

## PREVALENCE OF INFLAMMATORY PROCESSES IN THE REPRODUCTIVE TRACTS OF CROSSBRED DAIRY COWS

### PREVALÊNCIA DE PROCESSOS INFLAMATÓRIOS NO SISTEMA REPRODUTIVO DE VACAS LEITEIRAS MISTIÇAS

Paula Batista de ALVARENGA<sup>1</sup>; Danilo OLIVEIRA<sup>1</sup>; Thaisa Reis dos SANTOS<sup>1</sup>; Soraia Rage REZENDE<sup>1</sup>; Oglênia Pereira RAMOS<sup>1</sup>; Paula Mara Ribeiro TRONCHA<sup>1</sup>; Alessandra Aparecida MEDEIROS<sup>1</sup>; Pablo Gomes NOLETO<sup>1</sup>; Selwyn Arlington HEADLEY<sup>2</sup>; Ricarda Maria dos SANTOS<sup>1</sup>; João Paulo Elsen SAUT<sup>1</sup>

1. Large Animal Health Laboratory, Faculty of Veterinary Medicine, Universidade Federal de Uberlândia, Minas Gerais, Brazil. paulabatista\_alvarenga@hotmail.com; 2. Laboratory of Animal Pathology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Paraná, Brazil.

**ABSTRACT:** This study evaluated the prevalence of cervicitis and endometritis and their interrelations in crossbred dairy cows that were more than 60 days in milk (DIM), and the efficiency of the cytology technique (*cytobrush*) and histology as diagnostic methods for these diseases. The reproductive tracts ( $n=149$ ) derived from cows were collected from a slaughterhouse and grossly evaluated to determine uterine involution and the phase of the estrous cycle. Subsequently, cervical and uterine cytological evaluations were done to characterize the inflammatory response as cervicitis (with a neutrophilic count greater than 5%) or endometritis (with more than 6% neutrophils). Additionally, randomly obtained samples from the cervix and uterus were collected for histological evaluation. Cytological evaluation revealed that the frequency of cervicitis was 6% (9/149), and endometritis was diagnosed in 8.1% (12/149) of the samples; both inflammatory reactions were diagnosed 2.1% (3/149) in three of these. Histological evaluation revealed that of the cows diagnosed with cervicitis by cytology, 66.6% (6/9) had no inflammatory reaction in the cervical mucosa, and 33.3% (3/9) had mild inflammation in this region. Histological evaluation of the uterine horn revealed that of the 12 cows diagnosed with endometritis by cytology, 50% (6/12) of these had a histological diagnosis of mild and moderate inflammatory responses, 8.3% (1/12) of these demonstrated a severe inflammatory response, while no inflammatory reaction was identified in 41.7% (5/12) of the endometrial biopsies evaluated. In conclusion, the endometrial and cervical inflammatory response diagnosed by the cytobrush technique persisted in 12.1% of the cows. Comparatively, the frequency of the inflammatory responses at the cervix and uterus was reduced by histological analysis relative to cytological evaluation with the *cytobrush* technique.

**KEYWORDS:** Cervicitis. Cytology. *Cytobrush*. Endometritis. Histology

### INTRODUCTION

Postpartum conception in dairy cows is directly affected by the occurrence of cervicitis and/or endometritis diagnosed by cytological evaluation during the first weeks of postpartum with the *cytobrush* technique. These inflammatory processes affect 15-40% of postpartum cows (DEGUILLAUME et al., 2012) and, when present simultaneously, cervicitis and endometritis increase the risk of non-conception (AHMADI et al., 2005; DEGUILLAUME et al., 2012). Endometritis has been overestimated with regards to cervicitis (DEGUILLAUME et al., 2012), which results in increased purulent vaginal secretions and inflammatory cells in the reproductive tract.

Histologically, cervicitis is characterized by the presence of an inflammatory response within the cervical mucosa (SANTOS et al., 2011), while by cytology it is determined by a neutrophil count that is greater than 5%, a limit that is directly related to

losses in conception rate (DEGUILLAUME et al., 2012).

In Brazil, the information relative to the incidence of cervicitis in dairy herds and the relationship between cervicitis and cytological endometritis is sparse. This study compared the prevalence of cervicitis and endometritis and the utilization of cytology with the *cytobrush* technique and routine histology as diagnostic methods for these reproductive disorders in crossbred dairy cows that were more than 60 days on milk.

