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Assessment of the uterus in bovines with endometritis using Doppler ultrasound

Avaliação do útero bovino com endometrite pelo ultrassom Doppler

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Abstract

Postpartum uterine diseases, such as metritis and endometritis, are highly prevalent in dairy cows, exerting a significant detrimental impact on reproductive performance and, consequently, economic activity. Cows with puerperal endometritis may show alterations in the hemodynamics of the uterine tissue and arteries. In this study, we applied Doppler ultrasound to describe the hemodynamic changes in the uterus of cows diagnosed with endometritis between 25 and 35 days postpartum. A total of 89 cows were divided into two experimental groups, infected (n=56) or uninfected (n=33), based on endometrial cytology. Clinical examinations, vaginoscopy, and Doppler ultrasound were performed. Cows with endometritis exhibited a larger cervix (P=0.040) and a larger left uterine horn (P=0.020) compared to healthy cows. Additionally, 78.6% of the cows with endometritis exhibited abnormal uterine discharge, whereas only 57.6% of healthy cows had the same condition (P=0.0005). Spectral Doppler evaluation of the uterine arteries revealed no differences between groups. However, a higher score or increase in uterine vascularization on endometrial Doppler was associated with *Trueperella pyogenes* (P=0.0003) and the presence of intrauterine heterogeneous content (P=0.0047). Furthermore, mesometrial Doppler findings correlated with endometrial Doppler (P<0.001), uterine bacterial presence (*P*=0.001), and intrauterine heterogeneous content (*P*=0.049). Doppler ultrasound offers a rapid evaluation method for uterine alterations. Moreover, it is a less invasive alternative to uterine biopsy and endometrial cytology and provides insights into fertility and uterine health.

Resumo

Doenças uterinas pós-parto, como metrite e endometrite, são altamente prevalentes em vacas leiteiras. Essas doenças afetam negativamente o desempenho reprodutivo e, consequentemente, a atividade econômica. Vacas no período puerperal com endometrite podem apresentar alterações na hemodinâmica do tecido uterino e das artérias uterinas. Este estudo teve como objetivo utilizar a ultrassonografia Doppler para descrever as alterações hemodinâmicas no útero de vacas com endometrite diagnosticada entre 25 e 35 dias pós-parto. Um total de 89 vacas foram divididas em dois grupos experimentais, infectadas (n=56) ou não infectadas (n=33), de acordo com os resultados da citologia endometrial. Foram realizados exames clínicos, vaginoscopia e ultrassonografia Doppler. Vacas com endometrite apresentaram aumento no diâmetro do colo do útero (P=0,040) e no corno esquerdo (P=0,020) em comparação com vacas saudáveis. 78,6% das vacas com endometriose apresentaram corrimento uterino anormal, enquanto 57,6% das vacas saudáveis apresentaram a mesma condição (P=0,0005). A avaliação com Doppler espectral das artérias uterinas não revelou diferenças entre os grupos. A maior pontuação ou aumento da vascularização do útero do Doppler endometrial foi correlacionada com *Trueperella pyogenes* (P=0,0003) e conteúdo intrauterino heterogêneo (P=0,0047). Finalmente, Doppler mesometrial foi correlacionado com Doppler endometrial (P<0,001), bactérias uterinas (P=0,001) e conteúdo heterogêneo intrauterino (P=0,049). Em relação à evolução das alterações uterinas, a ultrassonografia Doppler fornece resultados rápidos e é uma técnica menos invasiva à biópsia do útero e à citologia endometrial e traz respostas sobre fertilidade e saúde do útero.

Palavras-chave: clínica de bovinos; clínica da reprodução; infertilidade.

1 | Introduction

High milk production results in significant negative energy balance and can cause changes in hormone levels, embryonic losses, and a higher incidence of reproductive disorders in dairy cows (Radostits et al. 2007; Bollwein et al., 2013). Unsatisfactory reproductive performance negatively affects productivity (Gröhn and Rajala-Schultz, 2000), leading to decreased milk production and fewer calvings (Couto Serrenho et al., 2021).

Uterine infections such as metritis and endometritis are the most important causes of infertility in dairy cows, causing a marked decrease in reproductive performance that can inflict significant economic losses (Couto Serrenho et al., 2021; LeBlanc, 2023). Metritis is a severe inflammation of all uterine layers and presents with reddish-brown, fetid uterine discharge and systemic clinical signs such as fever and reduced milk production within 21 days postpartum, as observed during clinical uterine examination (Dubuc et al., 2011). In contrast, endometritis occurs 21 days postpartum and is characterized by endometrial inflammation without any systemic clinical signs (Sheldon et al., 2006). Cytological endometritis may also develop without purulent discharge but is identified by a high number of neutrophils on an endometrial cytology slide (Sheldon et al., 2006; Leblanc, 2023). Doppler ultrasound, which provides visualization of blood flow presence, direction, and type, can be used to assess the hemodynamic changes crucial to understanding the morphophysiological aspects of the female reproductive tract (Carvalho et al., 2008). This technique is commonly used to classify fertility (Steer et al., 1994) and infertility in women. Doppler ultrasound shows changes in uterine circulation and is widely used in veterinary reproductive medicine (Bollwein et al., 2000; Bollwein et al., 2002).

