

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Subclinical Endometritis in Dairy Cattle

Luis Angel Quintela Arias, Marcos Vigo Fernández,
Juan José Becerra González, Mónica Barrio López,
Pedro José García Herradón and
Ana Isabel Peña Martínez

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.80229>

Abstract

Subclinical endometritis is recognized as a cause of poor reproductive performance in dairy cows. Inflammation of the endometrium persisting after postpartum uterine involution has been related with prolonged calving-conception intervals and low fertility in dairy cows. The subclinical nature of this condition makes it necessary in the use of endometrial cytology or biopsy for diagnosing it. There are some controversies among authors in relation to the postpartum period from which a physiological endometrial inflammation should be considered a pathological subclinical endometritis. Therefore, depending on the sampling period after calving, different studies establish a different degree of polymorphonuclear leukocyte infiltration as cutoff point to diagnose subclinical endometritis. Controversies also exist regarding the pathogenesis of the disease and its consequences on the fertility of dairy cattle. The aim of this chapter was to review the current knowledge on this uterine pathology.

Keywords: dairy cow, reproduction, uterine pathology, inflammation, infertility

1. Introduction

One of the main factors affecting reproductive performance of dairy cattle is postpartum uterine disease. Metritis and endometritis have been associated with delays in restarting ovarian activity postpartum, prolonged intervals from calving to first service, increased number of days open, decreased conception rates, and increased culling rates [1–5]. Affected animals are easily identified when they show clinical signs indicative of uterine disease. Though

symptoms of systemic illness are often absent, a purulent or mucopurulent vaginal discharge warrants further investigation, and therefore, clinical metritis and endometritis rarely remain undiagnosed.

Fourteen years ago, Kasimanickam et al. [6] found that many clinically normal postpartum cows had subclinical endometritis (SE). Those authors evaluated endometrial cytologies collected from 228 healthy cows at 21–33 days postpartum and related the cytological findings with the subsequent reproductive performance of cows. They used a receiver/response operating characteristic (ROC) curve to determine a threshold percentage of polymorphonuclear leukocytes (PMN%) in the cytological smears above which fertility was significantly reduced, and therefore, subclinical endometritis was diagnosed based on PMN% threshold. Since that pioneer work, many other studies have investigated the etiology, prevalence, and impact on reproduction of SE in dairy cows.

2. Etiopathogenesis

Subclinical endometritis is the inflammation of the endometrium without clinical signs and often without evidence of infection [7–9]. Alteration of the inflammatory response postpartum could be at the origin of this condition.

There is no doubt that uterine pathogens may negatively affect reproduction both by causing direct endometrial damage and by producing toxins [10, 11]. Bacterial endotoxins are known to have numerous effects on reproduction: (a) they may affect estradiol and progesterone secretion and alter follicular growth and the normal development of the corpus luteum [10–12], (b) may interfere with LH production and cause ovulation failure [13, 14], (c) may increase PGE₂ secretion and prolong the life span of corpus luteum [15], and (d) may induce embryo mortality [16].

In cows with metritis and clinical endometritis, recognized pathogens such as *E. coli*, *Trueperella pyogenes*, *Fusobacterium necrophorum*, or *Prevotella* spp. are commonly isolated from the uterus [17]. In the case of SE, in contrast, several studies [7–9] showed that bacterial populations isolated from the uterus of cows diagnosed with SE did not differ from those of healthy cows. Prunner et al. [18] found that presence of *Trueperella pyogenes* in the uterus postpartum was a risk factor for development of clinical endometritis, but neither *Trueperella pyogenes* nor *E. coli* were associated with SE. Results of the cited studies suggest that common pathogens associated with metritis and clinical endometritis do not have a significant role in the SE pathogenesis. It has been suggested that SE may be a response to unspecific uterine infections [19] or a prolonged inflammatory process that persists after bacterial elimination.

In several studies, cows with clinical and subclinical endometritis were shown to have increased endometrial mRNA expression and elevated serum concentrations of pro-inflammatory mediators as compared with healthy cows [20–24]. Situations of prolonged inflammation after elimination of bacterial contamination may occur when an exacerbated production of eicosanoids concurs with a low production of anti-inflammatory substances, originating a

delayed restoration of homeostasis in the affected tissues [25]. It has also been suggested that an unbalanced production of pro-inflammatory/anti-inflammatory cytokines during the first week postpartum could play a determinant role in the subsequent development of SE. A high ratio of pro-inflammatory/anti-inflammatory cytokines during the first week postpartum could lead to an excessive inflammatory response [26], whereas a low ratio of pro-inflammatory/anti-inflammatory cytokines might impair activation of inflammation and clearance of bacteria and lead to development of endometritis [22, 27].

On the other hand, diet fat levels and the type of fatty acids present in diet may affect cellular immune function [28]. Linoleic acid-enriched diets fed to dairy cows during the transition period [29] induced a pro-inflammatory status during the first week postpartum. Several studies have demonstrated that excess of adipose tissue and high serum concentrations of non-esterified fatty acids constitute risk factors for postpartum pro-inflammatory diseases in dairy cows, such as metritis or mastitis [30–32]. Innate immune response is activated when an aggressor agent is recognized by toll-like receptors (TLR). Different aggressor agents are recognized by specific TLR, which might also be activated by certain molecules in the absence of aggressor agents. For instance, lipopolysaccharides present in the cell wall of gram-negative bacteria are recognized by TLR4, which may also be activated by some fatty acids (lauric, palmitic, and oleic) [33]. Thus, an inflammatory response might be induced without the existence of infection.

In addition, the oxidative stress may contribute to an abnormal inflammatory response during postpartum [33, 34]. Increase of oxygen metabolism during postpartum would increase ROS production rate [33, 35]. Studies carried out in bovine endothelial cells evidenced that oxidative stress increased lipid hydroperoxide formation which enhanced a pro-inflammatory phenotype of these cells [36–38].

Independently of the cause of inflammation, the inflammatory status of the endometrium may have a major impact on reproduction. A direct negative effect of SE on embryo quality and survival has already been described [39, 40], which would affect conception rates. In addition, results from various studies suggest that SE may be associated to altered patterns of prostaglandin E_2 and $F_{2\alpha}$ synthesis [41, 42] which could compromise luteal function and pregnancy.

On the other hand, certain cytokines are known to play essential roles on the physiological regulation of ovarian function [43]. Cytokines are involved in regulation of follicular growth, ovulation, luteal formation, and regression [44, 45]. Inflammatory mediators, such as cytokines released in SE, may perturb this regulatory function.

3. Diagnosis

Cows with subclinical endometritis, by definition, do not show any clinical sign of endometritis, and therefore, the diagnosis of this condition requires the use of endometrial cytology, biopsy, or any other method able to evidence the presence of endometrial inflammation.

