyometra. In this study, no CEH-C bitch showed a RI below 0.72; thus, this threshold value could be used for the differential diagnosis between CEH-P and CEH-C. Further studies, including a larger number of animals, are still necessary to determine reference ranges as well as the prognosis value and the utility of Doppler for therapeutic elections. Furthermore, in women, Doppler ultrasound has been found to be a useful tool to evaluate medical treatment response [29]. The possibility to follow up the evolution of treated patients could improve the clinical outcome, avoiding unnecessary surgery and preserving the reproductive tract, in valuable bitches.

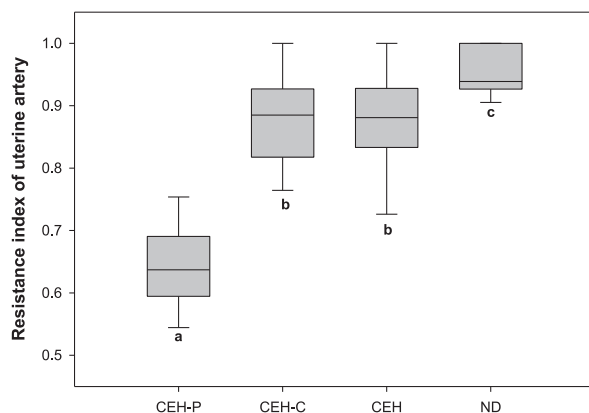


Fig. 3. Resistance index (mean \pm SEM) of the dogs of Figure 1. Different letters indicate differences ($P < 0.01$) among groups.

It is concluded that the uterine artery blood flow of bitches suffering from pyometra was higher, not only from normal bitches but also from females with endometrial hyperplasia. Furthermore, endometrial hyperplasia, accompanied or not by luminal contents, has a higher blood flow than normal uterus. Hemodynamic parameters appear as useful markers to differentiate canine uterine pathologic conditions.

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