### MATERIAL AND METHODS

#### Study area and criteria for inclusion of cows

The reproductive tracts of 149 cows were collected from a slaughterhouse in Uberlândia, Minas Gerais, Brazil. The inclusion criteria were as follows: crossbred adult dairy cows that were more than 60 days on milk (DIM), macroscopic identification of uterine involution, and cows in any

phase of the estrous cycle phase. All reproductive tracts were placed in individual plastic bags and processed within 2 hours of collection. Gross characterization of involution and the phase of the estrous cycle (luteal, follicular or anestrus) was done as described (IRELAND et al., 1980; PETER et al., 2009).

### Sample preparation for cytological and histological evaluations

All uteri were cleaned externally with 70% alcohol, after which samples from the cervical and uterine wall were obtained for cytological evaluation with the *cytobrush* technique, adapted by (KAUFMANN et al., 2009), with a disposable cervical brush (Labor import, Osasco, Brazil). The selected region at the endometrium and cervix was done randomly. All smears were made on glass slides and stained with Panotic Fast Kit (Newprov, Pinhais, Brazil) and evaluated by light microscope (magnification 400X) by two independent observers after which the average of these findings was used as the result. The following cells were evaluated: epithelial cells, neutrophils, lymphocytes, and macrophages. A cytological diagnosis of cervicitis was obtained when the number of neutrophils from the cervix was greater than 5% (AHMADI et al., 2006a; DEGUILLAUME et al., 2012), while cytological endometritis was determined when the percentage of neutrophils were more than 6% in endometrial samples (GILBERT et al., 2005; AHMADI et al., 2006b; DEGUILLAUME et al., 2012).

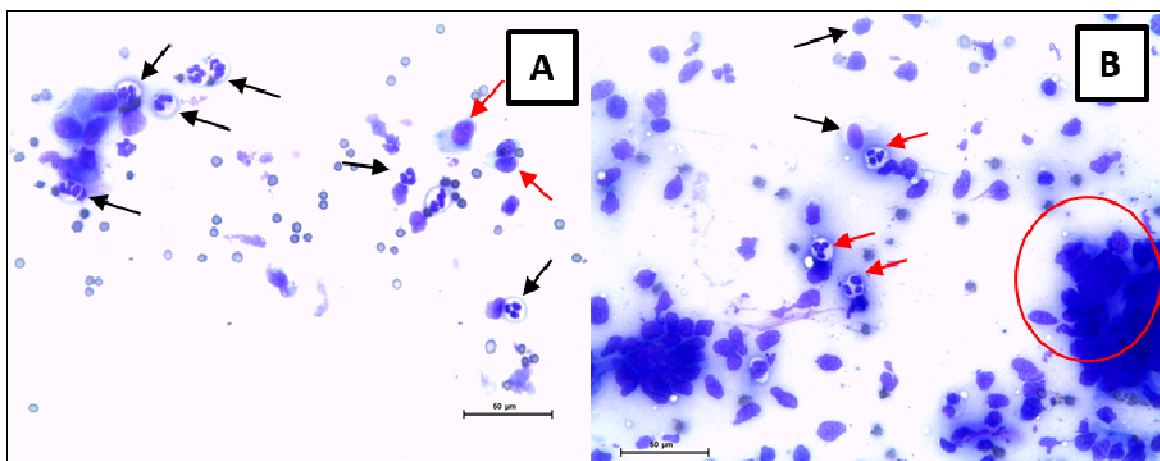
Samples of approximately 1cm<sup>2</sup> were collected from the uterine body and cervix of each

cow, taking care to ensure that all samples were collected at the intersection between grossly altered and normal regions. Each collected sample was placed in sterile vials containing 10% buffered formalin solution for 72 hours, routinely processed and stained with Hematoxylin and Eosin (H&E) for histological evaluation according to Tolosa et al., (2005). When gross lesions were not observed, histological samples were always collected from the intermediate portions of the uterine body and cervix. The inflammatory response in each slide was then classified as mild, moderate, and severe according to the degree of tissue involvement (GONZALEZ et al., 1985), with mild lesions indicating slight changes in tissue function (or morphology); in moderate lesions, there was involvement of the organ or tissue. Severe lesions resulted in disseminated alteration to tissue function, with potential impairment of the organism.

