Assessment of the bovine uterus with endometritis using Doppler ultrasound

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ABSTRACT

Postpartum uterine diseases such as metritis and endometritis are highly prevalent in dairy cows. These diseases negatively affect the reproductive performance and consequently economic activity. Cows in the puerperal period presenting endometritis may have alterations in the hemodynamics of the uterine tissue and the uterine arteries, which differ them from healthy cows. Therefore, this study aimed to use the Doppler ultrasonography to describe the hemodynamic changes in the uterus of cows showing endometritis diagnosed between 25 - 35 days postpartum. Eighty-nine Holstein Friesian females with 25 to 35 days postpartum were studied. Cows were assigned to two experimental groups, infected or not infected, according to the results of the endometrial cytology. Clinical examination, vaginoscopy, Doppler ultrasound and sample collection (saline solution was injected and recovered by endoscopie method aiming cytological and microbiological evaluation of the uterus) were also performed. Cows with endometritis had the cervix ($P = 0.040$) and the left horn ($P = 0.020$) increased compared to healthy cows. 78.6% of the endometritic cows showed abnormal uterine discharge, while 57.6% of healthy cows had this same condition ($P = 0.0005$). The spectral Doppler evaluation of the uterine arteries revealed no differences between groups. *Bacillus spp.*, *Trueperella pyogenes, Escherichia coli* and *Staphylococcus intermedius* were the most isolated bacteria among samples. Higher score or increase of uterus vascularization of the endometrial Doppler was correlated with *Trueperella pyogenes* (P = 0.0003) and intrauterine heterogeneous content (P = 0.0047). Finally, mesometrial Doppler was correlated with endometrial Doppler (P < 0.001), uterine bacteria (P = 0.001) and intrauterine heterogeneous content (P = 0.049). Regarding the evolution of uterine alterations, Doppler ultrasonography provides fast results and is a lesser invasive technique such as uterus biopsie and endometrial citology and gives answers about fertility and uterus health.

Keywords: Endometritis, clinical reproduction, infertility

1.INTRODUCTION

High milk production causes high negative energy balance and could cause changes in hormone levels, embryonic losses and a higher incidence of reproductive disorders in dairy cows [1,2]. Unsatisfactory reproductive performance negatively affects productivity [3], decreasing milk production and number of calves [4].

The uterine infection, like metritis and endometritis is the most important cause of infertility in dairy cows. Therefore causing decrease in reproductive performance with economic injuries [5,6]. Metritis is defined as a severe alteration of all layers of the uterus. In the clinical uterine examination the infected has reddish-brown fetid uterine discharge and systemic clinical signs such as fever and reduced milk production, normally within 21 days postpartum [7]. In contrast endometritis occurs after 21 days postpartum and is the inflammation of the endometrium. Animals show no systemic clinical signs [8]. Cytological endometritis may also occur, with absence of purulent discharge but high number of neutrophils on the endometrial cytological slide[8].

By showing the presence, direction and type of blood flow the Doppler ultrasonography technique determines hemodynamics changes wich are important to understand the morphophysiological aspects of the female reproductive tract [9]. This concept is used in women ginecology to tell a part fertile [10] and infertile women [11]. Doppler ultrasound shows up the changes in the uterine circulation and it is used widely in veterinary reproduction [12,13].

It is hypothesized that alterations in the hemodynamics of the uterus tissue and arteries may happened in cows presenting endometritis in the puerperal. Thus, the use of more than one diagnostic technique may help in the identification of uterine diseases. Therefore, this study aimed to use the Doppler ultrasonography to describe the hemodynamic changes in the uterus of cows showing endometritis diagnosed between 25 - 35 days postpartum.

2.MATERIALS AND METHODS

2.1 Area characterization and case definition

This study was carried out in dairy farms from the State of São Paulo (N = 2) and from the State of Minas Gerais (N=1), Brazil, under the strict regulations of CONCEA (Brazilian commetee for Animal use in experiments, protocol number 5135030214 and approved 27/08/2014 at CEUA, commetee for animal use in experiments at the Veterinary School in the University of São Paulo), equivalent to EU Directive 2010/63/EU for animal experiments. All farms have freestall breeding system and assisted calving. Eighty-nine Holstein Friesian females with 25 to 35 days postpartum were studied. Cows were assigned to two experimental groups according to the results of the endometrial cytology [14] as follows:

a) Control group (C): healthy cows (neutrophil polymorphonuclear count $< 10\%$).
b) Endometritis group (E): cows diagnosed by cytology (neutrophil polymorphonuclear count $\geq 10\%$).

Clinical examination, vaginoscopy, Doppler ultrasound and sample collection were also performed, all in the same day.

2.2 Clinical examination

Female genital tract was evaluated by rectal palpation as proposed by Grunert, Birgel and Vale (2005) [15]. Briefly, ovaries were evaluated searching for follicles and corpus luteum (CL). In the cervix, morphological alterations such as volume was evaluated. In the uterus, location, size, symmetry of the horns and consistency were also investigated. Changes were described according to Grunert, Birgel and Vale (2005) [15] using the classification: from I (not very thick) to VI (very bulky). The horns were classified as symmetrical (s) and asymmetrical (As). According to consistency (C), the uterus was classified as flaccid (CI), reactive (CII), and with vigorous and prolonged contraction (CIII).

2.3 Vaginoscopy

Vaginoscopy was performed after rectal palpation. The speculum was introduced in the vagina to observe the vaginal mucosa, cervix and the presence of discharge according to Grunert, Birgel and Vale (2005) [15].