Alterations in the hemodynamics of the uterine tissue and arteries have been hypothesized to occur in cows presenting puerperal endometritis. As such, the use of more than one diagnostic technique may help in the identification of uterine diseases. In this study, Doppler ultrasound was used to describe the hemodynamic changes in the uterus of cows with endometritis diagnosed between 25 and 35 days postpartum.

2 | Material and Methods

2.1 | Animals

In total, 100 cows underwent examinations, however, problems with the ultrasound device were encountered in 11 cases. Thus, 89 cows were included in this study, and divided into two groups according to the cytology results: a control group (C) (n=33) and an endometritis group (n=56).

2.2 | Study area and case definition

This study was carried out on dairy farms in the State of São Paulo (n=2) and Minas Gerais (n=1), Brazil, under the strict regulations of the Brazilian Committee for Animal Use in Experiments (CONCEA) protocol number 5135030214 and approved on August 27, 2014, by Committee for Animal Use in Experiments (CEUA) - at the School of Veterinary Medicine in the University of São Paulo, which is equivalent to the EU Directive 2010/63/EU for animal experiments. All farms operated a free-stall breeding system and performed assisted calving. 89 Holstein Friesian cows between 25 and 35 days postpartum were investigated. All cows were in their second and fifth lactation and were assigned to two experimental groups according to the endometrial cytology (Kasimanickam et al., 2005). The control group (C) consisted of 33 healthy cows (neutrophil polymorphonuclear count <10%), while the endometritis group (E) included 56 cows diagnosed by cytology (neutrophil polymorphonuclear count ≥10%). Clinical examination, vaginoscopy, Doppler ultrasound, and sample collection were all performed on the same day.

2.3 | *Clinical examination*

The female genital tract was evaluated by rectal palpation, as described by Grunert et al. (2005). Briefly, ovaries were examined for follicles and corpora lutea (CL). Morphological alterations, such as volume changes, were evaluated in the cervix. The location, size, and symmetry of the horns and the consistency of the uterus were also investigated. Changes were described according to Grunert et al. (2005), using the following classification: I (not very thick) to VI (very bulky). Horns were classified as symmetrical (S) or asymmetrical (As). According to consistency (C), the uterus was classified as flaccid (CI), reactive (CII), or with vigorous and prolonged contractions (CIII).

2.4 | Vaginoscopy

Vaginoscopy was performed following rectal palpation. The speculum was introduced into the vagina to observe the vaginal mucosa, cervix, and presence of discharge, according to Grunert et al. (2005).

2.5 | Ultrasound and Doppler mode

Ultrasound was performed using an M-TURBO FUJI FILM SONOSITE (Bothel, WA, USA) with a multifrequency micro convex probe (8,5-5 MHz), as proposed by Meira Jr. et al. (2012) and Heppelmann et al., (2013). Cervical and uterine diameters were classified as small or negative (<3.5cm), medium or suspect (between 3.5 and 5cm), and large or positive (>5cm), following the criteria proposed by Meira Jr. et al. (2012) (Figure 1). The presence of heterogeneous intrauterine content (HIC) and a hyperechoic uterine wall were associated with purulent discharge, according to Descoteaux et al. (2010) (Figure 2).

After conventional ultrasonographic evaluation, the hemodynamic patterns of the left and right uterine arteries were analyzed in the spectral Doppler mode (Bollwein et al., 2000). The maximum flow velocity, pulsatility index, and systole/diastole ratio were determined, as described by Heppelmann et al. (2013) (Figure 3).

