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Cytological examination of the cow uterus – description of the method and the preliminary results of its clinical application

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Summary

The aim of the study was to describe a new method used for diagnosing uterine disorders and to present preliminary results regarding the incidence of this kind of endometritis in dairy cows. For its diagnosis, samples must be taken from the lumen of the uterine horns. This requires a special tool which consists of a catheter open at both ends/on both sides, a mandrel and a brush for the collection of samples. After sampling the brush is rolled on cytological glass, and the smear is stained using one of the classic methods. Following this procedure, a number of polimorphonuclear leucocytes (PMNL), other white cells and epithelial cells are counted. In order to diagnose the disorder, the percentage of PMNL is considered. In our trials, conducted in two experimental herds, subclinical endometritis was diagnosed in 69.7% of cows in one herd and in 38.3% of cows in the other herd during the fourth week post partum. Two weeks later, the incidence of this disease dropped to 43.3% and 17%, respectively. Only in 12.1% and 8.7% of cows after parturition the number of PMNL grew with the passage of time. More research is needed to evaluate the influence of cytological endometritis on reproductive performance and to examine the pathological and physiological nature of this disorder.

Keywords: cattle, subclinical endometritis, cytobrush method

Uterine disorders are described as some of the main factors reducing the fertility of cows (3-5). In fact, these diseases are limited to inflammatory conditions of the organ, which occur in herds with high, yet varied, frequency (3%-40%). Recently, uterine inflammations have been re-classified differently, and the two main criteria of this new classification are the time when the condition occurs in the postpartum period and the diffusion of the inflammatory process in particular layers of the uterine wall. The inflammation of all layers of the uterine wall (*metritis*) usually occurs before the 21st day after parturition and is characterised by atony and a significant enlargement of the thin-walled uterus as well as reeking red-brownish watery discharge. These are often accompanied by general symptoms such as anorexia, increased temperature, a decrease in milk yield and dehydration (11). However, uterine inflammatory processes taking place after the 21st day post partum are related almost solely to the mucous membrane of that organ and described as *endometritis*. A characteristic feature of this form of the disease is its chronic course and the presence of inflammatory secretions of various amounts and types (mucous, mucous-purulent, purulent). Clinical symptoms accompanying the condition are of local character, limited to the uterus, and assume the form of more or less advanced changes in the size and consistency of the uterine horns and the cervix (7). This form may develop into another type of uterine inflammation, mainly pyometra, during which purulent secretion is accumulated inside the uterine lumen and the cervix is closed (10).

The forms of the uterine inflammatory conditions and their accompanying symptoms described above are usually enough for a clinical diagnosis. It should be stressed, however, that such a diagnosis requires a consistent application of a rectal examination, vaginoscopy and ultrasonography for there are reports saying that

the level of detectability of this disease depends on the type of diagnostic methods used (9).

Recently, a form of a uterine inflammatory condition called subclinical *endometritis* has created a huge diagnostic problem (5). The disease affects cases in which the inflammatory condition of varied intensity progresses in the uterine mucous layer, yet it is not accompanied by any of the aforementioned symptoms of clinical *endometritis*. Thus, commonly applied diagnostic methods are of no use in such cases. Identifying this form of a uterine inflammatory condition is possible only through a cytological examination of the uterine horn, and the diagnosis is based on detecting an increased number of polimorphonuclear leucocytes (PMNL) in the smear. Since the diagnosis requires a cytological examination, in the literature this form of *endometritis* is also called „cytological *endometritis*". So far, information on epidemiological aspects of this condition is very fragmentary, but it has been estimated that it may affect as many as 60% of cows examined (11). This disease is now considered as one of the causes of worse insemination results in dairy cow herds, which are observed in many countries (6, 11).

As already mentioned, diagnosing subclinical *endometritis* requires taking samples of mucus from the lumen of the uterine horns beyond the cervical barrier. It is worth noting that such a clinical and diagnostic procedure has not been used in cows to date, and all other identification methods have been limited to indirect conclusions on the uterus condition, based on the examination of the vaginal mucosa layer or the vaginal part of the cervix. From this point of view, diagnosing this form of a uterine inflammatory condition involves a significant element of novelty. It is also an example of a new condition identified in cows' reproduction connected with implementing a new diagnostic procedure. This article aims at presenting methodology that enables a thorough diagnosis of subclinical *endometritis* in cows.

Material and methods

This trial was performed in two herds of 230 and 160 Polish Holstein-Friesian cows. The age of the cows ranged from 2 to 9 years. The cows were kept in a loose-type barn and their feeding was based on grass and maize silage, concentrates, vitamins, and mineral supplements. TMR (Total Mixed Ration) was used as the feeding method in the first herd. In the second herd a PMR (Partial Mixed Ration) system was used, and additional concentrates were supplied in feeding stations. Cows in both herds calved in maternity boxes and stayed there until the end of the colostral period. Cows were milked twice a day in the milking parlour – fishbone and tandem type, respectively.