2.4 Ultrasonography and Doppler mode

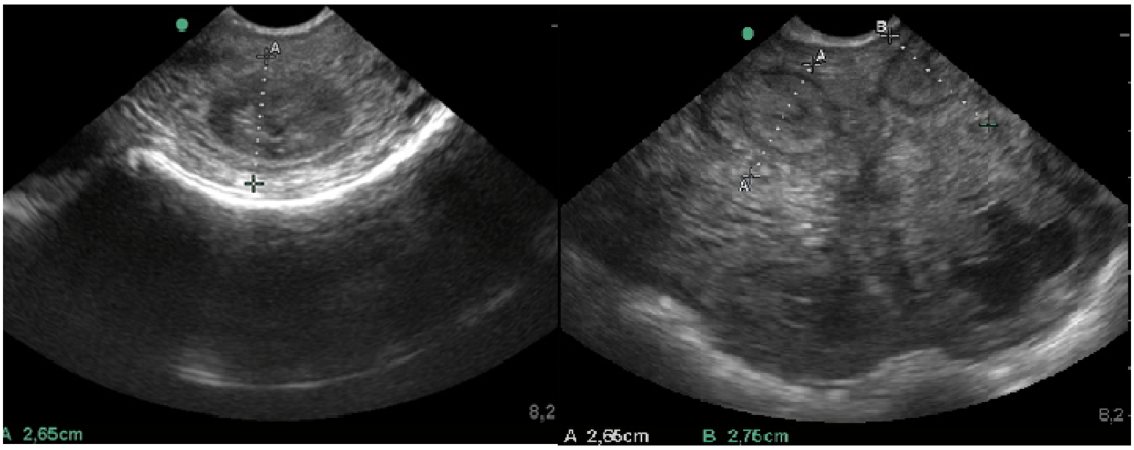
The ultrasound was performed with a m-turbo, Fuji film Sonosite (Bothel, WA, USA) with a micro convex probe (8-5 mhz), as proposed by Meira Jr et al. (2012) [16] and Heppelmann, Krüger and Leidl (2013)[17]. Diameters of the cervix and horns, ovarian structures and the presence of uterine discharge were evaluated. Cervical and uterine diameters were classified as small or negative (< 3.5 cm), medium or suspect (between 3.5 and 5 cm) and large or positive (> 5 cm) (Figure 1). Uterine dimensions were evaluated according to Meira Jr et al. 2012 [16] (Figure 1). The presence of heterogeneous intrauterine contents (hic) and the uterine wall with hyperechoic characteristics were associated with purulent discharge according to Descoteaux, Gnemmi and Colloton (2010) [18] (Figure 2).

After the conventional ultrasonographic evaluation, hemodynamic patterns of the left and right uterine arteries were analyzed using the spectral Doppler mode [12]. Maximum flow velocity, pulsatility index and systole/diastole ratio were determined as described by Heppelmann, Krüger and Leidl (2013) [17] (Figure 3).

Color Doppler mode was used to evaluate the hemodynamic pattern of the uterine tissue in the intercornual ligament. The colorimetric scale for reproductive patterns [19] was used to establish a parallel with the inflammatory process. The procedures involved a subjective evaluation of the endometrial vascularization (0 - without vascularization to 2 - very vascularized) and the mesometrium (0 - without vascularization to 4 - extremely vascularized), as described by Ginther (2007) [19] (Figures 4 to 8).

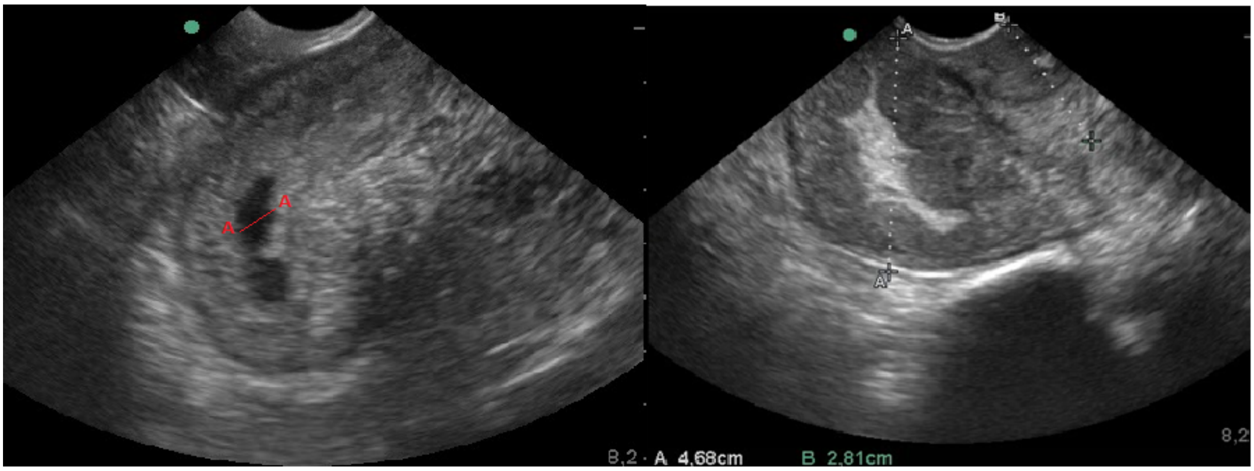
The ovaries were evaluated for their size, consistency and structures (CL, follicles and cysts). Morphological characteristics of the ovaries were assessed to verify the presence of abnormalities such as cysts or tumors, to determine the existence of cyclic luteal ovarian activity and to estimate the probable phase of the cycle, factors that may affect animal's fertility [20]. Particularly, the vascularization of the CL was assessed by colorimetric Doppler mode, and it was classified as containing or not containing vascularization (Figure 9).

Figure 1: Ultrasound image in transverse section of the cervix (left) and uterine horns (right), using micro probe convex 8.5 Mhz. Measurement in centimeters of the diameter of cervix and uterine horns in bovine.



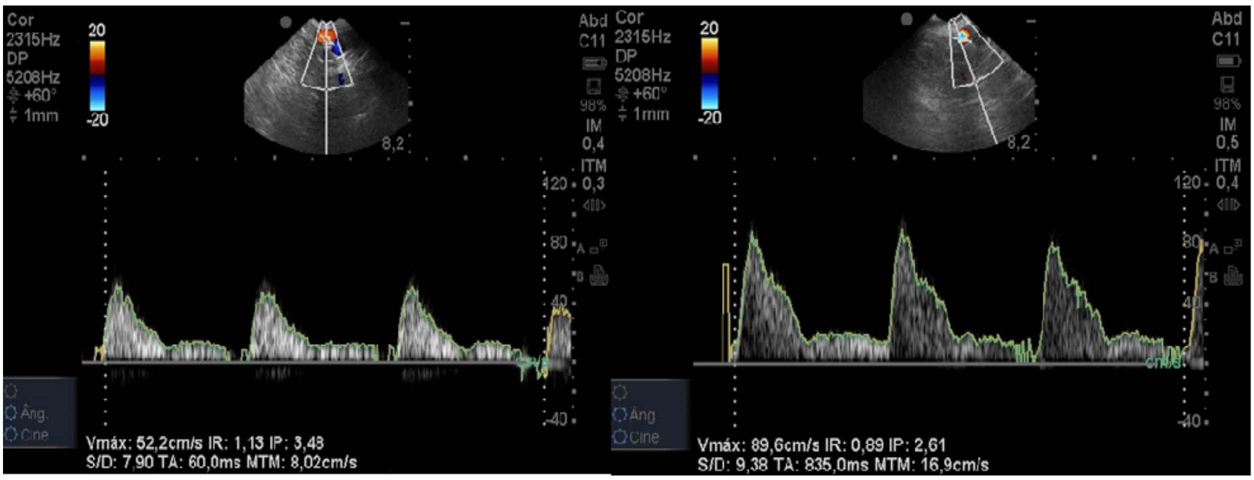
Source: (RIBEIRO, B.L.M. 2016) [21]