Ultrasonography has been used as a method to diagnose SE based on the presence of intra-uterine fluid and on the evaluation of uterine diameter. A small amount of fluid in the uterine lumen and/or thickened uterine walls can be considered signs of endometrial inflammation. However, in various studies ultrasound was found to be less sensitive than endometrial cytology [6, 46, 47] for SE diagnosis. Presence of intrauterine fluid and a thick uterine mucosa may be normal findings in physiological situations such as estrus or early postpartum [48], and perhaps the evaluation of fluid characteristics could improve the sensitivity of ultrasound diagnosis [49]. Mariño et al. [9] found a significant relationship between presence of abnormal intrauterine fluid and SE diagnosed by biopsy but not by cytology.

Doppler ultrasonography might be useful for the diagnosis of endometritis in cattle, but it is still an unexplored tool. Debertolis et al. [50] found significantly increased blood flow in uterine arteries of cows to which acute endometritis had been experimentally induced. Whether patterns of vascular flow may differ between healthy uterus and those with SE still has to be investigated.

Endometrial cytology is considered the most reliable method for the diagnosis of SE [46], and therefore, it is the one most frequently used. Samples for cytology can be obtained by two main techniques, cytobrush and uterine lavage.

The cytobrush technique consists of connecting a cytobrush to the plunger of an insemination catheter [6] and, protected by the catheter, introducing it into the uterus as for doing artificial insemination. Inside the uterus, the cytobrush is pushed out of the catheter, gently rotated against the uterine wall, guarded back inside the catheter, and removed from the uterus. The brush is rolled onto a microscopic slide and stained. Cytology samples can be obtained from the uterine body or from one of the uterine horns. Mariño et al. [9] compared cytology and biopsy findings between the two horns of 100 bovine uteri collected postmortem and observed that samples collected from the left horn were more representative of both uterine horns than those collected from the right one.

The uterine lavage technique consists of infusing sterile saline solution into the uterus with a catheter, gently massaging the uterus to allow fluid distribution within the lumen, and recovering some of the fluid by aspiration using the same catheter. The collected fluid is centrifuged, the supernatant discarded, and the sediment smeared onto a microscopic slide. Regardless of the collection technique, cytological smears are fixed and stained using conventional stains (e.g., Diff-Quick).

Kasimanickam et al. [51] did a comparative study of the two sampling techniques and concluded that cytobrush had some advantages over uterine lavage: it was less time-consuming, was easier to perform independently of the uterine size, did not produce endometrial irritation, and induced lower degree of cell structure distortion and lower presence of erythrocytes. One disadvantage of cytobrush is that the sample is collected from a specific area of the endometrium, whereas uterine lavage provides cells from the whole endometrial surface.

Recently, Pascottini [52] described a new method for sample collection that consisted of using a paper tape rolled around the top of an insemination catheter. With this method the author

observed less contamination with erythrocytes and a better preserved structure of epithelial cells than when using cytobrush. Moreover, this system would allow taking a sample for cytology at the same time of doing insemination.

Concerning the threshold used in different studies for the diagnosis of SE, the cutoff PMN% reported by the different authors has varied between 4 and 25% (**Table 1**) depending on the postpartum period at which the diagnosis was done.

Sampling method	Postpartum diagnosis period			
	Week 3–5	Week 5–7	≥7 week	PMN%
Cytobrush				
Lopdell et al. [53]	35.0%			>18.0%
Kasimanickam et al. [6]	35.1%			>18.0%
Heidarpour et al. [54]	13.5%			>18.0%
Kaufmann et al. [55]	12.4%			>18.0%
Barrio et al. [56]	17.6%			>18.0%
Madoz et al. [57]	21.5%			>8.0%
Dubuc et al. [58]	19.3%			>6.0%
Plöntzke et al. [59]	38.0%			>5.0%
Lopdell et al. [53]		7.0%		>18.0%
Kasimanickam et al. [6]		34.0%		>10.0%
Barlund et al. [46]		11.8%		>8.0%
Madoz et al. [57]		16.0%		>6.0%
Plöntzke et al. [59]		19.0%		>5.0%
Barrio et al. [60]		14.9%		>5.0%
Madoz et al. [57]			16.0%	>4.0%
Dubuc et al. [58]			11.1%	>4.0%
Uterine lavage				
Hammon et al. [61]	51.8%			>25.0%
Barlund et al. [46]	15.8%			>8.0%
Gilbert et al. [62]		53.0%		>5.0%
Cheong et al. [63]		25.9%		>10.0%
Cytotape				
Pascottini [52] (at AI, cows)			27.8%	≥1%
Pascottini [52] (at AI, heifers)			7.86%	≥1%

Table 1. Reported prevalence of subclinical endometritis in some studies that used different sampling methods, postpartum diagnosis periods, and PMN% cutoff values.

Uterine contamination at parturition or in the following days is unavoidable and normal, with 80–100% of animals having bacteria in the uterine lumen in the first 2 weeks postpartum [17]. Uterine contamination elicits neutrophil migration from peripheral blood to the uterine lumen and the subsequent phagocytosis of contaminating organisms by neutrophils. Prunner et al. [18] observed that uterine bacterial growth density increased from calving to 15 days postpartum and decreased from day 21 onwards and the PMN% in cytological samples decreased from calving to day 9, then increased around days 15–21, and decreased thereafter, but at each sampling period, the proportion of PMN strongly depended on bacterial counts.

Kasimanickam et al. [6] used ROC analysis to identify the PMN% above which fertility was significantly reduced, and this percentage was 18% for samples taken 20–33 days postpartum and 10% for those taken 34–47 days postpartum. Other authors also established the cutoff PMN% for diagnosing SE based on detrimental effects on subsequent reproductive performance [46, 57, 58, 64], and some [59, 61, 62] used arbitrary values. In general, most authors used PMN% thresholds of 15–18% for SE diagnosis at 21–30 days postpartum and values of 4–10% for diagnosis at later periods. Prunner et al. [18] categorized clinically normal cows at 21 days postpartum as having SE when $\text{PMN\%} \geq 5\%$ and found that SE-positive cytological samples had an average of 30% PMN; however, on day 28, cows previously categorized as having healthy uteri (i.e., $<5\%$ PMN on day 21) had a similar PMN% as those categorized as having SE, and for both groups, it averaged 15%. During the first month postpartum, healthy cows may show relatively high percentages of PMN in cytological samples, and therefore, diagnosis of SE during this period will be less accurate than a later diagnosis.

It has been suggested that the stage of the estrous cycle might have an effect on the proportion of PMN present in the cytology and, therefore, on the diagnosis of SE. During the follicular phase of the estrous cycle, there is an increased infiltration of PMN in the endometrium elicited under estrogenic influence [65]. Several studies [9, 52, 57] have found that the PMN% in cytological samples taken with cytobrush was not affected by the stage of the estrous cycle. However, when the SE diagnosis was done by biopsy, a higher degree of inflammatory infiltration could be observed in the follicular phase of the cycle [9]. This was because cytology only detects PMN infiltration in the superficial epithelium, whereas biopsy allows identifying inflammatory cells in deeper layers of the endometrium.

