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REVIEW

Uterine Infections in Cows and Effect on Reproductive Performance

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Abstract

Uterine infections affect the hypothalamus, hypophysis, ovaries and uterus. These infections inhibit the development of dominant follicles, prevent both the release of the luteinizing hormone (LH) and ovulation, reduce the size of the corpus luteum (CL), and decrease progesterone production. As these infections reduce fertility or cause infertility, they result in major economic losses. The diagnosis of uterine infections by means of various techniques and the detection of the severity of the infection enable the selection of the most appropriate treatment method and the estimation of the success rate that may be achieved with the application of the particular treatment method selected. This review aims to provide researchers and veterinary practitioners with practical information on the prevalence of postpartum uterine infections and currently applied diagnostic and treatment methods.

Keywords: *Cow, Uterine diseases , Reproductive performance*

İneklerde Uterus Enfeksiyonları ve Üreme Performansı Üzerine Etkisi

Özet

Uterus enfeksiyonları hipotalamus, hipofiz, ovaryum ve uterus üzerine etki eder. Bu enfeksiyonlar; dominant follikül gelişimini baskılar, luteinize edici hormon (LH) salınımını ve ovulasyonu engeller, korpus luteumun (CL) daha küçük ve progesteron üretiminin daha az olmasına neden olur. Fertilitenin düşmesine veya infertiliteye neden olarak ciddi ekonomik kayıplar oluşturur. Uterus enfeksiyonlarının çeşitli yöntemlerle teşhisi ve enfeksiyon şiddetinin belirlenmesi, uygun tedavi yönteminin belirlenmesine ve tedavi yöntemi başarısının tahminine olanak sağlar. Sunulan derleme ile postpartum uterus hastalıklarının yaygınlığı, güncel teşhis ve tedavi yöntemleri hakkında araştırmacılara ve veteriner hekimlere pratik bilgiler sunulmaya çalışıldı.

Anahtar sözcükler: *İnek, Uterus enfeksiyonları, Üreme performansı*

INTRODUCTION

The parturition period is characterized by high risk. During this period, the risk of the development of microbial uterine infections increases, due to several reasons including the possible physical damage of the birth canal, and retained placenta. The occurrence of a negative energy balance in this period further increases this risk. Uterine infections have a negative impact on animal welfare and reproductive performance, and lead to major economic losses [1]. This review aims to provide detailed literature information on the incidence, pathogenesis, classification and diagnosis of uterine infections, their effects on fertility, economic impact and available treatment options.

THE DEFENCE SYSTEM OF THE UTERUS

Prior to parturition, pathogenic microorganisms are not found in the uterus. The elimination of the mechanical protective barriers of the uterus with parturition enables the access of microorganisms to the organ [1]. For the bacterial contamination of the uterus to occur, pathogens must firstly pass the anatomical barriers of the uterus (the sphincter of the vulva and the cervix uteri). The microorganisms, which manage to pass the first barrier, are later tried to be eliminated by the regional and systemic

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defence mechanisms of the uterus. In uterine infections, the inflammatory cells, which first infiltrate to the site of infection, are the neutrophil leukocytes [2]. Apart from neutrophil leukocytes, lymphocytes also play a major role in uterine immunology. Pathogens, which are opsonized by the humoral defence system, are phagocytized by neutrophil leukocytes. The endometrium constitutes the first line of defence against bacteria, owing to the Toll-like receptors (TLRs) it bears and the antimicrobial proteins it secretes. The TLRs are capable of recognizing the molecules of bacterial pathogens (lipopolysaccharides, lipoteichoic acid and the deoxyribonucleic acid of the bacteria), and upon recognition, secrete prostaglandin E_2 (PGE_2) and antimicrobial proteins from uterine epithelial cells [3]. These proteins are secreted locally by the tumor necrosis factor, interleukin-6 and interleukin-8, following the recognition of the pathogens by the TLRs [4], and lead to the secretion of acute-phase proteins from the liver. Acute-phase proteins are molecules, which are effective in the elimination of uterine infections [5].

THE INCIDENCE OF METRITIS AND ENDOMETRITIS

The incidence of clinical metritis in cows and heifers has been reported as 18.6% and 30%, respectively [5]. Sheldon et al.[6] have determined the incidence of clinical metritis during the first two weeks of the postpartum period as 25-40%. It has been indicated that the incidence of clinical metritis at herd level ranges between 5% and 26%, and is on average 17% [7-9]. Furthermore, the incidence of subclinical endometritis has been reported to range from 19% to 74%, and to be 53% on average [10,11]. The large differences observed between the incidences determined in previous studies have been attributed to the diagnostic methods employed, the method used for the classification of uterine infections, the postpartum period in which the uterine infection was diagnosed, the general traits of the bovine animals included in previous research, their parity, and the management of the herds included in previous studies [12].