Figure 2 : Ultrasound imaging 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.



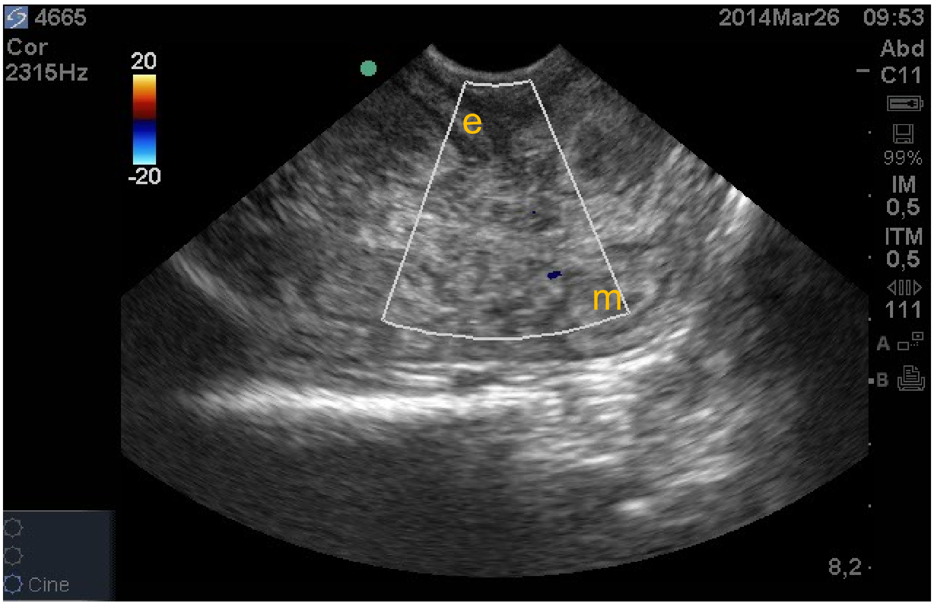
Source: (RIBEIRO, B.L.M. 2016) [21]

Figure 3: Ultrasound image in cross section of uterus, using 8.5 Mhz micro probe. Spectral Doppler of right left uterine arteries.



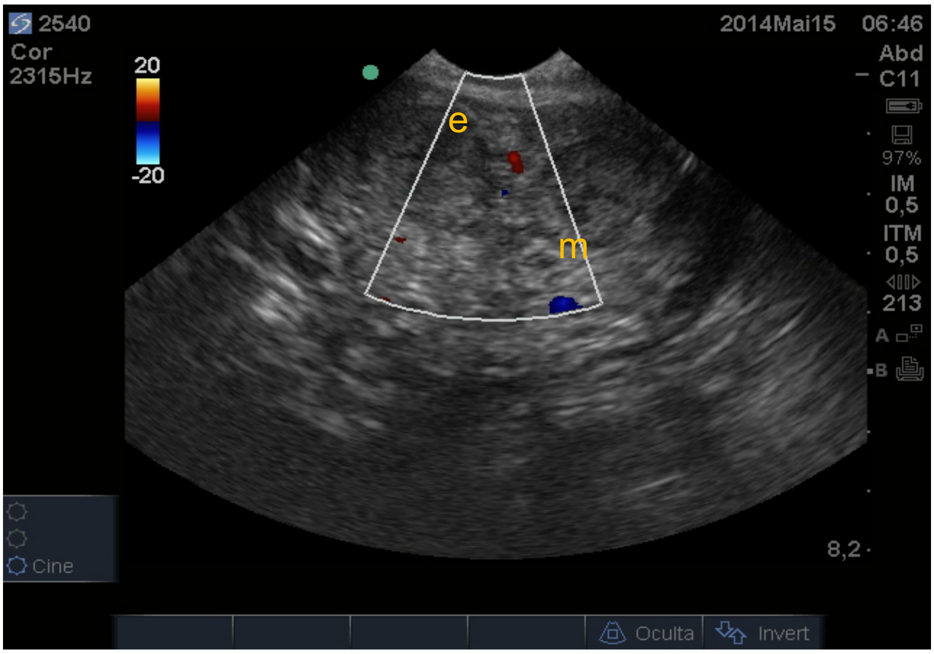
Source: (RIBEIRO, B.L.M. 2016) [21]