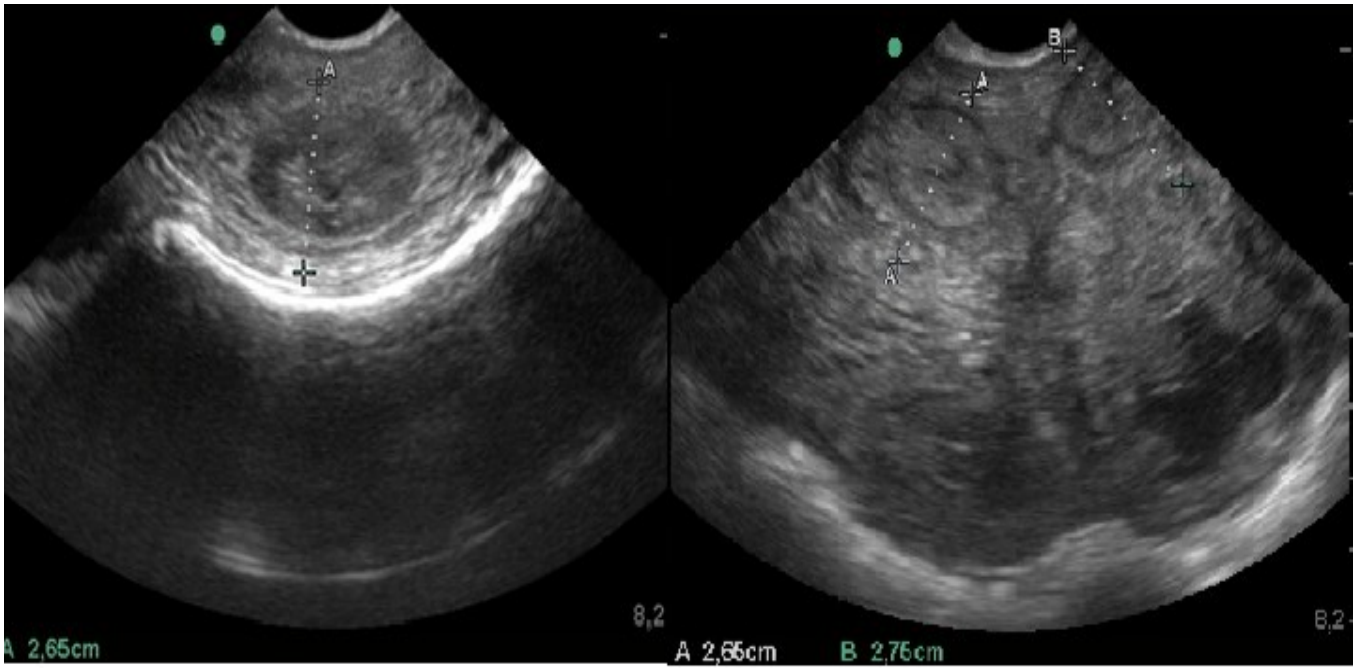

Figure 1. Ultrasound image in the transverse section of the cervix (left) and uterine horns (right), using 8.5 MHz micro probe convex. Measurement in centimeters of the cervical and uterine horn diameters in a cow.

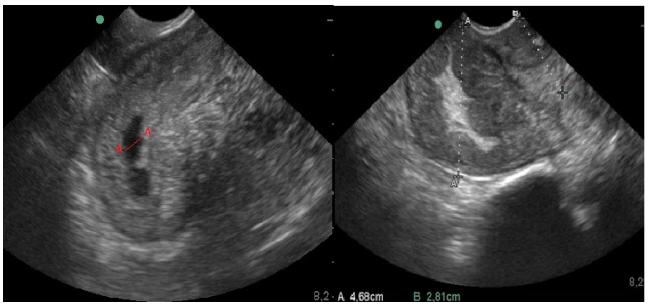

Figure 2. Ultrasound images in transverse section of the uterus, using a microconvex 8.5 MHz probe, characterizing intrauterine contents in abnormal proportions; intrauterine-fluid (left) and hyperechoic intrauterine content (right).

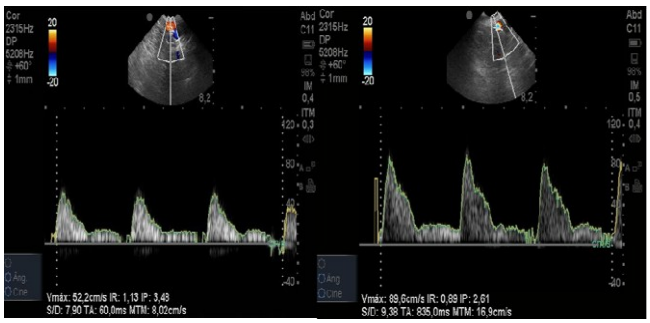

Figure 3. Ultrasound image of the cross section of the uterus, using 8.5 MHz microprobe. Spectral Doppler evaluation of right and left uterine arteries.

Color Doppler mode was used to evaluate the hemodynamic pattern of the uterine tissue at the intercornual ligament. A colorimetric scale for reproductive patterns (Ginther, 2007) was used to establish a correlation with the inflammatory processes. The procedures involved a subjective evaluation of endometrial vascularization (0, without vascularization to 2, very vascularized) and the mesometrium (0, without vascularization to 4, extremely vascularized), as described by Ginther (2007) (Figures 4 to 8).

