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# Cytological evaluation of inflammation of the uterus and influence of endometritis on selected reproductive parameters in dairy cows

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**Abstract**

There were two aims of this study. One was to evaluate the postpartum state of the reproductive system in cows based on ultrasonography, bacterial culture, and cytological examination of the uterus. The other was to determine whether postpartum endometritis affects the subsequent state of the endometrium, and, in consequence, selected reproductive parameters in cows. The study was conducted on 60 cows: the experimental group of 30 cows with endometritis, and 30 cows free of uterine inflammation (control). The percentage of leukocytes in both groups was similar only on day 5 of postpartum. In all subsequent tests (26, 40, 61 d postpartum), the percentage of leukocytes in the experimental group was statistically significantly higher than in the control ($P < 0.001$), both in samples collected with a brush and in lavage samples. Involution of the uterus in the experimental group was also slower ($P < 0.001$). The analysed reproductive parameters were markedly less favourable in the experimental group than in the control. The study showed that postpartum inflammation of the uterus can persist for a long time in the form of endometritis, causing substantial deterioration of reproductive parameters in cows. The authors suggest that cytological evaluation of the uterus, preferably using a brush, should be performed as soon as possible after parturition, even before day 21, up to which time puerperal metritis may still persist. Evaluation of the inflammatory process based on the number of leukocytes and the quality of endometrial cells is important.

**Key words:** cows, metritis, endometritis, endometrial cytology, reproductive parameters.

## Introduction

Uterine inflammation is a serious problem in dairy cow breeding all over the world, causing considerable economic losses (8, 11, 25). The inflammation is caused by microorganisms multiplying in the lumen and endometrium of the uterus. The most frequently isolated microorganisms after parturition are *Escherichia coli* (*E.coli*), which can induce inflammation as early as the first week after calving, and *Arcanobacterium pyogenes* (*Trueperella pyogenes)* (*T.pyogenes*), which can act synergistically with the anaerobic bacteria *Fusobacterium necrophorum* (*F. necrophorum*) and *Prevotella melaninogenica* (*P. melaninogenica*). *E. coli* is believed to function as a promoter for further infections induced by other pathogens (7, 10). Pathogenic bacteria induce mainly cellular local immune response, manifested by leukocyte infiltration visible in a cytological examination. Pathogenic microorganisms in the uterus can secrete substances weakening local immune mechanisms in the endometrium, allowing the inflammation to persist for a long time (7, 12). Impaired local immunity of the uterus is considered to

be of key importance in the development of postpartum uterine inflammation, which often persists for 60 d after parturition, negatively affecting reproductive parameters in cow herds (20, 29). One parameter frequently taken into account mainly during the postpartum period, is the diameter of the uterine horns, because it is an inferrer of the dynamics of uterus involution (4, 19, 23). Inflammation, particularly in the post-calving period can substantially delay involution of the uterus (14). The state of the endometrium can be assessed using new methods adopted from gynaecology, where they enter the practice continually (5, 14). One of them is cytological examination of the uterus, a method rarely used in field veterinary practice for diagnosing reproductive disorders in cattle. In recent years, however, interest in this type of diagnostics has increased considerably. One of three well-known methods described in the literature can be chosen: swab, cytological brush or lavage (14, 15, 26). Cytological examination is performed in cows mainly in order to exclude or confirm subclinical endometritis when no irregularities, or only slight changes, can be seen even by ultrasonography. Cytological examination can also be very useful in monitoring the postpartum period in cows (15, 26). It enables the fastest possible detection and treatment of metritis that has persisted too long (26, 28). The examination in such cases is limited to calculating in various ways the percentage of cells indicative of inflammatory infiltration, *i.e.* leukocytes, specifically in large part segmented neutrophils (PMN) and lymphocytes. There are several methods for counting the cells. According to some authors, 100 cells visible in the smear should be counted and the percentage of leukocytes calculated (14, 15). Another method is to count 150 cells visible in the smear and determine the percentage of leukocytes among them, or alternatively, to count the cells visible in 10 fields of view and determine the percentage of leukocytes (26).

There were two aims of the study: to evaluate the postpartum state of the reproductive system in cows based on ultrasonography, bacterial culture, and cytological examination of the uterus and to determine whether postpartum uterine inflammation affects the subsequent state of the endometrium, and, in consequence, selected reproductive parameters in cows. The cytological brush and lavage methods were used to perform the cytological examination, and the quality of the two methods was compared.