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



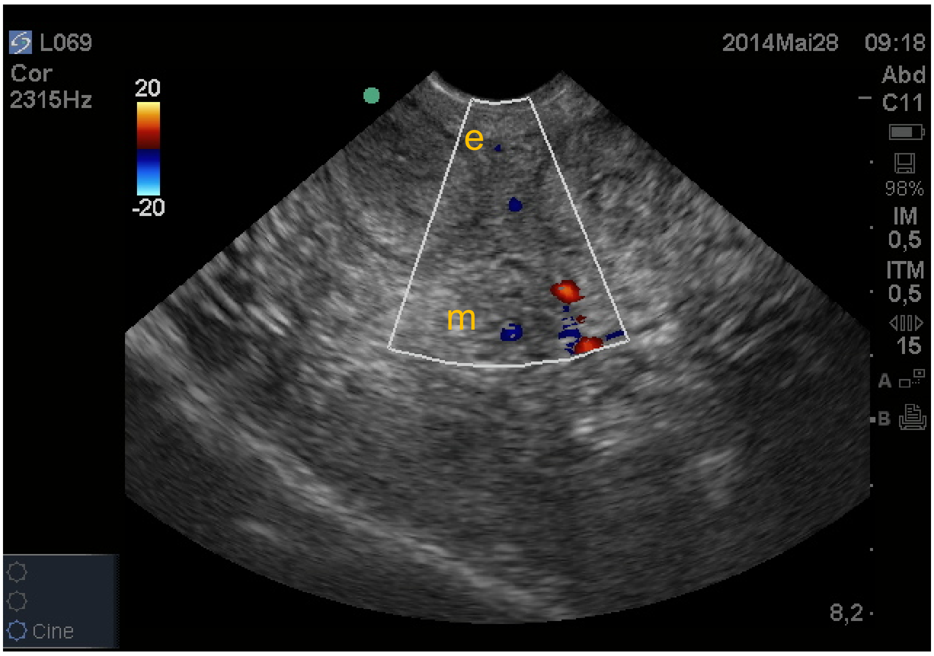
Source: (RIBEIRO, B.L.M. 2016) [21]

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



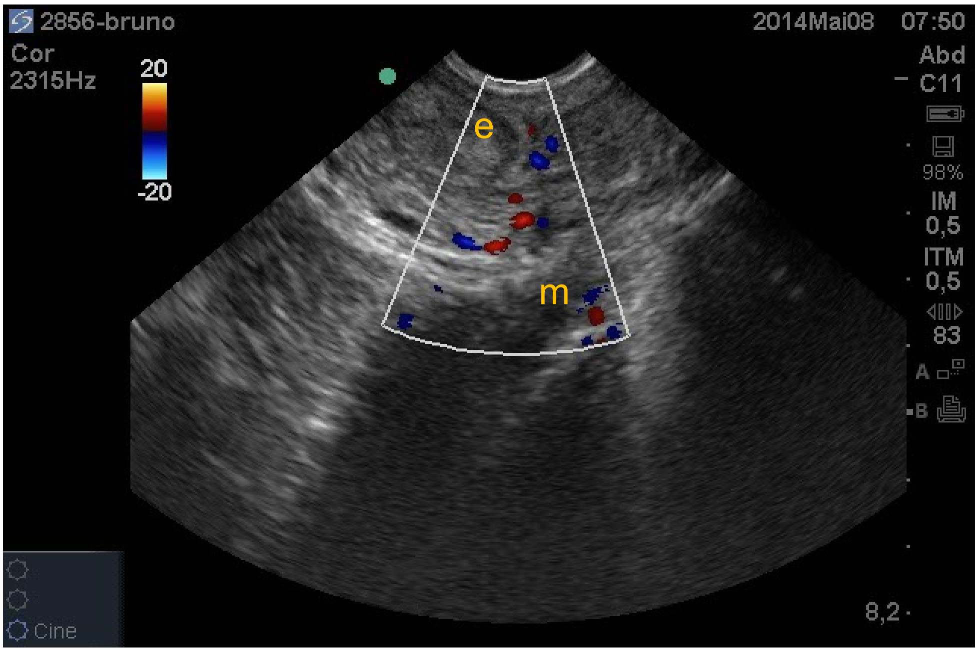
Source: (RIBEIRO, B.L.M. 2016) [21]

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.



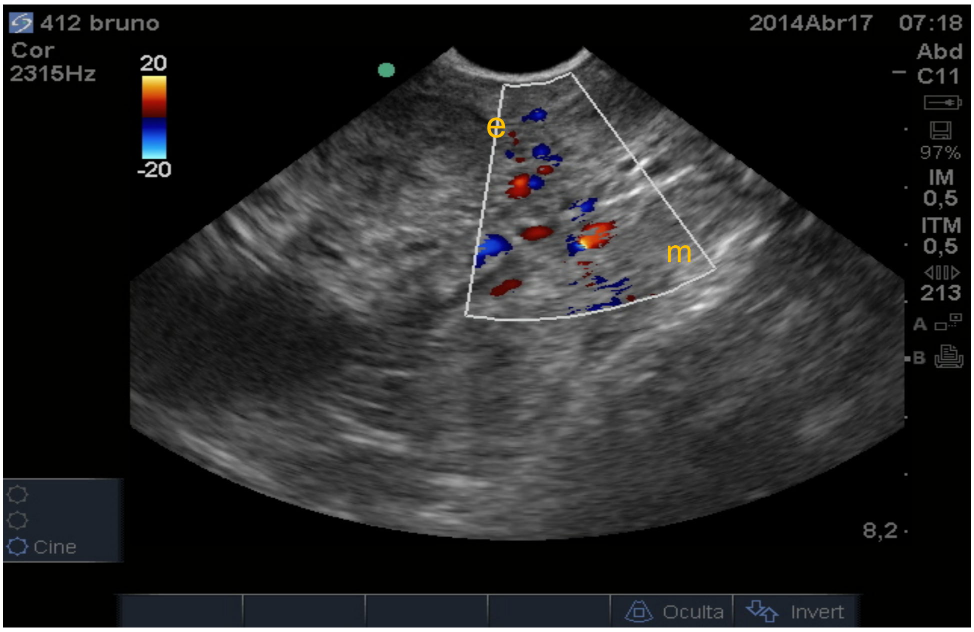
Source: (RIBEIRO, B.L.M. 2016) [21]

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



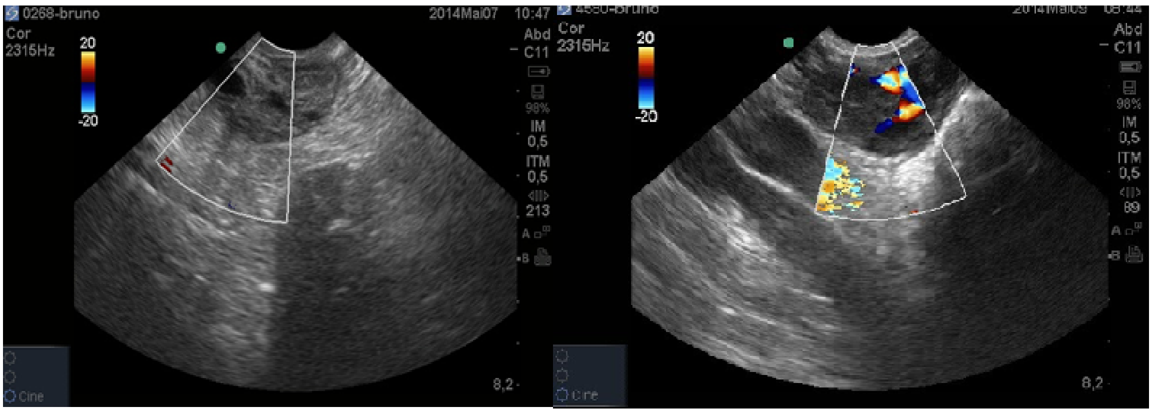
Source: (RIBEIRO, B.L.M. 2016) [21]

Figure 8: Characterization of the 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.