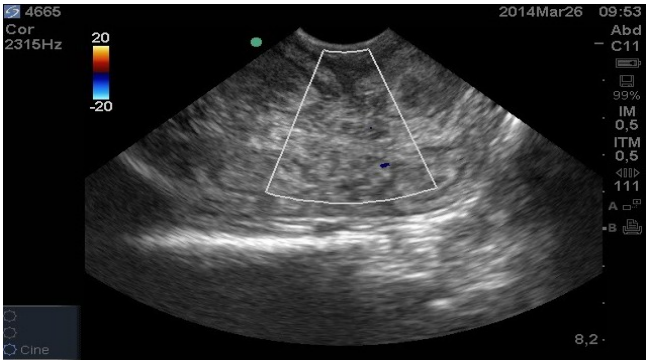

Figure 4. Characterization of the score 0 for the subjective evaluation of the vascularization pattern of mesometrium (m) and score 0 for endometrium (e) by colorimetric evaluation technique in the doppler color mode.

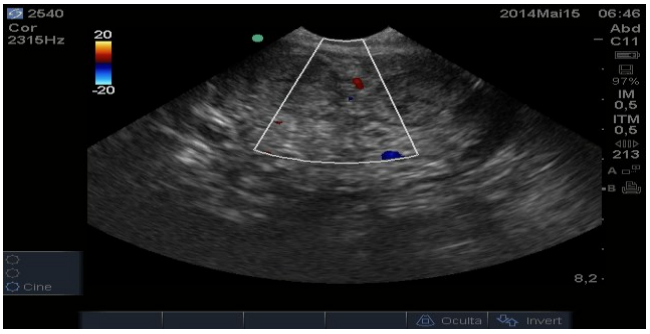

Figure 5. Characterization of score 1 for the subjective evaluation of the vascularization pattern of mesometrium 0 by colorimetric evaluation technique in the doppler color mode.

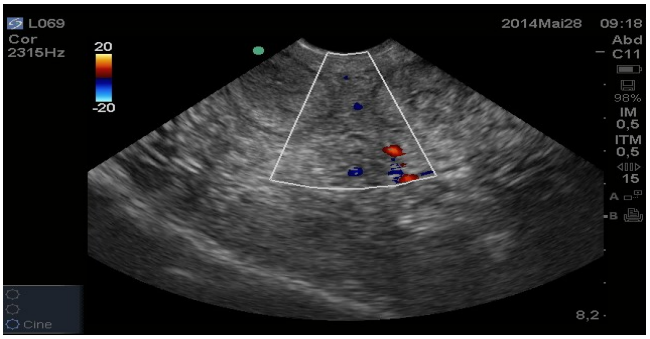

Figure 6. Characterization of score 2 for the subjective evaluation of the pattern of vascularization of the mesometrium and score 0 for endometrium by colorimetric evaluation in doppler color mode.

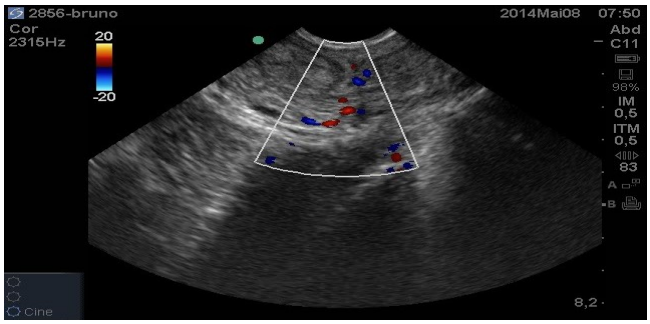

Figure 7. Characterization of score 3 for the subjective evaluation of the vascularization pattern of the mesometrium (m) and score 1 for the endometrium (e) by colorimetric evaluation technique in color doppler mode.

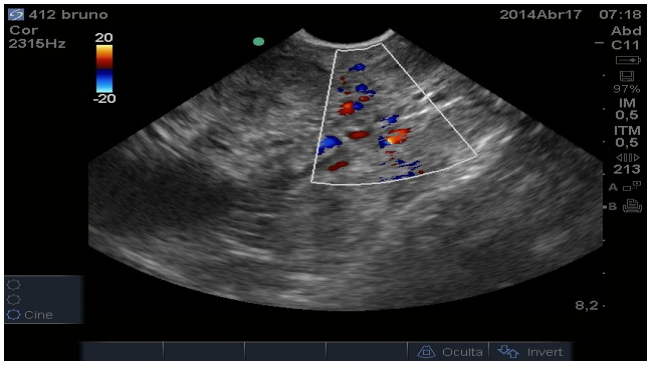

Figure 8. Characterization of score 4 for the subjective evaluation of the vascularization pattern of the mesometrium (m) and score 2 for the endometrium (e) by colorimetric evaluation in doppler color mode**.**

The ovaries were evaluated for size, consistency, and structure (CL, follicles, and cysts). The morphological characteristics of the ovaries were also assessed to verify the presence of abnormalities, such as cysts or tumors, to determine the presence of cyclic luteal ovarian activity, and to estimate the probable phase of the estrous cycle, factors that may affect animal fertility (Mello, 2014). In particular, CL vascularization was evaluated using color Doppler and categorized as either present or absent (Figure 9).