Another possibility to evidence the existence of endometrial inflammation is the use of urinary test stripes, which detect the presence of leukocytes in urine [66–68]. The diagnosis of endometritis can be done using uterine lavage fluids, or an endometrial cytobrush can be immersed in saline solution during 30 sec and then the strip introduced in the solution for 2 sec. It is a qualitative colorimetric test that showed a variable correlation with cytology. Santos et al. [66] reported a sensitivity of 96% and specificity of 98%. However, Cheong et al. [67] observed 77% sensitivity and 52% specificity. This test is rarely used in commercial dairy farms probably because it is not specifically designed for the diagnosis of endometritis.

Uterine biopsy is commonly used in human medicine as it is considered the gold standard for evaluating the human endometrium [69]. In domestic animals, uterine biopsy has been used since the 1960s to investigate causes of infertility in mares [70], and it is a routine diagnosis

method today [71]. However, in dairy cattle uterine biopsy is rarely performed by practitioners, and it is almost exclusively used for research purposes. The limited use of biopsy in clinical practice may be related with inconveniences associated to sampling time, requirement for laboratory skills, laboratory costs, and time to report [71] and also to the risk of inducing endometritis and the subsequent negative effects on fertility [72].

There are few studies using uterine biopsy for the diagnosis of SE in dairy cattle [73], and when biopsy and cytology findings were compared, the two diagnosis methods showed poor agreement [8, 9, 47]. The histopathological examination of biopsy samples gives detailed information about the degree of inflammation, distribution of the inflammatory infiltrate, or the lesions that may exist, whereas cytology only assesses the superficial layer of the endometrium [47]. Thus, it is not surprising that direct comparison between biopsy and cytology results showed low agreement. Evaluation criteria for SE diagnosis on biopsy samples, as for cytology, should be established based on detrimental effect on subsequent reproductive performance rather than on the presence of an arbitrary number of inflammatory cells [47].

4. Prevalence

The reported prevalence of SE in postpartum dairy cows has varied between 7 and 53% (Table 1). Such disparity among studies in SE prevalence may be due to differences in (i) postpartum period in which the diagnosis was made, (ii) PMN% established as threshold above which an endometrial cytology was considered positive for SE, and (iii) the method used to take the cytological sample, i.e., cytobrush, uterine flushing, or cytotape. In general, SE prevalence tended to be higher when the sample was collected by uterine lavage, the diagnosis was made before 30 days postpartum, and the cutoff PMN% applied was >5%.

5. Effects of SE on productive and reproductive performance

There are some discrepancies among authors concerning the effects of SE on reproductive performance of dairy cows. Whereas some authors [59, 74, 75] did not find significant effects on reproduction, many other studies described a variety of negative effects on fertility (Table 2). The disparity of results may not only be due to the different diagnosis criteria (e.g., postpartum period for SE diagnosis, threshold of PMN applied, etc.) used in the different studies but also to the numerous confounding factors that may have a negative effect on reproduction (e.g., poor heat detection, inadequate nutrition, insufficient cow comfort, old cows, poor semen quality, other diseases, etc.).

Subclinical endometritis has been related with the repeat breeder cow syndrome with controversial results. Whereas in some studies [78, 79] the prevalence of SE in repeat breeder cows was reported to be close to 50%, in another study [80] the observed prevalence was lower than 15%. The PMN% thresholds used in those studies differed from 3% [78] and 5% [80] to 10% [79].

Reference	Characteristics of the study	Reproductive impact	Affected parameters
Kasimanickam et al. [6]	n = 228; farms = 2; no cows with PVD; cytobrush; 20–33 DIM: >18% PMN; 34–47 DIM: >10%PMN	Adverse	Days open. Pregnancy rate
Gilbert et al. [62]	n = 141; farms = 5; no cows with PVD; uterine lavage; 40–60 DIM: ≈5%PMN	Adverse	Postpartum anestrus. First-service pregnancy rate. Services per conception. Days open. Pregnancy rate
Barlund et al. [46]	n = 221; farms = 8; no cows with PVD; cytobrush; 28–41 DIM: >8%PMN	Adverse	First-service pregnancy rate. Services per conception. Days open. Pregnancy rate
Dubuc et al. [58]	n = 1044; farms = 6; some cows with PVD; cytobrush; 35 ± 3 DIM: >6% PMN; 56 ± 3 DIM: >4% PMN	Adverse	Pregnancy rate
Plöntzke et al. [59]	n = 201; farms = 3; no cows with PVD; cytobrush; 18–38 DIM: >5%PMN; 32–52 DIM: >5%PMN	Without effect	Days to first service. Services per conception. Days open. Pregnancy rate
Burke et al. [76]	n = 78; farms = 1; no cows with PVD; cytobrush; 42 DIM: >6%PMN	Adverse	Postpartum anestrus
Green et al. [77]	n = 169; farms = 1; no cows with PVD; cytobrush; 21 ± 3 DIM: >18%PMN; 42 ± 3 DIM: >18%PMN	Adverse	Postpartum anestrus
McDougall et al. [64]	n = 303; farms = 1; some cows with PVD; cytobrush; 29 ± 2.4 DIM: >9%PMN; 43 ± 2.3 DIM: >7%PMN	Adverse	Postpartum anestrus. First-service pregnancy rate. Days open
Drillich et al. [39]	n = 48; farms = 1; no cows with PVD; cytobrush; IA: 0% PMN; embryo collection: 0% PMN	Adverse	Transferable embryo recovery rate

Reference	Characteristics of the study	Reproductive impact	Affected parameters
Fernandez-Sanchez et al. [40]	n = 41; farms = 1; no cows with PVD; cytobrush; no PMN% cutoff; donor cows in embryo transfer programs	Adverse	Transferable embryo recovery rate
Prunner et al. [74]	n = 383; farms = 10; no cows with PVD; cytobrush; 20–30 DIM: >5%PMN	Without effect	Days to first service. Services per conception. Days open. Pregnancy rate. Culling rate
Barrio et al. [56]	n = 467; farms = 1; no cows with PVD; cytobrush; 30 ± 2 DIM: >18%PMN	Adverse	First-service pregnancy rate
Barrio et al. [60]	n = 65; farms = 25; no cows with PVD; cytobrush; 30–45 DIM: >5%PMN	Adverse	Days open
Gobikrushanth et al. [75]	n = 126; farms = 1; no cows with PVD; cytobrush; 25 ± 1 DIM: >8%PMN	Without effect	Follicular development and ovulation. First-service pregnancy rate. Cows pregnant at 150 and 250 days postpartum

n, number of animals; PVD, purulent vaginal discharge; DIM, days in milk.