## Material and Methods

**Animals, experimental design and ultrasound examination.** The study was conducted on 60 Holstein-Friesian (HF) cows. The cows were kept in the same environmental conditions on the same farm and were fed there. The animals were selected from 140 cows, which underwent routine veterinary examinations during the postpartum period. In addition to an overall examination of the animals, a detailed examination of the reproductive system was performed in order to monitor the status of the endometrium. The uterus and ovaries were examined rectally, ultrasonography was performed with a Honda HS-1500 scanner (Honda Electronics Co., Ltd, Japan), and bacterial culture and cytological examination of the uterus were also performed. The ultrasound apparatus was also used to determine the diameter of the uterine horns, in order to evaluate the dynamics of involution of the uterus. These tests were first performed on the $15^{th}$ d of postpartum, and revealed that at this time *metritis clinica* was present in most of the cows. This was manifested in greater or lesser amounts of brown, bloody discharge from the genital tract, often with an unpleasant odour, while the uterus was substantially enlarged. In some of the cows, *metritis puerperalis* with generalised symptoms (elevated body temperature, appetite loss) was noted as well; these animals were eliminated from the experiment. At this stage, it was not possible to select the animals for the experimental groups. In the second examination, performed on the $26^{th}$ d after parturition, 30 cows with subclinical endometritis, evaluated on the basis of the cytological examination were selected (experimental-E group). Cows with clinical endometritis (purulent or mucopurulent discharge detectable in the vagina) were not included in the experiment. The remaining cows did not exhibit reproductive disorders, and 30 of these animals were selected for the control group (C). During the study neither group of animals exhibited disorders of other systems (metabolic disorders, mastitis, displaced abomasum, *etc*.), which could influence the results. In the cows selected for the experiment, the examinations described above were conducted on the $40^{th}$ and $61^{st}$ d after parturition. The tests conducted after the postpartum period (after the $21^{st}$ d following parturition) were performed at time intervals consistent with procedures adopted by other authors – the first examination between days 21 and 33 after parturition, the second between days 34 and 47, and the third after day 60 (14, 15, 28).

**Endometrial cytology and bacterial examination.** Cytological examination was performed using two methods – cytological brush and lavage. The first method was carried out using a transcervical brush (Jiangsu Yada Technology Group Co., Ltd, China) specially adapted for cows. Two separate brushes for cytological and bacteriological examination were used. The procedure for collecting the material was the same for all experimental animals. After the catheter was passed through the cervix to the basis of the uterine horn, the brush was released from the tube and rotated around its long axis within the uterus to collect the sample. Insertion of the catheter and collection of material were controlled by other hand through the rectum. After a few rotations, the brush was retracted into the catheter and removed from the uterus. All

elements used to collect the material were sterile, in a sanitary sleeve, which made it possible to perform bacterial cultures. The samples for bacteriological examination were placed in vials containing Amies transport medium (Meus s.r.l., Italy), cooled to 4-6°C, and transported to the laboratory within 1 h. Then the material was transferred directly from the brushes onto standard media – Columbia blood agar, Schaedler blood agar, McConkey agar, and Sabouraud dextrose agar (BioMérieux SA, France) to culture aerobic and anaerobic bacteria and fungi. Following incubation at 37°C for 24 h and 48 h under aerobic and anaerobic conditions, and at 28°C for 14 d (Sabouraud dextrose agar), bacterial growth density was evaluated. Bacterial species were identified based on colony morphology, Gram-stained smears, and biochemical characteristics, using API tests (Appareils et Procédés d'Identification: BioMérieux SA, France). Cytological smears were prepared from the second brushes, as imprint specimens on microscope slides. In the other method for collecting material, a Foley catheter (Jorgen Kruuse, Denmark) with a syringe was used to inject 50 mL of 0.9% saline solution into the uterus. The uterus was massaged through the rectum for a few minutes and then the saline solution was aspirated by drawing back the plunger of the syringe to create negative pressure in the catheter. In most cases, about 40 mL of the fluid was recovered, while some of it remained in the uterus. The recovered fluid was taken to the laboratory within a short time and centrifuged at 800 × g for 5 min. The fluid over the sediment was removed and slides were prepared from the sediment (13). When the smears obtained by both methods (brush and lavage) were dried, they were fixed and stained with Hemacolor rapid staining (Merck KGaA, Germany). The samples were examined under an Olympus CX 41 microscope (Olympus Corporation, Japan) at 1000 × magnification. Basler camera attached to the microscope sent the image to a computer so the smears could be documented. Cytological evaluation of the smears was based on the determination of the number of leukocytes. One hundred cells visible in different fields of view were counted in each smear and the percentage of leukocytes, mainly neutrophils and lymphocytes, was determined. After six months of postpartum, the most important parameters in the reproductive system evaluation were compared in the two groups of cows: first visible postpartum oestrus, calving-to-conception interval, and services per conception (number of inseminations needed to result in pregnancy).

