Reproductive performance of cows with subclinical endometritis diagnosed by different cytological thresholds in the postpartum period

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Key words
Cytology, endometritis, cow, reproductive parameters

Summary
Objective: To determine the impact of cytologically determined endometritis (CE) diagnosed according to three different cytological thresholds in the 4th and 6th week postpartum (p. p.) on the subsequent reproductive performance in dairy cows. Material and methods: Dairy cows (n = 222) were clinically examined in the 4th week (Exam I) and in the 6th week (Exam II) p. p. and endometrium samples were collected for cytological examination using the cytobrush method. Three cytological thresholds (percent of polymorphonuclear leucocytes to epithelial cells) were used for categorization of cows: 1) > 18% at Exam I and > 10% at Exam II; 2) > 8% at Exams I and II; 3) > 5% at Exams I and II. The animals were categorized as endometritis positive (CE+) or negative (CE-) according to all cytological thresholds at Exams I and II. For all six experimental groups the following reproductive parameters were calculated: first insemination pregnancy rate, number of services per conception, intercalving period, and total pregnancy rate on day 300 p. p. Results: According to different thresholds prevalence of CE ranged from 48.0% to 65.9% at Exam I and from 26.0% to 34.7% at Exam II. The first insemination pregnancy rate was statistically lower in all CE positive groups at Exam I. Number of inseminations per conception was higher (p < 0.05) in each CE positive cows with exception of group CE+ at Exam II. Intercalving periods were longer in CE cows, and significant (p < 0.05) difference was found for groups ICE+ and ICE- at Exam I. Overall pregnancy rates were similar in cytologically determined endometritis and control cows. Conclusion and clinical relevance: Impaired fertility at herd level may concern only particular reproductive indices. It seems that each herd with subclinical endometritis needs to be evaluated and managed individually, according to the different housing, feeding, and environmental conditions. The first of three thresholds used to cytologically diagnose endometritis seems to be the most reliable when applied in the 4th week p. p.

Schlüsselwörter
Zytologie, Endometritis, Kühe, Fruchtarbeitsindices

Zusammenfassung
Introduction

Endometritis is still considered an important cause of lower fertility in cows (8). According to the new classification of uterine disorders, one of their forms is subclinical endometritis (13). Subclinical endometritis is an inflammation of the uterus that results in a significant reduction of reproductive performance with absence of clinical signs of endometritis (13). For its diagnosis the elevated proportion of polymorphonuclear leucocytes (PMN’s) to epithelial cells in a uterine smear obtained mainly using the cytobrush method has been used (3, 5, 7). There is still debate about the threshold that should be taken into consideration to diagnose subclinical endometritis, and it ranges from 4% to 18% of PMN’s according to author and sampling time after parturition. In the majority of studies the samples were collected 4–6 weeks postpartum (3, 4, 7, 11). Because of these methodological aspects, the prevalence of cytologically determined endometritis (CE) in cows ranges from 9% to 55% (4, 5, 10).

The main aim of the many previous studies on CE was the establishment of cytological diagnostic criteria to diagnose endometritis and healthy cows. Retrospective evaluation of reproductive performance was used in those studies as a method for selection of animals with this disorder as well as estimation of its prevalence (3, 5, 7, 10, 12). In those studies mostly a single cytological threshold and its impact on chosen reproductive indices were used to evaluate the reproductive performance. There is a general opinion that subclinical endometritis impairs fertility in postpartum cows. In previous studies, cows diagnosed as having CE had extended days open, a reduced probability of conception after first artificial insemination (AI), and a lower total pregnancy rate compared with cows without this disorder (5, 7). Recently it has been reported that subclinical endometritis did not affect reproductive performance outcomes in extensive dairy farming based on pasture (10), as well as in beef cattle (12). Thus it may be claimed that the impact of CE on fertility parameters in cows is unclear and still controversial. Due to the different cytological thresholds and sampling time used in the various studies practical definition of CE remains problematic, as does evaluation of its impact on reproductive performance.

The main aim of this study was to determine the impact of CE diagnosed according to three different cytological thresholds in the 4th and 6th week postpartum on the subsequent reproductive performance.

Materials and methods

Animals

A total of 222 Polish Holstein-Friesian cows from a commercial herd were used for this study. Cows aged from 2 to 7 years. They were milked twice daily in a fishbone milking parlour. Average milk yields were around 9000 L. The cows were kept in a loose-type barn. Feeding was based on grass and maize silage, concentrates, and supplements of vitamins and minerals. Cows calved in maternity boxes and stayed there until the colostral period had been finished.

Cows were examined at the first visit (Exam I) between the 21st and 28th day postpartum. In each cow a clinical examination of the reproductive tract was performed by external inspection, vaginoscopy, transrectal palpation and ultrasound examination of the uterus and ovaries.