All results were used as inputs for descriptive statistical analyses utilizing the Biostat 5.0 software. All results were presented as percentages and the analysis of concordance between the cytological and histological findings was evaluated by the *Kappa* test (LANDIS; KOCH, 1977).

### RESULTS

Cytological evaluation with the *cytobrush* technique revealed that 12.1% (18/149) of all cows evaluated had an inflammatory reaction, in which endometritis (Fig. 1A) contributed to 8.1% (12/149) of these lesions, and 6% (9/149) was diagnosed as cervicitis (Fig. 1B).



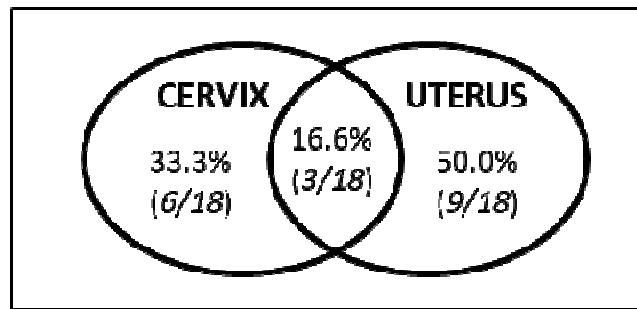
**Figure 1.** Cytological demonstration of cervicitis and endometritis in crossbred dairy cows.

Note: (A) cervical cytology: neutrophils (black arrows) and epithelial cells (red arrows); (B) uterine cytology: neutrophils (red arrows) scattered epithelial cells (black arrows) and grouped.

Of the 18 cows with an inflammatory response, 16.7% (3/18) of these had inflammation in

both anatomical locations evaluated, while 33.3% (6/18) were diagnosed with cervicitis and 50%

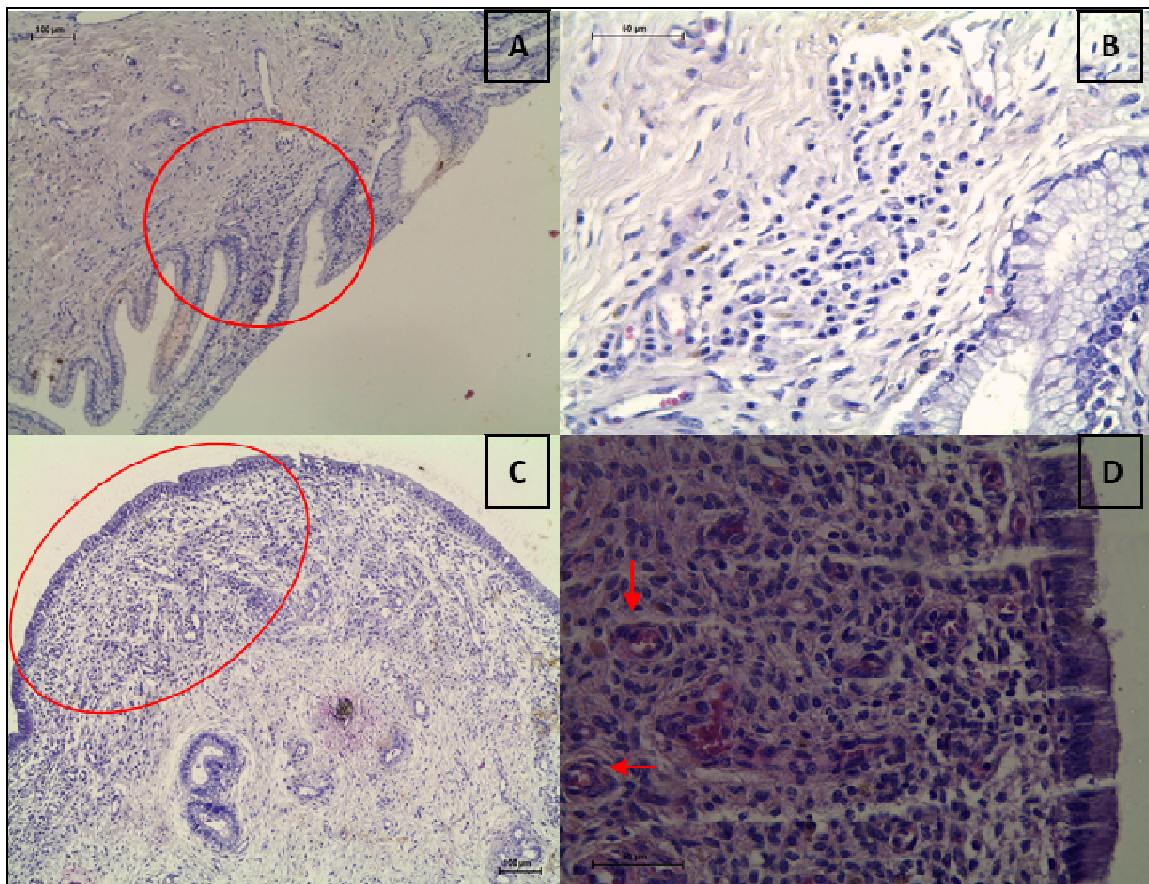
(9/18) with a cytological diagnosis of endometritis (Figure 2).