Source: (RIBEIRO, B.L.M. 2016) [21]

Figure 9: Ovarian evaluation with Doppler color mode: ovary with CL without vascularization (left); ovary vascularized (right).



Source: (RIBEIRO, B.L.M. 2016) [21]

2.5 Hysteroscopy

The hysteroscopic examination was performed using a rigid endoscope (430 X 6 mm) with 2 channels: one for the passage of 0.9% sterile saline for uterine lumen distension and another for cytological and microbiological samples collection. The optic part of the endoscope was introduced into the vagina, guided by transrectal palpation. Once inside the uterus, the sterile saline was infused to explore this anatomic site. The presence of mucopurulent or purulent inflammatory lesions in the mucosa were considered as signs of endometritis [22]. The images were classified as healthy or unhealthy (presence of pus, fibrin and hyperemia) mucosa. After exploration, a small portion of saline was recovered and used for macroscopic, cytological and microbiological evaluation of the uterus content. After each examination, the optic cable was washed with water only. The work channels were brushed with a flexible 75cm X 0.20mm brush immersed in enzymatic detergent (Hs-enzyme®, Strattner®) and, then, disinfected using paracetic acid (Peroxylife®), according to the manufacturer's instructions.

2.6 Uterine cytology

One hundred and fifty microliters of the recovered saline were transferred to a cytocentrifuge chamber and samples were fixed and centrifuged on a glass slide for microscopy at approximately 550 rpm for 6 minutes [6]. The slides were stained using the Panotipo Rapido® kit, and cytology was performed by counting 100 cells, under magnification of 400x using an optical microscope. The percentage of neutrophil polymorphonuclear cells (% pmn) (Figure 12) was determined. Cows with %Ne > 10% were considered positive to the cytology test [23].

2.7 Microbiological

Five hundred microliters of the recovered saline were added to thioglycolate broth (Difco®) and kept at 2°-10 °C until processing at the Laboratory of General Bacteriology, Biological Institute, São Paulo. Ten microliters were plated in 5% sheep blood agar and incubated for 72 hours at 37 °C under microaerophilic conditions. Morphological characteristics of each colony and Gram staining were performed. Bacteria species were determined using biochemical tests according to Winn et al. (2008).

2.8 Statistical analysis

Error normality and homogeneity of variance were analyzed by Shapiro-Wilk Test and Bartlett Test, respectively. Non-normal data were analyzed using a non-parametric test (Kruskal-Wallis test) (proc npar1way). Differences between the groups (control and endometritis) were evaluated by ANOVA, reaffirmed by a t-test (JMP 12.0 - SAS). Same conditions (proc glimmix from SAS) were used for binary data ("subjective evaluation score of endometrial perfusion" and "subjective evaluation score of mesometrium perfusion"). Correlation between variables were performed using Spearmann correlation test for variables that did not present normal distribution (proc corr from SAS). A significance level of 5% was used for all tests. All tests were performed in SAS.

3. Results

One hundred cows were initially examined, but due to problems with the ultrasound device, eleven samples were lost. So, 89 cows were enrolled in this study, and they were divided accordingly to citology results into two groups: control group (E) (N= 33) and endometritis group (N = 56).

3.1 Ultrasonographic exam

Cows with endometritis had the cervix (*P* = 0.040) and the left horn (*P* = 0.020) increased compared to healthy cows (Table 1). In the endometritis group, 78.6% of the cows showed abnormal uterine discharge, while in health group only 57.6% of the animals showed this same condition (*P* = 0.0005) (Table 2). Besides, intrauterine heterogeneous content were increased in sick cows (66.0%) compared to healthy cows (30.3%) (*P* = 0.0011) (Table 3). Evaluation of endometrial and mesometrium vascularization by the colorimetric method revealed differences in the vascularization score (vc) of the endometrium between healthy (C) and endometritis group (C) (*P* < 0.05) (Table 4 and 5). During ovarian examination, presence or absence of CL were not diferent between group (C) and group (E) (Table 6). In addition, the absence of vascularizated corpus luteum in the left ovary was increased in the endometritis group (*P* = 0.029) (Table 7). The spectral Doppler evaluation of the uterine arteries revealed no differences between groups (Table 8).

Table 1 - Average diameter between measurements of linear ultrasonography 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

Source: (RIBEIRO, B.L.M., 2016).

Table 2- Presence of intrauterine fluid (IUF) in cows with and without Endometritis

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

Source: (RIBEIRO, B.L.M., 2016).

Table 3 - Animals that presented intrauterine heterogeneous content (IUHC) in abnormal amounts throughout the experiment

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

Source: (RIBEIRO, B.L.M., 2016).

Table 4 - Evaluation of vascular pattern of endometrium

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%	

Source: (RIBEIRO, B.L.M., 2016).
Legend: 0 = non-vascularized; 1 = poorly vascularized; 2 = very vascularized

Table 5- Frequency of evaluation of the vascular pattern of Mesometrium

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%	

Source: (RIBEIRO, B.L.M., 2016).
Legend: 0 = non-vascularized; 1 = poorly vascularized; 2 = vascularized; 3 = very vascularized; 4 = extremely vascularized.

Table 6 - Frequency of Presence of CL as a function of Endometritis

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

Source: (RIBEIRO, B.L.M., 2016).

Table 7 - Presence of CLv as a function of Endometritis

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

Source: (RIBEIRO, B.L.M., 2016).