All animals were examined twice, in the fourth and in the sixth week after parturition. A vaginoscopy, rectal palpation, and ultrasound examination were performed. Cows with clinical signs of *endometritis* as well as cows treated with hormones or antibiotics a week prior to the examination were excluded from the trial. Thus, the trial was performed only on clinically healthy cows, and cytological samples of the endometrium were collected from the bigger horn. This method requires some additional equipment, including a stainless steel catheter 6 mm in diameter, open at both ends, a mandrel sharpened at one end and a brush for collecting samples (fig. 1).

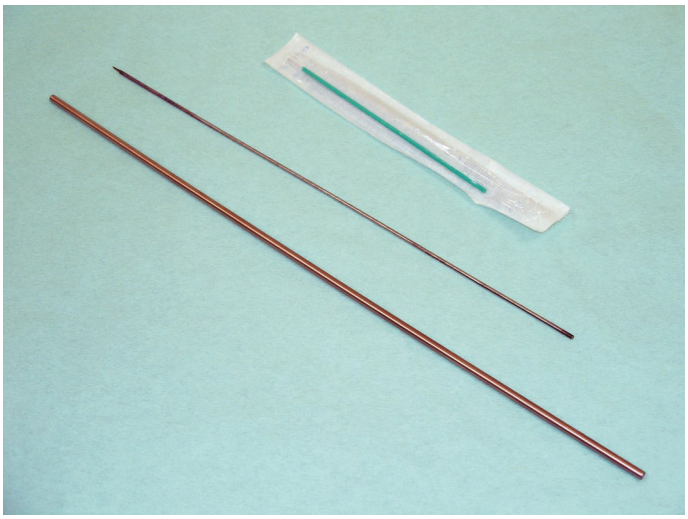

Fig. 1. A catheter opens from sides with a mandrel, and a brush for collecting smear from the cow's uterus

Sterile brushes applied in cytological examinations in human gynaecology were used for this purpose. The brush was combined with the mandrel, and both were inserted into the catheter and then into a glove for rectal examination so that the instrument would be protected from vaginal contaminations. This set of instruments was introduced into the genital tract of the cow under examination. Then, controlled by the hand in the rectum, the catheter was introduced into the vaginal opening of the cervix, after which the glove protecting the catheter was punctured. The next stage was to pass the catheter through the cervix, reaching the uterine horns. Upon introducing the catheter into the horn under examination, the brush was pushed forward, and a sample of material was taken by making three revolutions. Then, the brush was withdrawn into the catheter and the instrument was removed from the animal's genital tract.

Material collected by the cytobrush was transferred to a microscope slide by rolling the brush. The smear was then treated with cytological fixative (Cytofix®, Samko, Poland). The staining of the preparations was done by Papanicolau's method, and the smears were examined with a light microscope under 300 or 600 × magnification. Endometrial cells and polymorphonuclear leukocytes (fig. 2) were then counted to calculate their proportion and to include cows in one of two groups: healthy animals or those with subclinical *endometritis*.

When examining a smear, a hundred visible cells were counted, after which a proportion of white blood cells to the general number of epithelial cells was calculated without taking erythrocytes into account. The criteria for positive examination (subclinical *endometritis*) were ≥ of 18% of PMNL in the fourth week post partum and ≥ 10% in the sixth week or later.

Results and discussion

33 clinically healthy cows were examined in the first herd and 47 in the second herd. The author's own preliminary epidemiological results showed that the occurrence of this disease in the two herds was very different, with variations of up to 50% (69.7% vs. 38.3%) (tab. 1).

Also, the dynamics of changes in this phenomenon during the postpartum period in individual animals were observed. In one of the herds, in clinically healthy cows examined in the fourth week after calving, 69.7% were diagnosed as having an increased level of leukocytes in their smears (fig. 2). However, in the next examination carried out two weeks later, the percentage decreased to 42.4%. Changes in the percentage of leukocytes in individual cows during both examinations were also analyzed (tab. 2).

It appeared that in most cases (45.5% and 32.6% in particular herds) the number of PMNL had a tendency to decrease. An increase in the second examination, compared with the first, was observed only in a few cases (12.1% and 8.7% in the two herds, respectively).

This method of collecting samples from the bovine uterus, especially under field conditions, is quite new and supposedly not well known in Poland. The sampling procedure has been described in detail because, in the author's opinion, it can be used in practice. It should also be mentioned that this method makes it possible to observe and diagnose pathological processes inside the uterus directly. Previously, subclinical endometritis was diagnosed on the basis of indirect observations and examinations. The authors of the present study collected samples only from one area of the uterus, which may be considered as insufficient, but the samples were always collected from the bigger (supposedly previously gravid) horn of the uterus, which increased the chance of identifying the inflammatory process developing in the organ. An optimal diagnostic method would be to take samples from 2 or 3 places in the uterus (both horns and the body), yet this is not very likely under field conditions.