Table 2. Reported effects of subclinical endometritis on reproduction.

In addition to the potential effects on reproduction, endometritis may negatively affect milk production [81]. Clinical and subclinical endometritis have been related with a decrease in milk production of 0.6–1.03 kg/cow/day, reduction of milk fat and protein, and with increased somatic cell counts in milk [64, 76, 82]. Nevertheless, some authors [83] question these effects.

6. Treatment of subclinical endometritis

Antibiotics and prostaglandins $F_{2\alpha}$, combined or individually, have constituted the most common treatment for clinical endometritis postpartum. Haimerl et al. [84] and Lefebvre and Stock [85] did a critical evaluation of the scientific literature that in the last 20 years reported the use of $PGF_{2\alpha}$ alone or combined with antibiotics for the treatment of clinical endometritis in postpartum dairy cows. Both groups of researchers concluded that there was not enough clinical evidence that using $PGF_{2\alpha}$ in endometritis postpartum had a beneficial effect. And the only antibiotic that seemed to be effective for clinical endometritis was cephalirin (a first-generation cephalosporin).

In the case of SE, the treatment with $\text{PGF}_{2\alpha}$ and/or antibiotics was tested only in a few studies that cannot be easily compared as included different hormonal protocols for synchronization of estrus or ovulation, animals in different postpartum periods, and different diagnosis criteria for SE. In the studies of Kasimanickam et al. [51], Galvão et al. [86], and Denis-Robichaud and Dubuc [87], intrauterine infusion of cephalosporins was tested as treatment of SE, and Kasimanickam et al. [51], Galvão et al. [88], and Lima et al. [89] tested the use of prostaglandins. Kasimanickam et al. [51] and Denis-Robichaud and Dubuc [87] obtained higher pregnancy rates at first insemination in cows treated with intrauterine cephalosporins than in control cows, whereas Galvão et al. [86] did not observe any positive effect on reproduction when cows diagnosed with SE were treated with intrauterine ceftiofur infusion. Concerning the use of $\text{PGF}_{2\alpha}$ in cows with SE, Kasimanickam et al. [51] and Galvão et al. [88] observed positive effects on reproductive performance, whereas Lima et al. [89] did not find any effect. It should be pointed out that the magnitude of the positive effects observed in some of the cited studies was dependent on other factors such as existence of ovarian activity at the time of treatment [87] or body condition [88]. The scarce number of studies done so far and the different results obtained do not allow us to draw a conclusion about the efficacy of using $\text{PGF}_{2\alpha}$ and/or cephalosporins for the treatment of SE.

Because in many cases of SE there is no uterine content or positive bacterial culture, treatment with antibiotics or prostaglandins should be expected to be unsuccessful. However, there is an inflammatory response that very likely is the cause of the negative effects of SE, and therefore, the use of nonsteroidal anti-inflammatory drugs (NSAID) would be fully warranted. Priest [5] tested the use of the NSAID carprofen, three doses administered at 3-day intervals between 21 and 31 days postpartum, in cows diagnosed with SE when the cytology showed $>14\%$ PMN at 14 days postpartum. The treatment did not reduce the incidence of SE at day 42, but increased pregnancy rate as compared with untreated control cows. However, in a subsequent study [90], cows were treated with carprofen at 1 or at 3 weeks after calving, and the treatment did not improve milk production, indicators of health or reproductive performance.

Uterine lavage with sterile saline solution is a common treatment for endometrial inflammation in mares. Uterine lavage favors the elimination of inflammatory products, such as nonfunctional PMN, and induces uterine contractions that facilitate the evacuation of any content. In addition, elimination of nonfunctional PMN favors migration of new functional PMN that is able to counter the infection [91]. In bovine, potential usefulness of uterine lavage has been described in connection with treatment of repeat breeder cows, either as the only treatment or combined with prostaglandins and/or antibiotics, assuming that many repeat breeder cows may suffer chronic endometritis [92]. In cases of SE postpartum, uterine lavage with physiological saline at day 30 postpartum was associated with a reduction of the PMN% in cytological samples obtained at day 40, but its effect on reproduction was not evaluated [93].

Other protocols that have been used for the treatment of metritis or clinical endometritis, such as intrauterine infusion of dextrose [94], ozone [95], or N-acetylcysteine combined with amoxicillin and clavulanic [96], have not been tested in cows with subclinical endometritis. Nevertheless, the effect of those substances is mainly antibacterial or mucolytic, whereas in SE there is no mucopurulent secretion and, in most cases, no pathogen bacteria.

7. Conclusions

Subclinical endometritis is a uterine inflammation probably originated by the alteration of the inflammation regulatory mechanisms. The inflammatory status may abnormally persist after elimination of postpartum bacterial contamination, which may be associated with an unbalanced production of anti- and pro-inflammatory factors. Prevalence of subclinical endometritis in dairy farms may reflect the immune status of cows, which in turn would be indicative of the metabolic status of cows in transition and, eventually, of the nutritional management of farms.

Acknowledgements

The study was supported by Xunta de Galicia (Programa Sectorial de Medio Rural, Proyecto Ref. PGIDIT07MRU002E) and FEFRIGA, Santiago de Compostela, Spain.

Author details

Luis Angel Quintela Arias*, Marcos Vigo Fernández, Juan José Becerra González, Mónica Barrio López, Pedro José García Herradón and Ana Isabel Peña Martínez

*Address all correspondence to: luisangel.quintela@usc.es

Unit of Reproduction & Obstetrics, Faculty of Veterinary Medicine, Department of Animal Pathology, Lugo, Spain

References

- [1] Le Blanc S, Duffield T, Leslie K, Bateman K, Keefe G, Walton JS, et al. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal of Dairy Science*. 2002;**85**:2223-2236. DOI: 10.3168/jds.S0022-0302(02)74302-6
- [2] Gautam G, Nakao T, Yusuf M, Koike K. Prevalence of endometritis during the postpartum period and its impact on subsequent reproductive performance in two Japanese dairy herds. *Animal Reproduction Science*. 2009;**116**:175-187. DOI: 10.1016/j.anireprosci.2009.02.001
- [3] Sheldon IM, Price SB, Cronin J, Gilbert RO, Gadsby JE. Mechanisms of infertility associated with clinical and subclinical endometritis in high producing dairy cattle. *Reproduction in Domestic Animals*. 2009;**44**(Suppl. 3):1-9. DOI: 10.1111/j.1439-0531.2009.01465.x