Table 8 - Spectral Doppler average in 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

LEGEND: VMAX- MAXIMUM SPEED; IR- INDEX RESISTANCE; IP-INDEX PULSATILITY; S / D- SYSTOLE AND DIATST; MTM- AVERAGE TIME

3.3 Microbiological findings

Bacillus spp., *Trueperella pyogenes, Escherichia coli* and *Staphylococcus intermedius* were the most isolated bacteria among samples. Yeast was also detected in 25% of samples (Table 9).

Table 9 Identification of the microorganisms in 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.auerus	(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%

Source: (RIBEIRO, B.L.M., 2016).

3.4 Correlations with variables

A positive correlation between mixed culture (bacteria + yeasts) and endometritis was observed (P = 0.0003). In addition, weak correlations were observed between intrauterine heterogeneous content (r = 0.34), intrauterine fluid (r = 0.37) and endometritis. The disease was also correlated with Doppler ultrasonography ($P = 0.0027$) and endometrial Doppler ($P = 0.0383$). A weak and negative correlation with the presence of corpus luteum in the vascularized left ovary ($P = 0.0359$) and endometritis was also detected (Table 10).

Table 10 - 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

Source: (RIBEIRO, B.L.M., 2016).
Legend: IUHC = intrauterine heterogeneous content; IUF = intrauterine fluid; eclv = left vascularized corpus luteum.

Intrauterine fluid (IUF) was correlated with *Trueperella pyogenes* (r = 0.27), yeast (r = 0.25), *Staphylococcus intermedius* (r = 0.23), and *Escherichia coli* (r = 0.23) ($P < 0.05$) (Table 13). Strong correlations between intrauterine heterogeneous content (IUHC), and IUF ($P = 0.0001$) and the presence of bacteria (P = 0.0001) were detected. IUHC was also correlated with *Trueperella pyogenes* (r = 0.36), *Escherichia coli* (r = 0.30), *Bacillus spp.* (r = 0.25), *Staphylococcus intermedius* (r = 0.22) and yeast ($P < 0.05$) (Table 14). Endometrial Doppler was correlated with *Trueperella pyogenes* ($P = 0.0003$) and IUHC ($P = 0.0047$) (Table 15). Finally, mesometrial Doppler was correlated with endometrial Doppler ($P < 0.001$), uterine bacteria ($P = 0.001$) and IUHC ($P = 0.049$). A negative correlation with the presence of yeast ($P = 0.022$) was also observed (Table 16).

Table 11 - Correlation between Presence of Intrauterine Fluid (IUF) compared to 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

Source: (RIBEIRO, B.L.M., 2016).
Legend: *T. pyogenes= Trueperella pyogenes; S. intermedius= Staphylococcus intermedius E. coli = Escherichia coli*

Table 12 - 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

Source: (RIBEIRO, B.L.M., 2016).
Legend: IUF = Intrauterine fluid; *T. pyogenes = Trueperella pyogenes; S. intermedius= Staphylococcusintermedius;E.coli = Escherichia coli.*

Table 13 - 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

Source: (RIBEIRO, B.L.M., 2016).
Legend: IUHC = intrauterine heterogeneous content;*T. pyogenes = Trueperella pyogenes.*

Table 14 - Doppler color correlation of 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

Source: (RIBEIRO, B.L.M., 2016).
Legend: IUHC = intrauterine heterogeneous content.

4. Discussion

Endometritis is a uterine disease and is related to negative reproductive performance [8]. Besides physical examination, new diagnostic tools are important to improve the precocity in the diagnosis of this disease [24].

Our results showed differences between the size of the cervix and a larger uterus in cows with endometritis. After delivery, the uterus is greatly enlarged (approximately 8 to 10 kg). In general, the uterine macroscopic involution occurs between 3 to 5 weeks postpartum, when the uterus should weight approximately 0.9 kg and the diameter of the pre-gravid uterine horn should be less than 5 cm, total involution of the cervix occurs between 4 to 6 weeks postpartum [25]. A new pregnancy depends on the anatomical and functional return of the genital tract to a state similar to that before pregnancy [26]. In the present research, the increased left horn in animals with endometritis was observed. This information is in agreement with other researches that proved that a uterine infection provides a delayed involution [27, 26]. In this study, cows with endometritis presented larger cervix diameter compared to healthy animals. LeBlanc et al. (2002) [5] highlighted that cows with clinical endometritis showed cervical measurement ≥7.5 cm in diameter after 20 days postpartum and they presented mucopurulent or purulent discharge detected by vaginoscopy after 26 days postpartum. Our study highlights that endometritis was increased in cows showing abnormal intrauterine fluid accumulation, and it was in agreement with several studies [28, 29, 16]. Intrauterine fluid was correlated with *Trueperella pyogenes*, yeast, *Staphylococcus intermedius*, *Escherichia coli*, which suggests that these agents cause inflammation of the uterine mucosa and proliferation of the endometrial glands, leading to a greater accumulation of intrauterine fluid.

Endometritis was highly detected in cows showing intrauterine heterogeneous content (IHC). Meira Jr. et al. (2012) [16] also described high correlation between IHC and the diagnosis of endometritis. IHC was also associated with *Trueperella pyogenes*, *Escherichia coli*, *Bacillus spp*. and *Staphylococcus intermedius*, and yeast, demonstrating that these microorganisms may cause genital discharge [16].

The presence of vascularization in the endometrium (degrees 1 and 2) was increased in cows with endometritis, suggesting differences between healthy and unhealthy cows. The color Doppler mode provides colored images of the blood fow, allowing estimation of the tissue vascularization [34]. Ginther (2007) [19] proposed a model to evaluate the uterine hemodynamic associated with endometritis. This model was modified in the present research, and our results showed a few animals with alterations in the subjective evaluation score of endometrial vascularization, and a positive correlation with *Trueperella pyogenes*.

Ultrasonographic evaluation of the endometrium using color Doppler mode showed a positive correlation with endometritis. This information emphasizes the possible combination of this new noninvasive technique with the standard diagnostic methods to predict uterine disease. No difference was detected at data analisys of spectral Doppler mode. Herzog and Bollwein (2013) [2] reported increased pulsatility at 24 hours postpartum with a peak on the 28th day, decreasing progressively until 90th day. An inverse proportional relation was observed in blood flow, which already starts high in the postpartum and decreases until around the 28th day maintaining basal levels up to 90th day. Clinical and histologic uterine involution ranges from 21 to 50 postpartum, and this may differs from the recovery of hemodynamic patterns of the uterus that requires more time [30].