**Figure 2.** The distribution of cervicitis and endometritis in crossbred dairy cows diagnosed by cytology using the cytobrush technique.

When the cows with a cytological diagnosis of cervicitis were evaluated by histopathology (Figure 3A, 3B), cervical inflammation was not observed in 66.7% (6/9) of these, while moderate to mild cervicitis was diagnosed in 33.3% (3/9). When the uterine histology was compared with the cytological results, of the 12 animals with a cytological diagnosis of endometritis, 50% (6/12) of the cows had mild and/or moderate inflammatory

responses, 8.3% (1/12) were classified as severe, and 41.7% (5/12) had no histological evidence of endometrial inflammatory reaction (Figure 3C, 3D). Furthermore, when the cows with cytological evidence of inflammation in both anatomical locations were compared, two (66.7%; 2/3) of these demonstrated histological evidence of inflammatory reaction at the cervix and endometrium.



**Figure 3:** Photomicrograph of the cervical and uterine histology of crossbred dairy cows.

Note: (A) 100X magnification: preservation of cervical mucosa and mild inflammatory infiltrate (circle); (B) 400X magnification: mild inflammatory infiltrate in the cervical mucosa of A; (C) 100X magnification: mild inflammatory infiltrate in the endometrium (circle); (D) 400X magnification: congested and dilated vessels (arrows) and mild inflammatory infiltrate in endometrium of C.

The distribution of cervicitis and endometritis observed in the cows during this study relative to the phase of the estrous cycle is given in Table 1. When the concordance between the cytological and histological evaluations were

compared, it was shown that in the uterus the concordance was regular with 78.9% ( $k=0.596$ ), with a low correlation occurring in the cervix (55.6%;  $k=0.111$ ).

**Table 1.** Comparison of the inflammatory response observed by cytology and histology in the cervix and uterus of cows based on the phase of the estrous cycle.

	Cytological evaluation			Histological diagnosis		
	Follicular	Luteal	Anestrous	Follicular	Luteal	Anestrous
<b>Cervix</b>	2	5	2	1	1	1
<b>Uterus</b>	4	4	4	1	3	2
<b>Total</b>	6	9	6	2	4	3

Note: Classification of the phases of the estrous cycle according to Ireland et al. (1980) and Peter et al. (2009).

## DISCUSSION

In this population of cows, cytological evaluation revealed that 12.1% of the animals demonstrated an inflammatory response. Although the reason for the slaughter of these animals was not known and not investigated, reproductive failures are considered as the primary cause for the culling of dairy cows (GROOMS, 2011).

Although the results from this study have shown that cervicitis and endometritis can affect cows that are more than 60 DIM simultaneously, the occurrence of these alterations are independent to each other (AHMADI et al., 2005; DEGUILLAUME et al., 2012). However, LeBlanc (2014) has shown that 50-75% of postpartum cows had endometritis with concomitant cervicitis and *vice versa*.

The results from this study have suggested that cytology using the cytobrush technique may not be an adequate diagnostic method to evaluate cervicitis and endometritis in postpartum cows, since weak and regular concordance values were obtained between cytology and histology, respectively. It should be highlighted that the samples evaluated histologically may not have been adequate representations of the cytological outcome and may contribute to the differences observed by cytology and histology, since the samples obtained for cytology and histology were not collected simultaneously and from the same anatomic location. However, during this study the cytobrush technique was performed to simulate the routine collection method during which the technique is performed blindly.