**Statistical analysis.** The results were analysed statistically using Statistica 6.0 software (Statsoft, Poland). After arithmetic means and standard deviations were calculated in each group of cows, the results were compared between groups to determine statistical significance using Student's *t*-test. Statistical differences between the results for samples collected at different times in the two groups were calculated using a one-way analysis of variance (ANOVA) and Tukey's and Duncan's *post-hoc* tests, for probability $P < 0.01$. Pearson's correlation coefficient (r) between the percentage of leukocytes in the brush and lavage cytology and the uterine diameter was calculated for the two groups with $P < 0.05$.

## Results

Of 140 cows examined on day of 5 postpartum, 129 (92.1%) had clinical metritis, and 11 (7.9%) had puerperal metritis. On the 26$^{th}$ d after parturition, subclinical endometritis was noted in 30 (21.3%) cows and clinical endometritis in nine (6.4%) animals. The remaining 101 (72.3%) cows did not exhibit disorders in the reproductive system at this time.

The results of the bacteriological tests of swabs from the uterus of cows with endometritis are presented in Table 1. The data shows that on the 5$^{th}$ day after delivery, *Escherichia coli*, *Trueperella pyogenes*, *Staphylococcus* sp., *Streptococcus* sp., *Fusobacterium necrophorum* and *Prevotella melaninogenica* were isolated from experimental and control cows. Usually, there were mixed bacterial cultures. The differences in the percentage of each bacterial species in the cultures were found. At two-other time points (days 26 and 40), significant differences were observed between the two examined groups of animals. The same bacterial species were still isolated in all cows in the experimental group, whereas in the control group, *T. pyogenes* was isolated only in 6.6% and 3.3% of the cows and in the remaining cows the culture results were negative. On the last day of the study (day 61), four bacterial species, mostly *T. pyogenes*, could still be isolated from cows in the experimental group. The cultures were negative in the control cows.

The results of cytological examination of the uterus on day 5 of postpartum were very similar in both groups, despite some fluctuations. The percentage of leukocytes in the brush cytology for the experimental (E) group was 65.60 ± 7.73 and for the control (C) group it was 64.90 ± 7.43. In the lavage cytology the percentages were 67.70 ± 8.68 and 66.90 ± 8.73 respectively. During the second examination (day 26 postpartum), the percentage of leukocytes in the experimental group (Table 2) was statistically significantly higher than in the controls (Table 4), both in the smears prepared from the brush and from lavage ($P < 0.001$). On day 40 of postpartum, the percentage of leukocytes was still statistically significantly higher in the experimental group, both in the brush cytology and the lavage cytology ($P < 0.001$). In the last examination, performed 61 d of postpartum, the number of leukocytes in the experimental group remained statistically higher, both in the brush cytology (E - 13.86 ± 4.05; C - 3.16 ± 2.86; $P < 0.001$) and the lavage cytology (E - 14.80 ± 4.83; C - 4.23 ± 3.02; $P < 0.001$). Tukey's and Duncan's tests comparing the