Animals with purulent discharge and enlargement of the uterus were diagnosed as affected by clinical endometritis and excluded from the study. The same applied for cows which had received systemic or intrauterine antibiotic therapy or reproductive hormones within 7 days before the Exam I. Only animals without clinical signs of endometritis were used in this study. In these cows (n = 222) endometrial cells were obtained immediately after the clinical examination using a cytobrush. All animals were re-examined at the second visit (Exam II) between the 35th and 42nd day postpartum following the same examination protocol.

Cytology and categorization of cows

Cytological samples of the endometrium were collected from a larger horn as described earlier (2, 7). Material collected by a cytobrush was transferred to a microscope slide by rolling the brush onto the slide. The smear was treated with cytological fixative (Cytotix®, Samko, PL) and the preparations were stained using Papanicolaou’s method. Cytological assessment was performed by counting 100 cells (epithelial and immune system cells) under a light microscope at 300× and 600× magnification by a technician who was blinded to the cows’ population. The percent of leucocytes (%PMN’s) in the total number of cells counted was calculated.

Subclinical endometritis cases were defined according to the three previously established thresholds:
1. > 18% of PMN’s at Exam I and > 10% of PMN’s at Exam II (7)
2. > 8% of PMN’s at Exams I and II (3)
3. > 5% of PMN’s at Exams I and II (5)

On the basis of the cytological examination at Exams I and II all cows were assigned to one of six groups. The animals were categorized as cytologically determined endometritisis-positive (CE+) or negative (CE−) according to each threshold at Exam I (ICE+1, ICE−1, ICE+2, ICE−2, ICE+3, ICE−3) and at Exam II (IICE+1, IICE−1, IICE+2, IICE−2, IICE+3, IICE−3) and grouped as a combination of these two factors. Cows from cytologically determined endometritis-negative groups served as a control for animals from cytologically determined endometritis-positive groups.

Following Exam II, all experimental cows were managed according to the normal herd reproductive practices. Animals were inseminated in observed oestrous after 60 days of voluntary waiting period. Pregnancy diagnosis was performed using transrectal palpation and ultrasound around the 35th day after AI.

Reproductive data regarding all experimental groups were collected using an on-farm recording system until 300 days after the...
last cow had been taken into the study. For each of the six groups the following reproductive parameters were calculated: first insemination pregnancy rate (as a percent of pregnant cows after first AI), number of services per conception of all inseminated cows, intercalving period (as an interval between the two last pregnancies) and total pregnancy rate (as a percent of pregnant cows out of all inseminated cows).

Statistical analyses

Statistical analyses – Mann-Whitney test for intercalving period, number of inseminations, and Fisher test for first insemination pregnancy rate and total pregnancy rate – were performed using GraphPad Prism version 4.00 for Windows, (GraphPad Software, San Diego California, USA). Levels of significance were set at p < 0.05 and p < 0.01, respectively.

Results

The prevalence of CE was high and differed between experimental groups (Fig. 1). Moreover, at Exam II the incidence of CE decreased by about half compared with the occurrence at Exam I, reaching the minimum of 26%.

The data regarding reproductive performance in both endometritis-positive and negative cows are presented in Figs. 2, 3, and 4.

The first insemination pregnancy rate calculated for both endometritis-positive and negative cows was higher in all CE-negative groups (Fig. 2) These differences were statistically significant for all thresholds used at Exam I; however, there was a difference in the level of significance among groups ICE+1 (p < 0.01), ICE+2, and ICE+3 (p < 0.05).

The number of inseminations per conception was statistically significantly higher in all CE-positive cows than in CE-negative animals with exception of group IICE+. (Fig. 3).

Intercalving periods were generally longer in groups of CE-positive cows (Fig. 4). A statistically significant (p < 0.05) difference was found between cows with CE (ICE+1) and those unaffected (ICE-1), diagnosed on the basis of the first threshold at Exam I.

Overall pregnancy rates were similar in affected and control cows diagnosed by all thresholds at both examinations. They ranged from 83.2% to 96.7% for cytologically determined endometritis groups and from 85.2% to 93.4% for control groups. There were no statistical differences among the groups.

Discussion and conclusions

The current study is one of the few reports to evaluate the impact of subclinical endometritis on reproductive performance in clinically healthy postpartum dairy cows. A novel aspect of the study was the use of three already established cytological thresholds for the diagnosis of subclinical endometritis in the same population of cows.

The results obtained from the present study demonstrate that cows suffering from CE had a generally worse reproductive performance; however, this reduction was not easy to interpret. Al-
Repromotive indices of cows with endometritis

Though the pregnancy rate after first insemination was lower, the total number of inseminations was higher and the intercalving period was extended. These differences were weakly pronounced because only some of them were statistically significant. In spite of these differences, the overall pregnancy rate was similar in both affected and unaffected animals.