Advancing the understanding of uterine perfusion, Bollwein et al. (2002) [13] when evaluating pregnant animals, concluded that throughout pregnancy there is a strong increase of blood flow in the uterine arteries. However, they observed that the resistivity index decreased in the first 8 months of gestation and remained at a relatively constant level until the calf was born. It is known that during the diestro the blood flow velocity remains constant and are correlated to the plasma concentration estrogen and progesterone, indicating a moderate positive correlation Bollwein et al. (2016) [30]. These results indicate that there are other factors involved in the regulation of blood flow [31].

In healthy cows the uterine blood flow decreased after the complete uterine involution. However, cows with uterine disease presents slow uterine involution, occurring between 45 and 65 days postpartum. These results indicate an association between incomplete uterine involution and regeneration of the uterine vascular layer in cows with puerperal disease. Changes in uterine perfusion are greatly pronounced during the first 4 days after delivery. Uterine puerperal diseases such as retention fetal attachments and metritis have a negative impact on uterine involution [17]. Thus, uterine blood flow is affected by puerperal uterine diseases. Cows with retained placenta have higher resistivity index than healthy cows. On the 8th day postpartum, cows with metritis had higher blood flow and decreased pulsatility in the arteries compared to healthy cows [17].

Evaluation of the uterine and ovarian vascularization helps to determine the best moment to perform artificial insemination [31]. The vascular perfusion of corpus luteum and uterus is greater during the first follicular wave compared to the second follicular wave of the estrous cycle [31]. The color Doppler mode identifies follicles in normal development and predicts the next ovulate. In this research, the ovarian evaluation showed that some cows with endometritis had no corpus luteum in both the right and left ovary. However, animals with right and left CL presented 61.54% and 52.94% vascularized, respectively. Color Doppler mode showed that cows with no vascularized left and right corpus luteum had endometritis, demonstrating that there is a interference in reproduction performance even in endometritic cows exhibiting normal estrous cycle.

Microbiological results showed the presence of *Bacillus* spp., *Trueperella pyogenes*, *Staphylococcus intermedius, Escherichia coli, Streptococcus* spp., *Proteus* spp., *Staphylococcus aureus, Enterobacteria and Serratia* spp. besides fungus and yeast. Santos, Gilbert and Bicalho (2011) [32] reported that *Escherichia coli, Streptococcus* spp., *Trueperella pyogenes* and *Fusobacterium necrophorum* are the main bacteria that commonly contaminate the uterine lumen after childbirth. These microorganisms were also associated with some uterine diseases. *Trueperella pyogenes* acts in synergisms with *Fusobacterium necrophorum, Bacteroides* spp., and *Prevotella* spp. [33]. Potter et al. (2010) [34] reported that *Trueperella pyogenes* is recognized as an important pathogen associated with endometritis due to its persistence in the contaminated uterus. Recently, Boer et al. (2015) [35] found that cows with any bacterial growth at 21 days postpartum, regardless of bacterial species, had a lower conception rate. Using the 16S rRNA sequencing, Machado et al.

(2012) [36] detected *Trueperella pyogenes* increased in cows with endometritis. Yeasts have been associated with chronic endometritis in women [40]. In the present research, yeasts were detected in 22.5% of cows. The onset of *Trueperella pyogenes* was correlated with uterine inflammation seen by the colorimetric ultrasonographic method.

Although endometritis has a spontaneously healing condition, Dubuc et al. (2011) [7] found 63% of spontaneous cure, while LeBlanc et al. (2002a) [5] described 77% self-healing. It still causes many economic and reproductive damages in the dairy industry. In order to reduce this problem, it is important to use new tools to improve the precision and the precocity in the diagnostic, upgrading the reproductive indices.

5. Conclusions

It is detected the relationship between the diameter of the cervix and the uterine horns with endometritis. This relationship was notorious when the presence of intrauterine fluid and intrauterine heterogeneous content was verified. Color Doppler showed the association between the vascularization of inflamed tissue and endometritis. However, performing spectral Doppler ultrasound showed no differences between healthy and unhealthy animals. The microbiological examination showed that *Trueperella pyogenesis* and *Escherichia coli* have an important role in the development of endometritis, corroborating other researches. Less invasive techniques with fast results such as Doppler ultrasonography can provide satisfactory answers regarding to the evolution of uterine alterations, improving reproductive rates.

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REFERENCES

[1] Radostits OM; Gay CC; Hinchcliff KW; Constable PD. Veterinary medicine: a textbookof disease of cattle, sheep, pigs, goats and horses. 9. Ed. Philadelphia: WB. Sauders, 2007.

[2] Bollwein H; Luttgenau J; Herzog K. Bovine luteal blood flow: Basic mechanism and clinical relevance. Reprod, Feril, Dev. V.25, n.1, p.71-79, 2013.

[3] Grohn YT; Rajala-Schults PJ. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci, 60-61:605-614, 2000.

[4] Leblanc S. Economics if improving reproductive performance in dairy herds. WCDS advances in Dairy technology. 19:201-214, 2007.

[5] Leblanc SJ; Duffield TF; Leslie KE; Bateman KG; Keefe GP; Walton JS; Johnson WH. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci, 85(9):2223-2236, 2002.

[6] Gilbert RO; Shin ST; Guard CL; Erb HN; Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 64(9):1879-1888, 2005.

[7] Dubuc J; Duffield TF; Leslie KE; Walton JS; Leblanc SJ. Effects of postpartum uterine diseases on milk production and culling in dairy cows. J. dairy Sci. 94(3):1339-1346, 2011.

[8] Sheldon IM; Lewis GS; Leblanc S; Gilber RO. Defining postpartum uterine disease in cattle. Theriogenology. 65(8):1516-1530, 2006.

[9] Carvalho CF; Cjammas MC; Cerri GG. Princípios físicos do Doppler em ultra-sonografia. Cienc Rural. 38(3):872-879, 2008.

[10] Steer CV; Campbell S; Pampiglione JS; Kingsland CR; Mason BA; Collins WP. Transvaginal colour flow imaging of the uterine arteries during the ovarian and menstrual cycles. Hum Reprod. 5(4):391-395, 1990.