number of leukocytes revealed by the brush method to the same by the lavage method demonstrated that in both groups, the values obtained in each examination were statistically significantly lower than in the previous analysis. This last examination arrived at values at different levels – $P < 0.01$ for the experimental group and $P \leq 0.001$ for the control group. Selected photographs of the cytological smears from the inflamed uterus are presented to illustrate the data (Figs 1 and 2). Figure 3 presents the regeneration process of the endometrial epithelium during the recovery period. The uterine diameter was similar in the two groups only on day 5 of postpartum. In the subsequent examinations, the values were statistically significantly higher in the experimental group (Table 2) than in the control group (Table 4). For day 26 the values were E - 7.73 ± 1.94 and C - 5.56 ± 1.31 ($P < 0.001$); for day 40 E - 5.73 ± 1.26 and C - 3.76 ± 1.33 ($P < 0.001$); and for day 61 E - 4.33 ± 1.10 and C - 3.43 ± 0.91 ($P = 0.001$). In the experimental group, there was a statistically significant ($P < 0.001$) decrease in uterine diameter in successive examinations, while in the control group the uterine diameter was statistically significantly smaller ($P < 0.001$) in successive examinations up to day 40 of postpartum. Pearson's correlation coefficient r (Tables 3 and 5) between uterine diameter and the percentage of leukocytes obtained in the brush and lavage cytology, and between the number of leukocytes obtained in the cytology using the different methods, shows a very strong linear correlation (C - r = 0.91-0.99, E - r = 0.77-0.91), but the correlations between the parameters are stronger in the control group.

The basic reproductive parameters in the two groups are presented in Table 6. The data shows that the first visible postpartum oestrus, services per conception, and calving-to-conception interval were substantially less favourable in the experimental group than in the control. The values in the experimental group were statistically significantly higher ($P < 0.001$) than in the control.

**Table 1.** Percentage of bacteria isolated from the uterus of cows with endometritis on different days after parturition (n/%)

| Groups | Bacterial cultures | 5th day after parturition. | 26th day after parturition | 40th day after parturition | 61st day after parturition |
|---|---|---|---|---|---|
| Experimental (n = 30) | *E. coli* | 14/46.6% | 8/26.7% | 4/13.4% | 1/3.3% |
| | *T. pyogenes* | 7/23.3% | 12/40% | 17/56.6% | 23/76.7% |
| | *F. necrophorum* | 1/3.4% | 3/10% | 5/16.6% | 5/16.7% |
| | *P. melaninogenica* | 1/3.4% | 2/6.6% | 3/10% | 1/3.3% |
| | *Staphylococcus* sp. | 4/13.3% | 3/10% | 1/3.4% | - |
| | *Streptococcus* sp. | 3/10% | 2/6.7% | - | - |
| Control (n = 30) | *E. coli* | 7/23.3% | - | - | - |
| | *T. pyogenes* | 5/16.7% | 2/6.6% | 1/3.3% | - |
| | *F. necrophorum* | 1/3.4% | - | - | - |
| | *P. melaninogenica* | 1/3.4% | - | - | - |
| | *Staphylococcus* sp. | 10/33.2% | - | - | - |
| | *Streptococcus* sp. | 6/20% | - | - | - |

**Table 2.** Uterine cytology in cows with postpartum inflammation, using a brush and lavage, and uterine diameter during the same time intervals (n = 30)

| Type of test | Day 5 after parturition | Day 26 after parturition | Day 40 after parturition | Day 61 after parturition |
|---|---|---|---|---|
| Number of leukocytes (%), brush | $65.60^{a} \pm 7.73$ | $44.30^{b**} \pm 8.381$ | $33.43^{c**} \pm 8.96$ | $13.86^{d**} \pm 4.05$ |
| Number of leukocytes (%), lavage | $67.7^{a} \pm 8.68$ | $46.93^{b**} \pm 10.11$ | $35.73^{c**} \pm 9.97$ | $14.80^{d**} \pm 4.83$ |
| Uterine diameter (cm) | $12.73^{a} \pm 2.12$ | $7.73^{b**} \pm 1.94$ | $5.73^{c**} \pm 1.26$ | $4.33^{d**} \pm 1.10$ |

** Statistical significance at $P \leq 0.001$ with respect to the control

a-c Statistical differences between the results for the material collected at different times in the group for probability $P < 0.01$

**Table 3.** Pearson's correlation coefficients (r) between the percentage of leukocytes determined in the brush and lavage methods and the uterine diameter in cows with postpartum metritis (n = 30)

| | Number of leukocytes (%), brush | Number of leukocytes (%), lavage | Uterine diameter (cm) |
|---|---|---|---|
| Number of leukocytes (%), brush | X | 0.91* | 0.81* |
| Number of leukocytes (%), lavage | 0.91* | X | 0.77* |
| Uterine diameter (cm) | 0.81* | 0.77* | X |