Several previous studies have reported that subclinical endometritis impairs reproductive parameters in cattle (4, 5, 7). A substantial impairment in the reproductive process of affected cows was reflected in a reduction of pregnant cows by the end of the study, extended days open, and reduced results of first and subsequent inseminations (5, 7). The results of the current study are therefore only partially in line with the general opinion of a negative impact of CE on reproduction. A major contributor to impaired reproduction in the present study was a severe reduction in insemination results, while the total pregnancy rate was unaffected and the intercalving periods were similar. A pronounced first service pregnancy risk in CE cows has also been reported also by other authors; however, in those studies a simultaneously decreased number of pregnant cows and extended days open were found (5, 7).

On the other hand there are also studies in which the impact of subclinical endometritis on reproduction was not observed. These findings were obtained in beef cattle (12) or in dairy cows kept under an extensive housing system (10). It has also been speculated that a decrease in bacterial load in the uterus and a more effective immune system in cows in an extensive, pasture-based system improve the self-cure rate of CE compared with high-yielding cows (10). This suggestion does not fully explain the results obtained in the present study, because the experimental herd was kept under an intensive farming system; however the cows had the possibility of movement in loose-type barns.

In the current study three already established thresholds were used to allow a comprehensive evaluation of the impact of subclinical endometritis on reproductive performance. As expected, an increased prevalence of CE in relation to the severity of a given threshold was observed. The percentage ratio of affected cows varied therefore by up to 20%. The prevalence of subclinical endometritis in the present study was high at both examinations, and is in agreement with previous studies (5, 10).

An interesting observation was that such high a prevalence of CE, similar to reports about a negative effect on reproduction (5), showed a not clearly pronounced effect on the subsequent reproductive performance. This also underlines the difficulties to properly evaluate cytologically determined endometritis and its impact on reproductive performance. The similar incidence of this disorder in many herds, with a varying influence on fertility, suggests that CE is interrelated with many factors acting at herd level. Generally, it should be stressed that comparison of reproductive indices among the herds, either affected by CE or without this disorder, is difficult because the variations in herd fertility do not only reflect the impact of reproductive disorders but also the influence and interrelationship of many management and environmental factors (9).
Conclusion for practice

On the basis of the current study cytologically determined endometritis in cows in particular herds seems to have a slight impact on reproductive performance; however, this phenomenon remains poorly understood and requires further studies. It has been shown that impaired fertility at herd level may affect only particular reproductive indices. It seems that each herd with subclinical endometritis needs to be evaluated and managed individually, according to the different housing, feeding, and environmental conditions. Therefore it is not possible to design a general strategy for management of subclinical endometritis. It can be also stated that the first of three thresholds used to cytologically diagnose endometritis seems to be the most reliable, when applied in the 4th week postpartum (i.e. >18% of PMN).

From the current study it can also be claimed that the first threshold, out of the three used, was the most reliable, especially when applied at Exam I. The validity of endometrial cytology for diagnosing subclinical endometritis should be confirmed by its substantial impact on fertility (5, 13). Data obtained from the present study showed that mostly the first threshold was related to impaired fertility, confirmed by statistical analysis of the reproductive values. In cows diagnosed on the basis of other thresholds reproductive parameters were less affected; however, there was still a strong relationship with a decrease in insemination effectiveness. Surprisingly, examination at the earlier stage postpartum (Exam I) was better related to a reduced reproductive performance. This phenomenon is difficult to interpret.

In the current study the authors observed the disappearance of cytological inflammation in most of the cows, which reflects a decrease in CE prevalence with time after parturition. Other authors also observed a reduced proportion of CE cows in the later postpartum period (4, 10). This finding may suggest that uterine self-healing mechanisms are sufficient to cure this disorder. Because different treatment methods for subclinical endometritis gave unclear results (4, 6, 15), a prolonged voluntary waiting period before insemination seems to be a good alternative as a herd management strategy to minimize the negative effect of subclinical endometritis. An extended period from parturition to first insemination should allow the resolution of this disorder in the majority of cows and probably lead to an improvement in the general results of artificial insemination. This idea requires further studies; however, the similar pregnancy rate in both affected and unaffected cows with simultaneously reduced insemination results found in the current study, suggests that these fertility problems of CE cows were largely driven by the preterm service period.

This also indicates that in the case of a high herd prevalence of CE, management decision-making should prefer the change of herd level reproductive performance instead of treating individual animals. This proposal is also in line with the new breeding tendency for an extended voluntary waiting period and prolonged milking period (1, 14).

Conflict of interest
The authors confirm that they do not have any conflict of interest.

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