- [4] Sheldon IM, Rycroft AN, Dogan B, Craven M, Bromfield JJ, Chandler A, et al. Specific strains of *Escherichia coli* are pathogenic for the endometrium of cattle and cause pelvic inflammatory disease in cattle and mice. *PLoS One*. 2010;**5**(2):e9192. DOI: 10.1371/journal.pone.0009192
- [5] Priest N. The Effect of a Non-steroidal Anti-Inflammatory Drug on Subclinical Endometritis in Dairy Cows and the Identification of at-Risk Cows. [MSc Thesis]. New Zealand: Lincoln University; 2013
- [6] Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, et al. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. *Theriogenology*. 2004;**62**:9-23. DOI: 10.1016/j.theriogenology.2003.03.001
- [7] Lazzari G, Duchi R, Colleoni S, Baldazzi L, Benedetti V, Galli A, et al. Le patologie uterine cliniche e subcliniche come causa di infertilità nelle bovine da latte: Studio epidemiologico in due allevamenti della regione Lombardia. *Large Animal Review*. 2011;**17**:43-47
- [8] 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. *Journal of Dairy Science*. 2014;**97**:1-7. DOI: 10.3168/jds.2013-6836
- [9] Mariño B, Quintela LA, Becerra JJ, Barrio L, Mociños JE, Guillin JL, et al. Agreement between postmortem endometrial cytology, biopsy and bacteriology in culled dairy cows. *Animal Reproduction*. 2017;**14**(4):1024-1033. DOI: 10.21451/1984-3143-AR826
- [10] Herath S, Williams EJ, Lilly ST, Gilbert RO, Dobson H, Bryant CE, et al. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. *Reproduction*. 2007;**134**:683-693. DOI: 10.1530/REP-07-0229
- [11] Williams EJ, Fischer DP, Noakes DE, England GCW, Rycroft A, Dobson H, et al. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. *Theriogenology*. 2007;**68**:549-559. DOI: 10.1016/j.theriogenology.2007.04.056
- [12] Shimizu T, Miyauchi K, Shirasuna K, Bollwein H, Magata F, Murayama C, et al. Effects of lipopolysaccharide (LPS) and peptidoglycan (PGN) on estradiol production in bovine granulosa cells from small and large follicles. *Toxicology in Vitro*. 2012;**26**:1134-1142. DOI: 10.1016/j.tiv.2012.06.014
- [13] Lavon Y, Leitner G, Goshen T, Braw-Tal R, Jacoby S, Wolfenson D. Exposure to endotoxin during estrus alters the timing of ovulation and hormonal concentrations in cows. *Theriogenology*. 2008;**70**:956-967. DOI: 10.1016/j.theriogenology.2008.05.058
- [14] Lavon Y, Leitner G, Voet H, Wolfenson D. Naturally occurring mastitis effects on timing of ovulation, steroid and gonadotrophic hormone concentrations, and follicular and luteal growth in cows. *Journal of Dairy Science*. 2010;**93**:911-921. DOI: 10.3168/jds.2009-2112

- [15] Herath S, Lilly ST, Fischer DP, Williams EJ, Dobson H, Bryant CE, et al. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin F2 α to prostaglandin E2 in bovine endometrium. *Endocrinology*. 2009;**150**:1912-1920. DOI: 10.1210/en.2008-1379
- [16] Soto P, Natzke RP, Hansen PJ. Identification of possible mediators of embryonic mortality caused by mastitis: Actions of lipopolysaccharide, prostaglandin F2a, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. *American Journal of Reproductive Immunology*. 2003;**50**:263-272. DOI: 10.1034/j.1600-0897.2003.00085.x
- [17] Sheldon IM, Lewis GS, LeBlanc S, Gilbert RO. Defining postpartum uterine disease in cattle. *Theriogenology*. 2006;**65**:1516-1530. DOI: 10.1016/j.theriogenology.2005.08.021
- [18] Prunner I, Pothmann H, Wagener K, Giuliadori M, Huber J, Ehling-Schulz M, et al. Dynamics of bacteriologic and cytologic changes in the uterus of postpartum dairy cows. *Theriogenology*. 2014;**82**:1316-1322. DOI: 10.1016/j.theriogenology.2014.08.018
- [19] Gärtner MA, Peter S, Jung M, Drillich M, Einspanier R, Gabler C. Increased mRNA expression of selected pro-inflammatory factors in inflamed bovine endometrium in vivo as well as in endometrial epithelial cells exposed to *Bacillus pumilus* in vitro. *Reproduction, Fertility, and Development*. 2015;**28**:982-994. DOI: 10.1071/RD14219
- [20] Fischer C, Drillich M, Odau M, Heuwieser W, Einspanier R, Gabler C. Selected pro-inflammatory factor transcripts in bovine endometrial epithelial cells are regulated during the estrus cycle and elevated in case of subclinical or clinical endometritis. *Reproduction, Fertility, and Development*. 2010;**22**:818-829. DOI: 10.1071/RD09120
- [21] Gabler C, Fischer C, Drillich M, Einspanier R, Heuwieser W. Time-dependent mRNA expression of selected pro-inflammatory factors in the endometrium of primiparous cows postpartum. *Reproductive Biology and Endocrinology*. 2010;**8**:152. DOI: 10.1186/1477-7827-8-152
- [22] Galvão KN, Santos NR, Galvão JS, Gilbert RO. Association between endometritis and endometrial cytokine expression in postpartum Holstein cows. *Theriogenology*. 2011;**76**: 290-299. DOI: 10.1016/j.theriogenology.2011.02.006
- [23] Ghasemi F, Gonzalez-Cano P, Griebel PJ, Palmer C. Proinflammatory cytokine gene expression in endometrial cytobrush samples harvested from cows with and without subclinical endometritis. *Theriogenology*. 2012;**78**:1538-1547. DOI: 10.1016/j.theriogenology.2012.06.022
- [24] Kasimanickam R, Kasimanickam V, Kastelic JP. Mucin 1 and cytokines mRNA in endometrium of dairy cows with postpartum uterine disease or repeat breeding. *Theriogenology*. 2014;**81**:952-958. DOI: 10.1016/j.theriogenology.2014.01.018
- [25] Mattmiller SA, Carlson BA, Sordillo LM. Regulation of inflammation by selenium and selenoproteins: Impact on eicosanoid biosynthesis. *Journal of Nutritional Science*. 2013;**2**(e28):1-13. DOI: 10.1017/jns.2013.17