* Statistically significant differences for probability $P < 0.05$

**Table 4.** Uterine cytology in cows with a normal postpartum period, using a brush and lavage, and uterine diameter during the same time intervals (n = 30)

| Type of test | Day 5 postpartum | Day 26 postpartum | Day 40 postpartum | Day 61 postpartum |
|---|---|---|---|---|
| Number of leukocytes (%), brush | 64.90 [a] ± 7.43 | 14.93 [b] ± 3.86 | 9.36 [c] ± 3.02 | 3.16 [d] ± 2.86 |
| Number of leukocytes (%), lavage | 66.90 [a] ± 8.73 | 16.96 [b] ± 4.56 | 11.46 [c] ± 4.58 | 4.23 [d] ± 3.02 |
| Uterine diameter (cm) | 12.10 [a] ± 1.64 | 5.56 [b] ± 1.31 | 3.76 [c] ± 1.33 | 3.43 [c] ± 0.91 |

[a-c] Statistical differences between the results for the material collected at different times in the group for probability P < 0.01

**Table 5.** Pearson's correlation coefficients (r) between the percentage of leukocytes in the brush and lavage methods and the uterine diameter in healthy cows from the control group (n = 30)

| | Number of leukocytes (%), brush | Number of leukocytes (%), lavage | Uterine diameter (cm) |
|---|---|---|---|
| Number of leukocytes (%), brush | X | 0.99* | 0.91* |
| Number of leukocytes (%), lavage | 0.99* | X | 0.91* |
| Uterine diameter (cm) | 0.91* | 0.91* | X |

* Statistically significant differences for probability P < 0.05

**Table 6.** Reproductive parameters in the experimental group and control group 6 months of postpartum

| Group | Parameter | | |
|---|---|---|---|
| | First postpartum oestrus (days since parturition) | Services per conception | Calving-to-conception interval |
| Control (n = 30) | 41.73 ± 8.11 | 1.86 ± 1.07 | 90.96 ± 22.88 |
| Experimental (n = 30) | 71.12 ± 10.24** | 3.93 ± 1.65** | 140.96 ± 31.79** |

** Statistical significance at P < 0.001 with respect to the control

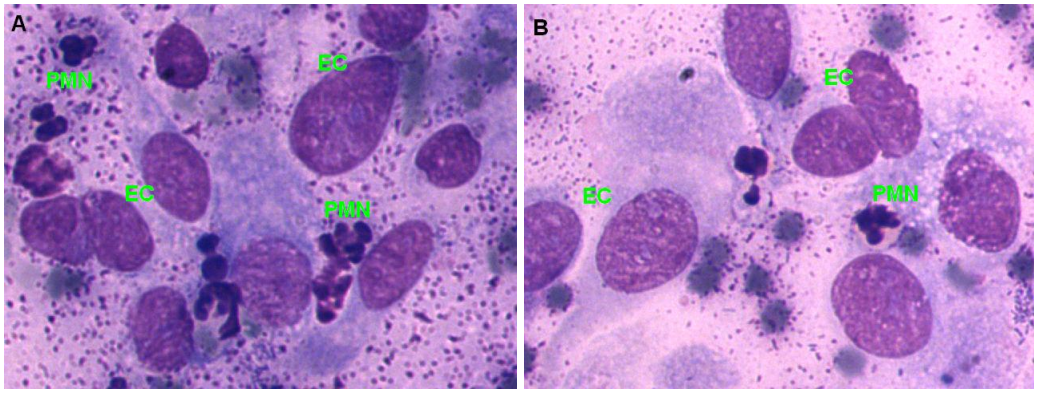



**Fig. 1.** Brush cytology. A - inflammation of the uterus 26 d of postpartum; B - inflammation of the uterus 40 d of postpartum; EC - endometrial cells; PMN - polymorphonuclear cells. Hemacolor staining (1000 x)