Figure 9. Ovarian evaluation using the Doppler color mode of an ovary with CL without vascularization (left) and an ovary with CL vascularized (right).

2.6 | Hysteroscopy

A hysteroscopic examination was performed using a rigid endoscope (430×6mm) with two channels: one for the infusion of 0.9 % sterile saline for uterine lumen distension and the other for cytological and microbiological sample collection. The optical portion of the endoscope was introduced into the vagina and guided by transrectal palpation. Sterile saline was infused into the uterus to allow anatomical exploration. The presence of mucopurulent or purulent inflammatory lesions in the mucosa was considered a sign of endometritis (Madoz et al., 2014). Mucosal appearance was classified as either healthy or unhealthy, with the latter characterized by the presence of pus, fibrin, or hyperemia. After exploration, a small portion of the saline was recovered and used for macroscopic, cytological, and microbiological evaluation of the uterine contents.

2.7 | Uterine cytology

A total of 150μL of the recovered saline was transferred to a cytocentrifuge chamber and centrifuged at approximately 550 rpm for six minutes. Then, the samples were fixed onto a glass slide for microscopy (Gilbert et al., 2005). The slides were stained using the Panótico Rápido® kit, and cytology was performed by counting 100 cells under 400x magnification using an optical microscope. The percentage of polymorphonuclear neutrophil (%PMN) was subsequently determined. Cows with %PMN>10% were considered positive in the cytology test (Kasimanickam et al., 2005).

2.8 | Microbiological

For this assay, 500 μL of the recovered saline was added to thioglycolate broth (Difco®) and stored at 2°-10°C until processing at the Laboratory of General Bacteriology, Biological Institute, São Paulo. Subsequently, 10 μL of this mixture was plated onto 5% sheep blood agar and incubated for 72 hours at 37°C under microaerophilic conditions. The morphological characteristics of each colony were recorded, and Gram staining was performed. Bacterial species were identified using biochemical tests according to Sauer et al. (2008).

2.9 | Statistical analysis

Error normality and homogeneity of variances were analyzed using the Shapiro–Wilk and Bartlett tests, respectively. Data that were not normally distributed were analyzed using the non-parametric Kruskal-Wallis test. Inter-group differences (control and endometritis) were evaluated by ANOVA and confirmed with a t-test (JMP 12.0, SAS). The same conditions (PROC GLIMMIX from SAS) were used for binary data ("subjective evaluation score of endometrial perfusion" and "subjective evaluation score of mesometrium perfusion"). Correlations between variables were assessed using the Spearman correlation test for non-normally distributed variables (PROC CORR in SAS). A significance level of 5 % was used for all the tests. All tests were performed using SAS software.

3 | Results

A total of 100 cows underwent examinations, however, technical issues with the ultrasound device prevented complete data collection in 11 cases. Thus, 89 cows were included in this study and divided into two groups according to cytology results: the control group (C) (n=33) and the endometritis group (E) (n=56).

3.1 | Ultrasonographic evaluation

Cows with endometritis showed a larger cervix (P=0.040) and left horn (P=0.020) than healthy cows (Table 1). In the endometritis group, 78.6% of cows showed abnormal uterine discharge, whereas only 57.6% of cows showed this symptom in the control group (*P*=0.0005) (Table 2). In addition, the intrauterine heterogeneous content was more prevalent in the endometritis group (66.0%) than in the control group (30.3%) *(P*=0.0011) (Table 3). Evaluation of endometrial and mesometrial vascularization (Table 4 and 5) using the colorimetric method revealed differences in the vascularization score (VS) of the endometrium between the healthy and endometritis group (P<0.05) (Tables 6 and 7). During ovarian examination, the presence or absence of the CL did not differ between the groups (Table 8). The absence of a vascularized corpus luteum in the left ovary was more frequent in the endometritis group (P=0.029) (Table 9). Spectral doppler evaluation of the uterine arteries revealed no differences between the groups (Table 10).

Table 1. Average diameter between measurements of linear ultrasound of the cervix and uterine horns with endometritis

Uterine structures	Control	Endometritis	P value
Cervical (cm)	3.55 (±0,13)	3.89 (±0,01)	0.040
Right horn (cm)	2.56 (±0,11)	2.77 (±0,08)	0.100
Left horn (cm)	2.48 (±0,13)	2.89 (±0,10)	0.020

Table 2. Presence of intrauterine fluid (IUF) in cows with and without endometritis

	Control n (%)		Endometritis n (%)		P value
Presence of IUF	(14/33)	42.42%	(44/56)	78.57%	0,0005
Absence of IUF	(19/33)	57.58%	(12/56)	21.43%	

Table 3. Animals with abnormal intrauterine heterogeneous content (IUHC) throughout the experiment

	Control n (%)		Endometritis n (%)		P value
Presence of IUHC	(10/33)	30.30%	(37/56)	66.07%	0,0011
Absence of IUHC	(23/33)	69.70%	(19/56)	33.93%	

Table 4. Comparison of the endometrium color Doppler correlation compared to other variables

	Doppler Endometrium -1<r>1	P value
T. pyogenes	0.38	0.0003
Bacterium	0.38	0.0003
IUHC	0.36	0.0047

IUHC - intrauterine heterogeneous content; T. pyogenes - Trueperella pyogenes.