Several authors have correlated low reproductive performance with the presence of cervicitis and/or cytological endometritis in cows during immediate postpartum (AHMADI et al., 2006a; YAVARI et al., 2009; DEGUILLAUME et

al., 2012). Nevertheless, additional studies are necessary to confirm that the poor correlation observed between the cytological and histological evaluations in cows over than 60 DIM may also occur in immediate postpartum, during which cytology is widely accepted as the method of choice to diagnose cervicitis and endometritis.

The largest number of animals with inflammatory reaction detected in the luteal phase has been related with the highest concentration of progesterone with a consequent reduction of the immune response of the endometrium to infection (LEWIS 2003; 2004). This is because elevated concentrations of progesterone (P4) prevent the uterus from resisting infections by suppressing the synthesis of eicosanoids, such as prostaglandin  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) and leukotriene ( $LTB_4$ ) (LEWIS, 2004). As luteal function develops and P4 concentrations begin to increase, the uterine production of  $PGF_{2\alpha}$  and  $LTB_4$  are reduced to baseline within a few days, after which the uterus then becomes susceptible to infections. This is because  $PGF_{2\alpha}$  stimulates neutrophil chemotaxis and improves its capacity to induce phagocytosis; whereas  $LTB_4$ , in addition to assist in increased chemotaxis, increases cellular migration and a wide variety of cells recruited, and acts in antibody-independent cell mediated cytotoxicity (HOEDEMAKER et al., 1992; LEWIS, 2004). In addition to acting directly on neutrophils,  $PGF_{2\alpha}$ , being a pro-inflammatory molecule, can stimulate the production of cytokines, which in turn, improve phagocytosis and lymphocytic function (KELLY et al., 2001; SEALS et al., 2003). These inflammatory processes can be related to chronic uterine infections, during which there was uterine involution, lochia expulsion with the elimination or not of bacterial agents, with the persistence of inflammation within the lumen of the cervix and/or uterus.

## CONCLUSION

The endometrial and cervical inflammation diagnosed by cytology technique, persisted in 12.1% of the cows over 60 days in milk. A lower percentage of inflammatory processes was observed when the histological evaluations of the cervix and uterus were compared to cytology analyses with the *cytobrush* technique.

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**RESUMO:** O objetivo deste estudo foi determinar a prevalência de cervicite e endometrite citológicas e suas inter-relações em vacas leiteiras mestiças com mais de 60 dias pós-parto (DPP), bem como avaliar as técnicas de citologia (*cytobrush*) e histologia como métodos diagnósticos para estas doenças. Foram utilizados 149 tratos reprodutivos de vacas com mais de 60 DPP de abatedouro e avaliados macroscopicamente para determinar a involução uterina e a fase do ciclo estral. Posteriormente, realizou-se citologia cervical e uterina para o diagnóstico de inflamação utilizando como ponto de corte, contagem de polimorfonucleares (PMN) superior a 5% e 6%, respectivamente, para cervicite e endometrite citológica. Também foram coletadas amostras de cérvix e útero para histologia. Do total de vacas, identificaram-se 6% (9/149) com cervicite e 8,1% (12/149) com endometrite citológica, sendo três destes animais (2,1%) com ambas as inflamações. Histologicamente, dos animais com cervicite citológica, 66,6% (6/9) não apresentaram infiltrado inflamatório na mucosa cervical e 33,3% (3/9) apresentaram inflamação leve. A histologia uterina mostrou que de 12 vacas com endometrite citológica, 50% (6/12) apresentaram infiltrados inflamatórios leves a moderados, 8,3% (1/12) grave e 41,7% (5/12) não tinham inflamação endometrial. Concluiu-se que a inflamação endometrial e cervical, por meio da técnica de citologia, persistiu em 12,1% das vacas com mais de 60 DPP. Quando se utiliza a histologia como método de diagnóstico, a detecção de inflamação cervical e uterina é inferior ao detectado pela técnica de citologia por meio do esfregaço endometrial (*cytobrush*).

**PALAVRAS CHAVE:** Cervicite. Citologia. *Cytobrush*. Endometrite. Histologia

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