- [26] Herath S, Lilly ST, Santos NR, Gilbert RO, Goetze I, Bryant CE, et al. Expression of genes associated with immunity in the endometrium of cattle with disparate postpartum uterine disease and fertility. *Reproductive Biology and Endocrinology*. 2009;**7**:1-13. DOI: 10.1186/1477-7827-7-55
- [27] Brodzki P, Kostro K, Brodzki A, Wawron W, Marczuk J, Kurek L. Inflammatory cytokines and acute-phase proteins concentrations in the peripheral blood and uterus of cows that developed endometritis during early postpartum. *Theriogenology*. 2015;**84**:11-18. DOI: 10.1016/j.theriogenology.2015.02.006
- [28] Salehi R, Colazo MG, Gobikrushanth M, Basu U, Ambrose DJ. Effects of prepartum oilseed supplements on subclinical endometritis, pro- and anti-inflammatory cytokine transcripts in endometrial cells and postpartum ovarian function in dairy cows. *Reproduction, Fertility, and Development*. 2017;**29**(4):747-758. DOI: 10.1071/RD15334
- [29] Silvestre FT, Carvalho TS, Crawford PC, Santos JE, Staples CR, Jenkins T, et al. Effects of differential supplementation of fatty acids during the peripartum and breeding periods of Holstein cows: II. Neutrophil fatty acids and function, and acute-phase proteins. *Journal of Dairy Science*. 2011;**94**:2285-2301. DOI: 10.3168/jds.2010-3371
- [30] Bernabucci U, Ronchi B, Lacetera N, Nardone A. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. *Journal of Dairy Science*. 2005;**88**:2017-2026. DOI: 10.3168/jds.S0022-0302(05)72878-2
- [31] Goff JP. Major advances in our understanding of nutritional influences on bovine health. *Journal of Dairy Science*. 2006;**89**:1292-1301. DOI: 10.3168/jds.S0022-0302(06)72197-X
- [32] Douglas GN, Rehage J, Beaulieu AD, Bahaa AO, Drackley JK. Prepartum nutrition alters fatty acid composition in plasma, adipose tissue, and liver lipids of periparturient dairy cows. *Journal of Dairy Science*. 2007;**90**:2941-2959. DOI: 10.3168/jds.2006-225
- [33] Sordillo LM, Aitken SL. Impact of oxidative stress on the health and immune function of dairy cattle. *Veterinary Immunology and Immunopathology*. 2009;**128**:104-109. DOI: 10.1016/j.vetimm.2008.10.305
- [34] Miller JK, Brzezinska-Slebodzinska E, Madsen FC. Oxidative stress, antioxidants, and animal function. *Journal of Dairy Science*. 1993;**76**:2812-2823. DOI: 10.3168/jds.S0022-0302(93)77620-1
- [35] Sordillo LM, O'Boyle N, Gandy JC, Corl CM, Hamilton E. Shifts in thioredoxin reductase activity and oxidant status in mononuclear cells obtained from transition dairy cattle. *Journal of Dairy Science*. 2007;**90**:1186-1192. DOI: 10.3168/jds.S0022-0302(07)71605-3
- [36] Weaver JA, Maddox JF, Cao YZ, Mullarky IK, Sordillo LM. Increased 15-HPETE production decreases prostacyclin synthase activity during oxidant stress in aortic endothelial cells. *Free Radical Biology & Medicine*. 2001;**30**:299-308. DOI: 10.1016/S0891-5849(00)00466-4
- [37] Sordillo LM, Weaver JA, Cao Y-Z, Corl C, Sylte MJ, Mullarky IK. Enhanced 15-HPETE production during oxidant stress induces apoptosis of endothelial cells. *Prostaglandins & Other Lipid Mediators*. 2005;**76**:19-34. DOI: 10.1016/j.prostaglandins.2004.10.007

- [38] Sordillo LM, Streicher KL, Mullarky IK, Gandy JC, Trigona W, Corl CM. Selenium inhibits 15-hydroperoxyoctadecadienoic acid-induced intracellular adhesion molecule expression in aortic endothelial cells. *Free Radical Biology & Medicine*. 2008;**44**:34-43. DOI: 10.1016/j.freeradbiomed.2007.09.002
- [39] Drillich M, Tesfayec D, Ringsc F, Schellanderc K, Heuwiesera W, Hoelker M. Effects of polymorphonuclear neutrophil infiltration into the endometrial environment on embryonic development in superovulated cows. *Theriogenology*. 2012;**77**:570-578. DOI: 10.1016/j.theriogenology.2011.08.033
- [40] Fernandez-Sanchez FI, Barrio-Lopez M, Quintela-Arias LA, Becerra-Gonzalez JJ, Peña-Martinez AI, Martinez-Bello D, et al. Use of endometrial cytology and metabolic profiles for selection of embryo donor cows. *Spanish Journal of Agricultural Research*. 2014;**12**(3):664-671. DOI: 10.5424/sjar/2014123-4948
- [41] Gabler C, Drillich M, Fischer C, Holder C, Heuwieser W, Einspanier R. Endometrial expression of selected transcripts involved in prostaglandin synthesis in cows with endometritis. *Theriogenology*. 2009;**71**:993-1004. DOI: 10.1016/j.theriogenology.2008.11.009
- [42] Baranski W, Lukasik K, Skarzynski D, Sztachanska M, Zdunczyk S, Janowski T. Secretion of prostaglandins and leukotrienes by endometrial cells in cows with subclinical and clinical endometritis. *Theriogenology*. 2013;**80**:766-772. DOI: 10.1016/j.theriogenology.2013.07.001
- [43] Sakumoto R, Shibaya M, Okuda K. Tumor necrosis factor- α (TNF- α) inhibits progesterone and estradiol 17- β production from cultured granulosa cells: Presence of TNF- α receptors in bovine granulosa and theca cells. *The Journal of Reproduction and Development*. 2003;**49**(6):441-449. DOI: 10.1262/jrd.49.441
- [44] Sakumoto R, Okuda K. Possible actions of tumor necrosis factor- α in ovarian function. *The Journal of Reproduction and Development*. 2004;**50**(1):39-46. DOI: 10.1262/jrd.50.39
- [45] Field SL, Dasgupta T, Cummings M, Orsi NM. Cytokines in ovarian folliculogenesis, oocyte maturation and luteinisation. *Molecular Reproduction and Development*. 2013;**81**(4):284-314. DOI: 10.1002/mrd.22285
- [46] Barlund CS, Carruthers TD, Waldner CL, Palmer CW. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. *Theriogenology*. 2008;**69**:714-723. DOI: 10.1016/j.theriogenology.2007.12.005
- [47] Meira EBS Jr, Henriques LCS, Sá LRM, Gregory L. Comparison of ultrasonography and histopathology for the diagnosis of endometritis in Holstein-Friesian cows. *Journal of Dairy Science*. 2012;**95**:6969-6973. DOI: 10.3168/jds.2011-4950
- [48] Quintela LA, Becerra JJ, Herradón PG. *Essential Guides on Cattle Farming: Uterine Diseases*. Servet: Zaragoza; 2016. 69 p
- [49] López-Helguera I, López-Gatius F, García-Ispiero I. The influence of genital tract status in postpartum period on the subsequent reproductive performance in high producing dairy cows. *Theriogenology*. 2012;**77**(7):1334-1342. DOI: 10.1016/j.theriogenology.2011.10.038