Table 5.-Doppler color correlation of the Mesometrium compared with other variables

	Doppler Mesometrium	P value
Doppler Endometrium	0.52	0.000
Bacterium	0.35	0.001
Yeast	-0.25	0.022
IUHC	0.21	0.049

IUHC - intrauterine heterogeneous content.

Table 6. Evaluation of the endometrial vascular pattern

EV- Doppler Endometrium	Control n (%)		Endometritis n (%)		P value
0	(31/33)	93.94%	(42/56)	75.00%	0,0246
1+2	(2/33)	6.06%	(14/56)	25.00%	

0 - non-vascularized, 1 - poorly vascularized, 2 - very vascularized.

Table 7. Frequency of the evaluation of the mesometrium vascular pattern

EV- Doppler Mesometrium	Control n (%)		Endometritis n (%)		P value
0	(10/33)	30.30%	(8/56)	14.29%	
1	(11/33)	33.33%	(9/56)	16.07%	
2	(5/33)	15.15%	(14/56)	25.00%	0,0344
3	(5/33)	15.15%	(12/56)	21.43%	
4	(2/33)	6.06%	(13/56)	23.21%	

0 - non-vascularized, 1 - poorly vascularized, 2 - vascularized; 3 - very vascularized; 4 - extremely vascularized.

Table 8. Frequency of CL as a function of endometritis

	Control n (%)		Endometritis n (%)		P value
Presence of CL Right	(15/33)	45.45%	(24/56)	42.86%	0.811
Absence of CL Right	(18/33)	54.55%	(32/56)	57.14%	
Presence of CL Left	(16/33)	48.48%	(18/56)	32.14%	0.125
Absence of CL Left	(17/33)	51.52%	(38/56)	67.86%	

CL - corpora lutea

Table 9. Presence of vascularized CL as a function of endometritis

	Control n (%)		Endometritis n (%)		P value
Presence of CLv Right	(12/33)	36.36%	(19/56)	33.93%	0.815
Absence of CLv Right	(21/33)	63.64%	(37/56)	66.07%	
Presence of CLv Left	(15/33)	45.45%	(13/56)	23.21%	0.029
Absence of CLv Left	(18/33)	54.55%	(43/56)	76.79%	

CL - corpora lutea

Table 10. Average Spectral Doppler values in the uterine arteries

Doppler spectral	Control	Endometritis	P value
Vmax-Right	88.47 (±5.33)	93.84 (±4.14)	0.428
IR- Right	0.85 (±0.35)	0.84 (±0.02)	0.766
IP-Right	2.57 (±0.20)	2.66 (±0.16)	0.744
S/D Right	22.16 (±6.39)	12.63 (±4.96)	0.242
MTM-Right	20.01 (±2.35)	21.84 (±1.83)	0.541
Flow- Right	0.94(0.11)	1.02 (0.086)	0.540
Vmax-Left	90.19 (±4.47)	85.77 (±3.38)	0.433
IR- Left	0.80 (±0.03)	0.85 (±0.03)	0.217
IP-Left	2.62 (±0.27)	2.70 (±0.20)	0.809
S/D- Left	19.47 (±5.67)	12.19 (±4.30)	0.309
MTM-Left	21.50 (±2.05)	18.93 (±1.55)	0.321
Flow- Left	1.01(0.09)	0.89 (0.07)	0.321

VMAX- maximum speed; IR- Index resistance; IP-Index pulsatility; S/D- systole and diatst; MTM- average time

3.2 | Microbiological findings

Bacillus spp., *Trueperella pyogenes*, *Escherichia coli* and *Staphylococcus intermedius* were the most frequently isolated bacteria. Yeasts were detected in 25% of the samples (Table 11).

Table 11. Identification of microorganisms isolated in the collected samples.

	Endometritis n (%)	
Bacterium+Yeast	(6/85)	7.06%
Yeast	(21/85)	24.71%
Bacterium	(38/85)	44.71%
T. pyogenes	(10/85)	11.76%
S. intermedius	(7/85)	8.24%
S. aureus	(1/85)	1.18%
Streptococcus sp.	(1/85)	1.18%
Bacillus sp.;	(13/85)	15.29%
E. coli	(7/85)	8.24%
Proteus sp.	(2/85)	3.53%

4 | Discussion

Endometritis is a uterine disease associated with a negative reproductive performance (Sheldon et al. 2006). In addition to physical examinations, new diagnostic tools are important to improve the early detection of this condition (Dubuc et al., 2010).