The prevalence of subclinical (cytological) *endometritis* in the two herds examined was comparable to the results of other authors (5, 11). It should be stressed here that all cows were diagnosed as clinically healthy on the basis of routinely used methods. The difference between the two herds was surprising – a phenomenon which is difficult to explain. Both calving conditions (maternity pens) and the level of hygiene were similar in both herds. It is a well known fact that the prevalence of clinical endometritis differs significantly from herd to herd, which is caused by many and not always clearly identified factors (10). In the experimental herds we assumed a similar situation regarding subclinical *endometritis*. In order to better explain this phenomenon, a more detailed investigation should be conducted in the future.

Tab. 1. Prevalence of subclinical *endometritis* in the herds examined during the first six weeks post partum

No. of examination	Herd I		Herd II	
	n	%	n	%
First	23	69.7	18	38.3
Second	14	42.4	8	17.0

Tab. 2. Changes in the percentage of leucocytes in two smears (two weeks apart) from the same cows during the first six weeks post partum

Examinations	Herd I	Herd II
Both negative n (%)	6 (18.2)	25 (54.4)
Only first positive n (%)	15 (45.5)	15 (32.6)
Only second positive n (%)	4 (12.1)	4 (8.7)
Both positive n (%)	8 (24.2)	3 (6.5)

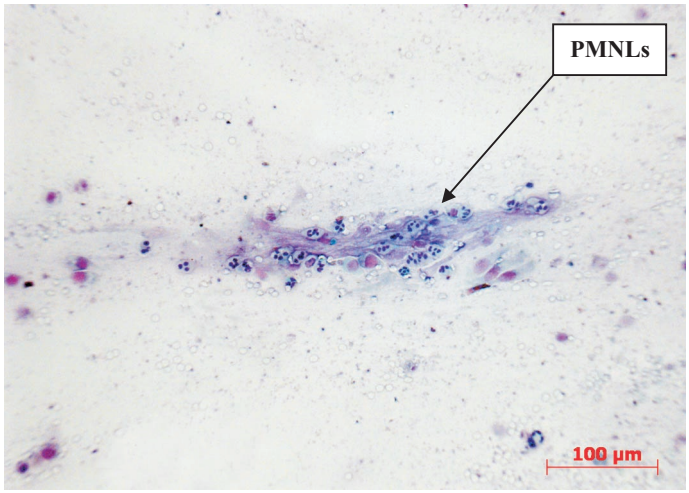

Fig. 2. Cytological smear from the cow's uterus. Papanicolau staining. There are visible polimorphonuclear leukocytes and epithelial cells

The above-described criteria were used in our trial so as to make it possible to compare our results with others that had already been published (5, 6). These rely on the number of leukocytes in the endometrium and the time after parturition. According to some sources, the time of sampling should not be taken into consideration. Some authors claim that the threshold value for this form of *endometritis* is 8% of leukocytes; others bring this value down to 5% (1, 8). With the present level of knowledge, it is difficult to firmly decide which of these values should be accepted as correct. There is still insufficient information on the fertility of cows with an increased number of leukocytes in their smears to verify these criteria under field conditions. To the author's knowledge, only Kassimanickam et al. (6) presented data which showed worse reproduction indices of cows with an elevated percentage of PMNL in the uterus. Currently, there is not enough information to unequivocally identify this phenomenon as pathological. It is also possible that

the condition under discussion is one of the processes of the postpartum involution and regeneration of the uterus, which manifest themselves with various intensity and at different times in particular cows.

Apart from the aforementioned cells, important for the diagnosis, many red blood cells were observed in the smears, especially during the first examination. This may reflect the regeneration of the endometrium after parturition and its progressive recovery. It should be stressed here that the technique applied was safe and the cows showed no negative consequences of the examination, such as pathological uterine discharge, adhesions, or pain during rectal examination. Moreover, no such consequences are reported in the literature.

The dynamics of these processes in individual cows during puerperium have not been described until now. When analysing changes during the postpartum period, a tendency for the decrease of leucocytes was observed in most cows. Yet, there were also animals in which an increased level of leukocytes was observed all the time. Only in few cases leukocytes actually increased from a physiological level to the one which showed inflammatory condition. Therefore, for a correct interpretation of the biological importance of this type of *endometritis*, it is essential to follow its dynamics in the first two months post partum. Extensive epidemiological research should provide an answer to the question about the pathological and physiological meaning of this process during the post-partum period. Moreover, further investigations and an examination of the influence of ovaries on this phenomenon are needed to determine the influence of its intensified occurrences on the course of reproductive processes.

Cytological examination of uterine smears is a new method of examining the reproductive tract in cows. This examination is relatively simple to carry out and interpret so the method can be widely used, also in field conditions. Thus, it may form an alternative to the biopsy method, which is a very useful diagnostic tool for the identification of degenerative and inflammatory changes within the uterus but a more difficult one to apply (2). In the cytological examinations described above, open questions remain the choice of criteria which should be adopted to interpret the results obtained and the most appropriate methods of treating subclinical inflammation.

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