- [50] Debertolis L, Gaetano M, Barbara M, Sabine M, Eleonora I, Heinrich B. Effects of an induced endometritis on uterine blood flow in cows evaluated by transrectal Doppler sonography. *Journal of Veterinary Science*. 2015;**17**(2):189-197. DOI: 10.4142/jvs.2016.17.2.189
- [51] Kasimanickam R, Duffield TF, Foster RA, Gartley CJ, Leslie KE, Walton JS, et al. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. *The Canadian Veterinary Journal*. 2005;**46**:255-259
- [52] Pascottini OB. Subclinical Endometritis in Dairy Cattle: A Practical Approach. [PhD Thesis]. Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University; 2016
- [53] Lopdell T, Berg MC, Green MP, Back PJ. Effect of sub-clinical uterine infection on plasma aminoacid concentrations in early lactation dairy cows. *Proc NZ Soc Anim Prod*. 2011;**71**:291-295
- [54] Heidarpour M, Mohri M, Fallah Rad AH, Shahreza FD, Mohammadi M. Acute-phase protein concentration and metabolic status affect the outcome of treatment in cows with clinical and subclinical endometritis. *The Veterinary Record*. 2012;**171**:1-5. DOI: 10.1136/vr.100947
- [55] Kaufmann T, Drillich M, Tenhagen B-A, Heuwieser W. Correlations between periparturient serum concentrations of non-esterified fatty acids, beta-hydroxybutyric acid, bilirubin, and urea and the occurrence of clinical and subclinical postpartum bovine endometritis. *BMC Veterinary Research*. 2010;**6**:47. DOI: 10.1186/1746-6148-6-47
- [56] Barrio M, Becerra JJ, Herradon PG, Sebastian F, Fernandez M, Quintela LA. Fertility in cows with endometritis diagnosed by cytology at day 30 postpartum. *Proc 12th Cong AERA*; 2014. Abstract
- [57] Madoz LV, Giuliadori MJ, Plontzke J, Drillich M, de la Sota RL. The relationship between endometrial cytology during estrus cycle and cutoff points for the diagnosis of subclinical endometritis in grazing dairy cows. *Journal of Dairy Science*. 2013;**96**(7):4333-4337. DOI: 10.3168/jds.2012-6269
- [58] Dubuc J, Duffield TF, Leslie KE, Walton JS, LeBlanc SJ. Definitions and diagnosis of postpartum endometritis in dairy cows. *Journal of Dairy Science*. 2010;**93**:5225-5234. DOI: 10.3168/jds.2010-3428
- [59] Plöntzke J, Madoz LV, De la Sota RL, Drillich M, Heuwieser W. Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in Argentina. *Animal Reproduction Science*. 2010;**122**:52-57. DOI: 10.1016/j.anireprosci.2010.07.006
- [60] Barrio M, Vigo M, Quintela LA, Becerra JJ, García-Herradón PJ, Martínez-Bello D, et al. Influence of subclinical endometritis on the reproductive performance of dairy cows. *Spanish Journal of Agricultural Research*. 2015;**13**(4):05-02. DOI: 10.5424/sjar/2015134-8129

- [61] Hammon DS, Evjen IM, Dhiman TR, Goff JP, Walters JL. Neutrophil function and energy status in Holstein cows with uterine health disorders. *Veterinary Immunology and Immunopathology*. 2006;**113**:21-29. DOI: 10.1016/j.vetimm.2006.03.022
- [62] Gilbert RO, Shin ST, Guard CL, Erb HN, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. *Theriogenology*. 2005;**64**:1879-1888. DOI: 10.1016/j.theriogenology.2005.04.022
- [63] Cheong SH, Nydam DV, Galvao KN, Croiser BM, Ricci A, Caixeta LS, et al. Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. *Journal of Dairy Science*. 2011;**94**:762-770. DOI: 10.3168/jds.2010-3439
- [64] McDougall S, Hussein H, Aberdein D, Buckle K, Roche J, Burke C, et al. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. *Theriogenology*. 2011;**76**:229-240. DOI: 10.1016/j.theriogenology.2010.12.024
- [65] Ohtani S, Okuda K, Nishimura K, Mohri S. Histological changes in bovine endometrium during the estrous cycle. *Theriogenology*. 1993;**39**:1033-1042. DOI: 10.1016/0093-691X(93)90004-O
- [66] Santos NR, Roman HB, Gilbert RO. The use of leukocyte esterase reagent strips for diagnosis of subclinical endometritis in dairy cows. *Theriogenology*. 2006;**66**:666-667. DOI: 10.1016/j.theriogenology.2006.04.027
- [67] Cheong SH, Nydam DV, Galvão KN, Crosier BM, Ricci A, Caixeta LS, et al. Use of reagent test strips for diagnosis of endometritis in dairy cows. *Theriogenology*. 2012;**77**:858-864. DOI: 10.1016/j.theriogenology.2011.09.009
- [68] Couto GB, Vaillancourt DH, Lefebvre RC. Comparison of a leukocyte esterase test with endometrial cytology for diagnosis of subclinical endometritis in postpartum dairy cows. *Theriogenology*. 2013;**79**:103-107. DOI: 10.1016/j.theriogenology.2012.09.014
- [69] Strowitzki T, Germeyer A, Popovici R, von Wolff M. The human endometrium as a fertility-determining factor. *Human Reproduction Update*. 2006;**12**:617-630. DOI: 10.1093/humupd/dml033
- [70] Brandt GW, Manning JP. Improved uterine biopsy technics for diagnosing infertility in the mare. *Veterinary Medicine, Small Animal Clinician*. 1969;**64**:977-983
- [71] Nielsen JM. Endometritis in the mare: A diagnostic study comparing cultures from swab and biopsy. *Theriogenology*. 2005;**64**:510-518
- [72] Zaayer D, van der Horst CJ. Non-fertility in cows: Treatment with PGF and investigation of uterine biopsies. *Cytobios*. 1986;**45**:55-70
- [73] De Boer NW, LeBlanc SJ, Dubuc J, Meier S, Heuwieser W, Arlt S, et al. Systematic review of diagnostic tests for reproductive-tract infection and inflammation in dairy cows. *Journal of Dairy Science*. 2014;**97**:3983-3999. DOI: 10.3168/jds.2013-7450