The current study revealed differences in cervical and uterine size between cows with endometritis and healthy cows (Table 12). After parturition, the uterus was significantly enlarged, reaching approximately 8 – 10 kg. In general, uterine macroscopic involution occurs between three to five weeks postpartum, when the uterus should weigh approximately 0.9kg and the diameter of the pre-gravid uterine horn should be less than 5cm. Involution of the cervix is completed between four to six weeks postpartum (Morrow et al., 1966). The establishment of a new pregnancy depends on the anatomical and functional return of the genital tract to a pre-pregnancy state (Mateus et al., 2002). In the present study, an increase in the volume of the left uterine horn was observed in animals with endometritis (Table 12). This finding aligns with other studies demonstrating that uterine infection delays uterine involution (Slama et al., 1991; Mateus et al., 2002). Additionally, cows with endometritis had larger cervical diameters than healthy animals. LeBlanc et al. (2002) highlighted that cows with clinical endometritis showed a cervical 'diameter ≥7.5 after 20 days postpartum and presented mucopurulent or purulent discharge detected by vaginoscopy after 26 days postpartum. The current study highlights that endometritis increased in cows with abnormal intrauterine fluid accumulation, which agrees with the results of several studies (Barlund et al., 2008; Oral et al., 2009; Heppelmann et al., 2013). The presence of intrauterine fluid was correlated with *Trueperella pyogenes*, yeast, *Staphylococcus intermedius*, *Escherichia coli*, indicating that these agents cause inflammation of the uterine mucosa and proliferation of the endometrial glands, leading to a greater accumulation of intrauterine fluid (Table 13 and 14).

Table 12. Correlation between endometritis and other variables.

	Endometritis -1<r>1	P value
Bacterium+ Yeast	0.38	0.0003
IUHC	0.34	0.0012
IUF	0.37	0.0005
Doppler Mesometrium	0.32	0.0027
Eclv	-0.23	0.0359
Doppler Endometrium	0.22	0.0383

IUHC - intrauterine heterogeneous content; IUF - intrauterine fluid; Eclv - left vascularized corpus luteum.

Endometritis was detected in cows with heterogeneous intrauterine contents (IUHC). Meira Jr. et al. (2012) further reported a strong correlation between IUHC results and the diagnosis of endometritis. IUHC was likewise associated with *Trueperella pyogenes*, *Escherichia coli*, *Bacillus spp*., *Staphylococcus intermedius*, and yeast, demonstrating that these microorganisms may cause genital discharge (Table 13 and 14) (Meira Jr. et al., 2012).

Table 13. Correlation between the presence of intrauterine fluid (IUF) and the presence of microorganisms

	IUF -1<r>1	P value
Bacterium	0.52	0.0001
T. pyogenes	0.27	0.0109
Yeast	0.25	0.0215
S. intermedius	0.23	0.0344
E. coli	0.23	0.0345

T. pyogenes - Trueperella pyogenes; S. intermedius - Staphylococcus intermedius E. coli - Escherichia coli

Table 14. Correlation of animals with heterogeneous content compared to other variables

	IUHC -1<r>1	P value
IUF	0.72	0.0001
Bacterium	0.64	0.0001
T. pyogenes	0.36	0.0007
E. coli	0.3	0.0052
Yeast	0.27	0.0135
Bacillus sp.	0.25	0.0223
S. intermedius	0.22	0.0434

IUF - Intrauterine fluid*; T. pyogenes - Trueperella pyogenes; S. intermedius - Staphylococcus intermedius E. coli - Escherichia coli*

Endometrial vascularization scores (Grades 1 and 2) were elevated in cows with endometritis, suggesting differences between healthy and unhealthy cows (Table 4). Color Doppler mode provides colored images of blood flow, allowing the estimation of tissue vascularization (Potter et al., 2010). Ginther et al. (2007) previously proposed a model to evaluate uterine hemodynamics associated with endometritis. This model was modified in the present study, revealing a few animals with alterations in the subjective evaluation score of endometrial vascularization, as well as a positive correlation with *Trueperella pyogenes*.

Ultrasonographic evaluation of the endometrium using color doppler confirmed a positive correlation with endometritis. This information emphasizes the possible combination of this new non-invasive technique with standard diagnostic methods to predict uterine disease. In contrast, no differences were detected between the groups in the spectral doppler mode data. Piersanti et al. (2019) described that transrectal ultrasound revealed the accumulation of echogenic fluid and inflammatory process in the uterus of heifers following bacterial infusion (*Trueperella pyogenes* and *E. coli*), which was absent in control heifers. Bollwein et al. (2013) reported increased pulsatility at 24 hours postpartum, with a peak on the 28th day, decreasing progressively until the 90th day. An inversely proportional relationship was further observed in blood flow, which was initially high in the postpartum period and decreased until around the 28th day, subsequently maintaining basal levels up to 90th day. Clinical and histological uterine involution ranges from 21 to 50 days postpartum, which may differ from the recovery of hemodynamic patterns of the uterus, which requires more time (Bollwein et al., 2016).