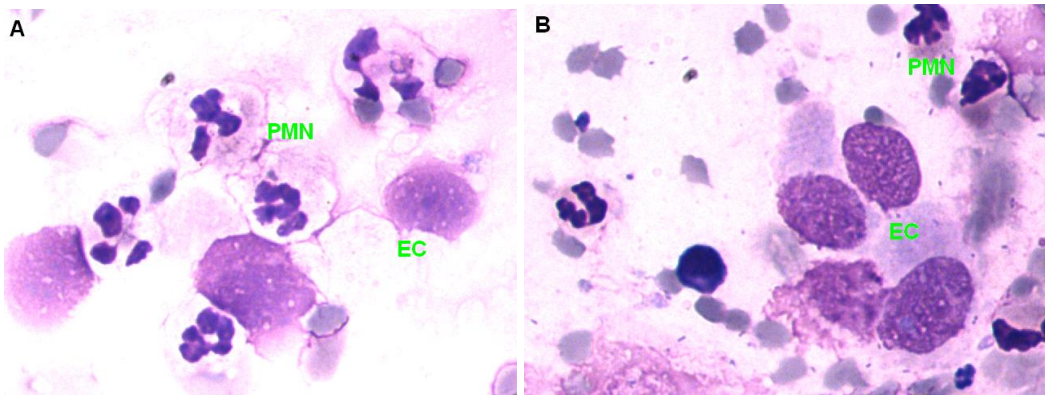



**Fig. 2**. Lavage cytology. A - inflammation of the uterus 26 d of postpartum; B - inflammation of the uterus 40 d of postpartum; EC - endometrial cells; PMN - polymorphonuclear cells. Hemacolor staining (1000 x)

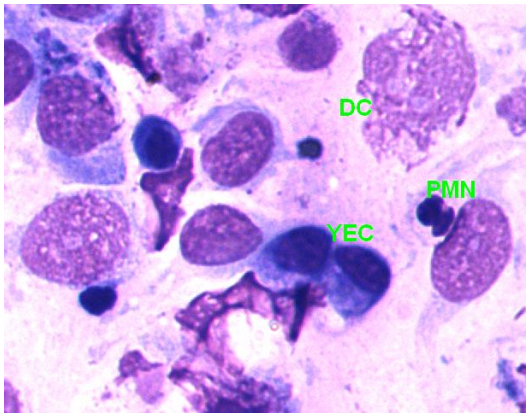



**Fig. 3.** Brush cytology. Inflammation of the uterus 15 d of postpartum in the cows with normal puerperium. YEC - young endometrial cells with cytoplasm and small round cell nucleus, PMN - polymorphonuclear cells; DC - disintegrated cells

## Discussion

Endometrial cytology in cows is restricted in practice to confirm or exclude uterine inflammation (14, 16, 30). Such examinations are currently decisive in diagnosing subclinical endometritis, and provide the basis for Sheldon's new classification of uterine inflammation, which is still in use (16, 28, 30). In the present study, cytological examination detected uterine inflammation in 21.3% of the cows in the experimental herd. This is consistent with the observations of other authors, who have noted even 23.6% incidence of endometritis in individual herds (2, 10). Uterine inflammation is the main cause of fertility disorders in dairy cows. The present study shows that postpartum metritis has a very strong influence on the subsequent fertility of cows, and that in some cows untreated postpartum metritis takes the form of endometritis and can persist for a very long time, reducing fertility. Many authors confirm that postpartum inflammation later takes a subclinical form and can persist even for months after parturition (7, 10, 17). Subclinical inflammatory status has no external symptoms, which means that it is often not detected by herd owners or even herd veterinarians. A standard clinical examination of the reproductive system is not sufficient for effective diagnosis of subclinical endometritis even with ultrasonography. Only by means of cytological, bacteriological, and histopathological testing this inflammatory status can be diagnosed (6, 9, 21). Unfortunately, these diagnostic methods are not yet standard in veterinary practice in the management of reproduction in cows. Depending on local immune mechanisms in the uterus, ovarian activity, and treatment, persistent uterine inflammation can lead to short- or long-term fertility disorders. All of these immune mechanisms are known to be closely related. A persistent corpus luteum on the ovary secretes progesterone, impairing the local immunity of the uterus and sustaining the inflammatory status. The inflamed uterus does not produce normal quantities of PGF2α, which has luteolytic properties, but produces PGE2α, which has luteotropic properties. The effect of this abnormal production is to sustain the presence of the corpus luteum and the influence of progesterone on endometrial functions, which is unfavourable at this time (13, 30). Hormonal changes taking place and local immunosuppression favour the multiplication of bacteria, mainly aerobic in the early postpartum period (puerperal metritis) and facultatively anaerobic and anaerobic bacteria in the later period (endometritis), as shown in the present study. Studies by other authors confirm the dominance of aerobic bacteria (mainly *E. coli*) in the early postpartum period and of *T. pyogenes*, *F. necroforum*, and *B. melanino-genica* in the later period (7, 10). Uterine inflammation causes impairment of endometrial function, preventing fertilisation and implantation, and can even be the cause of early abortion in cows. The condition also leads to disturbances in the ovarian cycle involving the formation of ovarian cysts, prolongation of the luteal phase, and delay to the first visible postpartum oestrus. In consequence, it leads to the return to cyclic ovarian activity, resulting in poor reproductive parameters (13, 27). This is confirmed by our observations. The cytologically diagnosed metritis presented in the study led to substantially less favourable reproductive parameters in the experimental group than in the control. The deterioration of these parameters resulted mainly from disturbances in the oestrous cycle – mainly less pronounced oestrus symptoms, which made oestrus more difficult to detect, lack of or delayed ovulation, preventing the successful insemination procedures, and cysts arising from anovulatory follicles. These block the oestrous cycle after insemination. Studies by other authors, confirming the results of the present study, discuss a possible mechanism of these disorders (27, 30). They indicate that endometritis can slow the growth of ovarian follicles and reduce the size of preovulatory follicles, lowering the concentration of oestrogens. It can also reduce the mass of the corpus luteum and lower the progesterone level. Due to the diminution in the production of these hormones, they inadequately stimulate the hypothalamus and pituitary gland, leading to specific irregularities in the oestrous cycle: the absence of or less pronounced signs of oestrus, lack of or delayed ovulation, or the ovarian cysts mentioned above (17, 27). The effect of other factors, such as the cows' milk productivity, feeding, and housing, was eliminated as the experimental and control groups were from the same herd. The animals were selected to minimise other factors which could influence fertility (3, 18, 31).