- [74] Prunner I, Wagener K, Pothmann H, Ehling-Schulz M, Drillich M. Risk factors for uterine diseases on small- and medium-sized dairy farms determined by clinical, bacteriological, and cytological examinations. *Theriogenology*. 2014;**82**:857-865. DOI: 10.1016/j.theriogenology.2014.06.015
- [75] Gobikrushanth M, Salehi R, Ambrose DJ, Colazo MG. Categorization of endometritis and its association with ovarian follicular growth and ovulation, reproductive performance, dry matter intake, and milk yield in dairy cattle. *Theriogenology*. 2016;**86**:1842-1849. DOI: 10.1016/j.theriogenology.2016.06.003
- [76] Burke CR, Meier S, McDougall S, Compton C, Mitchell M, Roche JR. Relationships between endometritis and metabolic state during the transition period in pasture-grazed dairy cows. *Journal of Dairy Science*. 2010;**93**:5363-5373. DOI: 10.3168/jds.2010-3356
- [77] Green MP, Ledgard AM, Beaumont SE, Berg MC, McNatty KP, Peterson AJ, et al. Long-term alteration of follicular steroid concentrations in relation to subclinical endometritis in postpartum dairy cows. *Journal of Animal Science*. 2011;**89**:3551-3560. DOI: 10.2527/jas.2011-3958
- [78] Salasel B, Mokhtari A, Taktaz T. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. *Theriogenology*. 2010;**74**:1271-1278. DOI: 10.1016/j.theriogenology.2010.05.033
- [79] Janowski T, Barański W, Łukasik K, Skarżyński D, Rudowska M, Zduńczyk S. Prevalence of subclinical endometritis in repeat breeding cows and mRNA expression of tumor necrosis factor α and inducible nitric oxide synthase in the endometrium of repeat breeding cows with and without subclinical endometritis. *Polish Journal of Veterinary Sciences*. 2013;**16**(4):693-699
- [80] Pothmann H, Prunner I, Wagener K, Jaureguiberry M, de la Sota RL, Erber R, et al. The prevalence of subclinical endometritis and intrauterine infections in repeat breeder cows. *Theriogenology*. 2015;**83**:1249-1253. DOI: 10.1016/j.theriogenology.2015.01.013
- [81] Bell MJ, Roberts DJ. The impact of uterine infection on a dairy cow's performance. *Theriogenology*. 2007;**68**(7):1074-1079. DOI: 10.1016/j.theriogenology.2007.08.010
- [82] Green MP, Ledgard AM, Berg MC, Peterson AJ, Back PJ. Prevalence and identification of systemic markers of sub-clinical endometritis in postpartum dairy cows. *Proceedings of the New Zealand Society of Animal Production*. 2009;**69**:37-42
- [83] Dubuc J, Duffield TF, Leslie KE, Walton JS, Leblanc SJ. Effects of postpartum uterine diseases on milk production and culling in dairy cows. *Journal of Dairy Science*. 2011;**94**:1339-1346. DOI: 10.3168/jds.2010-3758
- [84] Haimerl P, Arlt S, Heuwieser W. Evidence-based medicine: Quality and comparability of clinical trials investigating the efficacy of prostaglandin $F_{2\alpha}$ for the treatment of bovine endometritis. *The Journal of Dairy Research*. 2012;**79**:287-296. DOI: 10.1017/S002202991200012X
- [85] Lefebvre RC, Stock AE. Therapeutic efficiency of antibiotics and prostaglandin $F_{2\alpha}$ in postpartum dairy cows with clinical endometritis: An evidence-based evaluation.

- Veterinary Clinics of North America. Food Animal Practice. 2012;**28**:79-96. DOI: 10.1016/j.cvfa.2012.01.002
- [86] Galvão KN, Greci LF, Vilela JM, Sá Filho MF, Santos JEP. Effect of intrauterine infusion of ceftiofur on uterine health and fertility in dairy cows. *Journal of Dairy Science*. 2009;**92**:1532-1542. DOI: 10.3168/jds.2008-1615
- [87] Denis-Robichaud J, Dubuc J. Randomized clinical trial of intrauterine cephalixin infusion in dairy cows for the treatment of purulent vaginal discharge and cytological endometritis. *Journal of Dairy Science*. 2015;**98**:6856-6864. DOI: 10.3168/jds.2014-9129
- [88] Galvão KN, Frajblat M, Brittin SB, Butler ER, Guard CL, Gilbert RO. Effect of prostaglandin $F_{2\alpha}$ on subclinical endometritis and fertility in dairy cows. *Journal of Dairy Science*. 2009;**92**:4906-4913. DOI: 10.3168/jds.2008-1984
- [89] Lima FS, Bisinotto RS, Ribeiro ES, Greco LF, Ayres H, Favoreto MG, et al. Effects of 1 or 2 treatments with prostaglandin $F_{2\alpha}$ on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. *Journal of Dairy Science*. 2013;**96**:6480-6488. DOI: 10.3168/jds.2013-6850
- [90] Meier S, Priest NV, Burke CR, Kay JK, McDougall S, Mitchell MD, et al. Treatment with a nonsteroidal anti-inflammatory drug after calving did not improve milk production, health or reproduction parameters in pasture-grazed dairy cows. *Journal of Dairy Science*. 2014;**97**(5):2932-2943. DOI: 10.3168/jds.2013-7838
- [91] Brinsko SP, Blanchard TL, Varner DD, Schumacher J, Love CC, Hinrichs K, Hartman DL. Endometritis. In: Brinsko SP, Blanchard TL, Varner DD, Schumacher J, Love CC, Hinrichs K, Hartman DL, editors. *Manual of Equine Reproduction*. 3rd ed. St Louis: Mosby; 2011. pp. 73-84
- [92] Ahmadi MR, Dehghan SA. Evaluation of the treatment of repeat breeder dairy cows with uterine lavage plus PGF $_{2\alpha}$ with or without cephalixin. *Turkish Journal of Veterinary and Animal Sciences*. 2007;**31**:125-129
- [93] Dini P, Farhoodi M, Hostens M, Van Eetvelde M, Pascottini OB, Fazeli MH, et al. Effect of uterine lavage on neutrophil counts in postpartum dairy cows. *Animal Reproduction Science*. 2015;**158**:25-30. DOI: 10.1016/j.anireprosci.2015.04.005
- [94] Brick TA, Schuenemann GM, Bas S, Daniels JB, Pinto CR, Rings DM, et al. Effect of intrauterine dextrose or antibiotic therapy on reproductive performance of lactating dairy cows diagnosed with clinical endometritis. *Journal of Dairy Science*. 2012;**95**:1894-1905. DOI: 10.3168/jds.2011-4892
- [95] Djuricic D, Vince S, Ablondi M, Dobranic T, Samardzija M. Effect of preventive intrauterine ozone application on reproductive efficiency in Holstein cows. *Reproduction in Domestic Animals*. 2012;**47**:87-91. DOI: 10.1111/j.1439-0531.2011.01805.x
- [96] Tras B, Dinc DA, Uney K. The effect of N-acetylcysteine on the treatment of clinical endometritis and pregnancy rate in dairy cows. *Eurasian Journal of Veterinary Sciences*. 2014;**30**(3):133-137