In a study regarding uterine perfusion, Bollwein et al. (2002) observed a strong increase in blood flow in the uterine arteries throughout pregnancy. However, the resistivity index decreased in the first eight months of gestation and remained relatively stable until calving. During diestrus, blood flow velocity remains constant, and is correlated with the plasma concentrations of estrogen and progesterone, indicating a moderate positive correlation (Bollwein et al., 2016). These results indicate that other factors are involved in the regulation of uterine blood flow (Honnens et al., 2008).

In healthy cows, uterine blood flow decreases following complete uterine involution. However, cows with uterine disease present slow uterine involution, which occurs between 45 and 65 days postpartum, indicating an association between incomplete uterine involution and regeneration of the uterine vascular layer in cows with puerperal alterations. Changes in uterine perfusion were pronounced during the first four days after parturition. Uterine puerperal diseases, such as retention of fetal membranes and metritis exert a negative impact on uterine involution. Thus, uterine blood flow is affected by puerperal uterine diseases. Cows with a retained placenta have a higher resistivity index than healthy cows. On the 8th day postpartum, cows with metritis had higher blood flow and decreased pulsatility in the arteries than healthy cows (Heppelmann et al., 2013). Guido et al. (2020) studied the use of color doppler ultrasound to obtain the endometrial perfusion score and proved it to be efficient for the diagnosis of subclinical endometritis in dairy cows, considering its strong correlation with PMNs.

The evaluation of uterine and ovarian vascularization helps to determine the best time to perform artificial insemination. For example, vascular perfusion of the corpus luteum and uterus is greater during the first follicular wave of the estrous cycle than in the second (Honnens et al., 2008). Color doppler mode identifies follicles during normal development and predicts the next ovulation stage. In this study, ovarian evaluation revealed that some cows with endometritis lacked CL in both ovaries. However, animals with vascularized right and left CL were 61.54% and 52.94%, respectively. Color Doppler mode showed that cows without vascularized left or right corpora lutea had endometritis, demonstrating that there is interference in reproductive performance, even in cows with endometritis exhibiting a normal estrous cycle.

Microbiological analysis revealed the presence of *Bacillus* spp., *Trueperella pyogenes*, *Staphylococcus intermedius, Escherichia coli, Streptococcus* spp*., Proteus* spp*., Staphylococcus aureus, Enterobacteria* spp., *Serratia* spp., yeast, and other fungi. Santos et al. (2011) and Piesanti et al. (2019) reported that *Escherichia coli, Streptococcus* spp*., Trueperella pyogenes* and *Fusobacterium necrophorum* are the main bacteria that commonly contaminate the uterine lumen after childbirth and are associated with uterine diseases. *Trueperella pyogenes* act synergistically with *Fusobacterium necrophorum, Bacteroides* spp*.,* and *Prevotella* spp. (Bicalho et al., 2012). Potter et al. (2010) previously reported that *Trueperella pyogenes* is an important pathogen associated with endometritis because of its persistence in a contaminated uterus. Boer et al. (2015) found that cows with bacterial growth at 21 days postpartum had a lower conception rate, regardless of the bacterial species. Using 16S rRNA sequencing, Bicalho et al., 2012 previously detected an increased prevalence of *Trueperella pyogenes* in cows with endometritis, while yeast infection has been shown to be associated with chronic endometritis in women. In the present study, yeast

was detected in 22.5% of cows. The presence of *Trueperella pyogenes* was correlated with uterine inflammation, as seen by colorimetric ultrasound.

In some cases, endometritis can be resolved spontaneously. Dubuc et al. (2011) reported a spontaneous cure rate of 63%, whereas LeBlanc et al. (2023) found a self-healing rate of 77%. However, this condition still causes significant economic and reproductive losses in the dairy industry. To mitigate this problem, it is important to use new tools to improve the precision and earliness of the diagnostic process, thereby improving reproductive performance.

5 | Conclusion

The diameters of the cervix and uterine horns were related to endometritis. This relation was most evident in the presence of intrauterine fluid and heterogeneous content. Color doppler imaging can be used to reveal an association between the vascularization of the inflamed tissue and endometritis. In contrast, spectral doppler ultrasound showed no significant differences between healthy and affected animals. Based on microbiological examination, *Trueperella pyogenes* and *Escherichia coli* play important roles in the development of endometritis, corroborating the results of other studies. Less invasive techniques with rapid results such as doppler ultrasound can provide valuable insights regarding the evolution of uterine alterations and improve reproductive rates.

6 | Conflict of interests

The authors declare no conflict of interest.

7 | Ethical Approval

The submitted article was approved by the Bioethics Commission of the Faculty of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA Nº 5135030214).

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