[11] Steer CV; RTan SL; Mason BA; Campbell S. Midluteal-phase vaginal color Doppler assessment of uterine artery impedance in subfertile population. Fertil Steril. 61(1):53-58, 1994.

[12] Bollwein H; Mayer HH; Maierl J; Weber F; Baumgartner U; Stolla R. Transrectal color Dopplersonography of the A uterine in cyclic mares. Theriogenology. 49:1483-1488, 2000.

[13] Bollwein H; Baumgartner U; Stolla R. Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. Theriogenology. 57(8):2053-2061, 2002.

[14] Kasimanickam R; Duffield TF; Foster RA; Gartley CJ; Leslie KE; Walton JS; Johnson WH. The effect of a single administration of cephapirin or cloprostenol on the reproductive performance of dairy cows with subclinical endometritis. Theriogenology. 63(3):818-830, 2005.

[15] Grunert E; Birgel EH; Vale WG. Patologia e clínica da reprodução dos animais mamíferos domésticos: ginecologia. São Paulo: Varela,2005. 551p.

[16] Meira JR EBS; Henriques LCS; Sá LRM; Gregory L. Comparison of ultrasonography and histopathology for diagnosis of endometritis in Holstein-Friesian cows. J Dairy Sci. 95(2):6969-6973, 2012.

[17] Heppelmann M; Kruger L; Leidl HB. Science transrectal Doppler sonography of uterine blood flow during the first two weeks after parturition in Simmenthal heifers. J Vet Sci 14(3):323-327, 2013.

[18] Descoteaux L; Gnemmi G; Colloton J. Ruminant and camelid reproductive ultrasonography. Ames:Blackwell Publishing, 2010.

[19]Ginter OJ. Ultrasonic imaging and animal reproduction: color-Doppler ultrasonography. Cross Plains, WI: equiservices publishing, 2007, v.4, 258p.

[20] Mello RC. Perdas reprodutivas em femeas bovinas. Agropecuária científica no semiárido. 10(4):07-23, 2014.

[21] Ribeiro,BLM. Avaliação do útero bovino com endometrite utilizando a técnica de ultrassonografia Doppler. Dissertação de Mestrado, Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia 72 f., 2016.

[22] Madoz LV; Giuliodori MJ; Migliorisi AL; Jaureguiberry M; De la Sota RL. Endometrial cytology, biopsy and bacteriology for the diagnosis of subclinical endometritis in grazing dairy cows. J Dairy Sci. 97(1):195-201, 2014.

[23] Kasimanickam R; Duffield TF; Foster RA; Gartley CJ; Leslie KE; Walton JS; Johnson WH. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology. 62(1-2): 09-23, 2004.

[24] Dubuc J; Duffield TF; Leslie KE; Walton JS; Leblanc SJ. Definitions and diagnosis of postpartum endometritis in dairy cows. J Dairy Sci. 93(11):5225-5233, 2010.

[25] Morrow DA; Roberts SJ; Mcentee K; Gray HG. Postpartum ovarian activity and uterine involution in dairy cattle. J Am Vet Med Assoc. 149:1596-1609, 1966.

[26] Mateus, L; da Costa LL; Bernardo F; Silva JR. Influence of puerperal uterine infection on uterine involution and postpartum ovarian activity in dairy cows. Reprod Domest Anim. 37(1):31-35, 2002.

[27] Slama H.; Vaillancourt D; Goff AK. Pathophysiology of puerperal period: relationship between prostaglandin E2 (PGE@) and uterine involution in the cow. Theriogenology. 36(6):1071-1090, 1991.

[28] Barlund, C S; Carruthers, T D; Waldner, C L; Palmer, CWA. Comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology, v. 69, n. 6, p. 714-723, 2008.

[29] Oral , H; Sozmen, M.; Serin, G.; Kaya, S. Comparison of the cytobrush technique, vaginoscopy and transrectal ultrasonography methods for the diagnosis of postpartum endometritis in cows. J

Anim Vet Adv, v. 8, n. 7, p. 1252-1255, 2009.

[30] Bollwein, H; Heppelmann, M; Johannes, L. Ultrasonographic Doppler use for female reproduction management. Vet Clin North Am Small Anim Pract, v. 32, n. 1, p. 149-164, 2016.

[31] Honnens, A; Voss, C; Herzog, K; Niemann, H; Rath, D; Bollwein, H. Uterine blood flow during the first 3 weeks of pregnancy in dairy cows. Theriogenology, v. 70, p. 1048–56, 2008.

[32] Santos TM, Gilbert RO, Bicalho RC. Metagenomic analysis of the uterine bacterial microbiota in healthy and metritic postpartum dairy cows. J Dairy Sci 94(1): 291–302, 2011.

[33] Bicalho ML, Machado VS, Oikonomou G, Gilbert RO, Bicalho RC. Association between virulence factors of *Escherichia coli*, *Fusobacterium necrophorum*, and *Arcanobacterium pyogenes* and uterine diseases of dairy cows. Vet Microbiol 57(1-2):125-31, 2011.

[34] Potter, TJ; Guitian, J; Fishwick, J; Gordon, PJ; Sheldon, IM. Risk factors for clinical endometritis in postpartum dairy cattle.Theriogenology, v.74, p.127–134, 2010.

[35] Boer, M; Buddle, BM; Heuer, C; Hussein, H; Zheng, T; Leblanc, SJ; McDougall, S. Associations between intrauterine bacterial infection, reproductive tract inflammation, and reproductive performance in pasture-based dairy cows. Theriogenology, v. 83, p. 1514-1515, 2015.

[36] Machado, VS; Oikonomou, G; Bicalho, MLS; Knauer, WA; Gilbert, R; Bicalho, R C Investigation of postpartum dairy cows' uterine microbial diversity using metagenomicpyrosequencing of the 16S rRNA gene. Vet Microbiol, v. 159, n. 3, p. 460-469, 2012.

[37] Cicinelli, E; De Ziegler, D; Nicoletti, R; Colafiglio, G; Saliani, N; Resta, L; Rizzi, D; De Vito, D. Chronic endometritis: correlation among hysteroscopic, histologic, and bacteriologic findings in a prospective trial with 2190 consecutive office hysteroscopies. Fertil Steril, v. 89, n. 3, p. 677-684, 2008.