Lopez-Gatius *et al.* (22) clearly demonstrated that cows with metritis are 2-3 times more likely to lose an

embryo or foetus than healthy cows. The aim of this study was not to test whether uterine inflammation can cause loss of pregnancy due to early abortion, but if similar pathologies cannot be excluded, since in seven cows from the experimental group pregnancy was not diagnosed until five months of postpartum, and two cows from this group were found still not pregnant up to the 6$^{th}$ month after parturition. Oestrus was repeated despite insemination, and anoestrus periods were prolonged. Other authors confirm that postpartum uterine inflammations can contribute to fertility disorders and loss of pregnancy, even resulting in elimination of the cow from the herd (8, 11, 25). However some authors did not observe a significant impact of endometritis on fertility in cows (1, 24). Comparison of cytology performed by two different methods showed that both of them are suitable for evaluation of advanced endometritis. Despite visible differences in the number of leukocytes between the two methods, no statistically significant differences were noted. The study demonstrated that cytology can be used both for postpartum evaluation of the status of the endometrium and to diagnose subclinical inflammation long after parturition, in cows which postpartum period was not monitored. Studies by Kasimanickam *et al*. (15) and Prieto *et al*. (26) are consistent with our study and confirm that both methods are suitable for evaluation of endometritis. As regards the quality of the cytological smears themselves, it should be emphasised that cytology performed with a brush is of greater value than lavage cytology, because the sample contains far more epithelial cells for a better evaluation of the state of the endometrium. Based on the cytological smears, the authors were also able to observe the regeneration of the endometrial epithelium during the transition from postpartum inflammation to subclinical inflammation. A far greater number of young cells with round nuclei and distinct cytoplasm appear in the cytological image. The authors were unable to find studies in the available literature relevant to these observations. Lavage cytology contains fewer cells because only surface material from the uterine cavity is collected (material is not collected from the uterine wall as in the first method). Moreover, the lavage method collects material from a larger area of the uterus, which always contains more leukocytes and more artefacts, making evaluation of smears more difficult.

The study demonstrated that uterine inflammation in some cows (21.3% of the herd) can persist long after parturition as subclinical endometritis, causing significant deterioration of reproductive parameters. Based on this study the authors suggest that cytological evaluation of the uterus, preferably using a brush, should be performed as soon as possible after parturition, even before day 21, up to which puerperal metritis may still persist. The estimation of the status of inflammation based on the number of leukocytes and the quality of endometrial cells is important at this time. In cows with a normal puerperium, the number of leukocytes should gradually decrease, but new (young) endometrial cells should appear as well, indicating that the recovery process has begun. This vital aspect has not been described in the available literature regarding cytological examinations. Most importantly, the inflammatory status and failure of the endometrium tendency towards regeneration should be diagnosed as soon as possible after parturition, so that necessary treatment can be begun to prevent the development of endometritis and its consequences – a decline in reproduction in the cows, or even their elimination from the herd.

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