PATHOGENESIS OF UTERINE INFECTIONS

Following parturition, in 80 to 100% of animals, the uterus becomes contaminated with bacteria [13]. Uterine infections develop upon the establishment of pathogenic microorganisms onto the mucosa, their colonisation and penetration of the epithelial layer, and the production of toxins by these microorganisms [14]. The development of uterine infections depends on the immune status of the animal, the species and number of pathogenic microorganisms contaminating the uterus, and several other environmental and individual factors [1,2]. Different opinions exist on the impact of nutrition on the development of uterine infections. The increase in insulin resistance, decrease in feed consumption, ocurrence of a negative energy balance, and loss of body weight in animals in advanced gestation lead to the weakening of the defence system [15]. It has been ascertained that the rate of uterine bacterial contamination is above 90% during the first few days after calving [12], and decreases to 78% on day 30 postpartum, to 50% on day 45 postpartum, and to 9% by day 60 postpartum [16,17].

The most common bacterium encountered in uterine infections during the first few days of the postpartum period is *Escherichia coli*. After the first week postpartum, *Fusobacterium necrophorum* and *Trueperella pyogenes* also become involved in uterine infections [18,19]. Certain bacteria, including *Campylobacter fetus* and *Trichomonas fetus*, are specific to cases of endometritis [20]. Nevertheless, bacteria of the genera *Clostridia, Pepticoccus* and *Peptostreptococcus,* as well as several unidentified *Gram positive* and *Gram negative anaerobic bacteria* and aerobic and facultative anaerobic pathogens such as *alpha-haemolytic streptococci, Arcanobacterium pyogenes* and *Escherichia coli* may also cause endometritis [2,16,17,20]. Uterine infections are most commonly observed following twin births, dystocia, surgical interventions employed to aid in cases of dystocia, retained placenta, stillbirth, prolapsus uteri, artificial insemination, copulation, and the use of intrauterine irritants [1,2,14].

In the postpartum period, the puerperal discharge (remains of the fetal membranes, endometrial sloughing, fetal fluids, etc.) constitutes an ideal environment for microbial growth. The elimination of the puerperal discharge from the uterus reduces the bacterial load of the organ [21]. Furthermore, during the postpartum period, the oestrogen level increases in cattle. Oestrogen increases the blood supply and enables the infiltration of white blood cells, including neutrophil leukocytes, to the uterus. This hormone also induces the secretion of vaginal mucus. This mucus enables the reduction and discharge of the bacterial load of the uterus [2]. Oestrogen also increases the number of oxytocin receptors in the uterus and the susceptibility of the myometrium to contractions. Muscle contractions both accelerate uterine involution and decrease the bacterial load of the uterus by enabling the elimination of bacteria in the puerperal discharge [22]. Previous studies have suggested that ovulation in the early postpartum period has no effect on the incidence of subclinical metritis [23].

CLASSIFICATION AND DIAGNOSIS OF UTERUS INFECTIONS

Metritis is defined as the inflammation of the superficial and deep layers of the uterus. Depending on the severity

of metritis, degeneration and leukocyte infiltration may be observed in some or all layers of the uterus [14]. Acute puerperal metritis occurs during the first two weeks after parturition (mostly during days 4-10 postpartum). Animals suffering from acute puerperal metritis, in general, present with toxaemia, septicaemia and pyemia. Cases of acute puerperal metritis are characterized by an enlarged uterus with a a tapered wall, reddish brown coloured and malodorous vaginal discharge, and systemic signs including fever (40-41°C), increased heart and respiratory rates, anorexia, diarrhoea and shock [2,13]. Clinical metritis is defined as a uterine infection, which develops until day 21 postpartum with no systemic disorder, but displays purulent or mucopurulent discharge [24]. Clinical endometritis develops as from the third week postpartum. In such cases, the uterus is filled with a purulent or mucopurulent content, and if the cervix uteri is open, a vaginal discharge of a character the same with that of the uterine content may be observed [8,25]. Subclinical endometritis is the inflammation of the uterus, which does not present with any of the signs observed in clinical endometritis, but causes reproductive disorders [26]. The excessive infiltration of neutrophil leukocytes is observed in subclinical cases [14]. Subclinical endometritis is reported to develop between days 35-60 postpartum in 35 to 50% of animals, and to prolong the parturition-conception interval and to reduce fertility [10,26]. Pyometra is characterized by the accumulation of a purulent exudate in the uterus and the persistence of the corpus luteum (CL) [2,27]. If the ovulation occurs before the elimination of the microorganisms in the uterus, then the CL is maintained [16,20]. During the period in which the uterus is under the influence of progesterone, generally the cervix uteri is tightly closed and the purulent exudate begins to accumulate in the cornu uteri. In some cases, a small amount of purulent vaginal discharge may be observed [2]. Pyometra is very rarely associated with systemic findings [20]. In general, pyometra progresses into chronic endometritis [2].

Puerperal metritis presents with both local and systemic clinical findings. Fever is common and both the heart rate and respiratory rate are altered. A malodorous reddish coloured uterine discharge is observed [2]. Animals with clinical metritis do not present with any impairment of the general condition. An enlarged and incompletely involuted uterus [14] and a purulent or mucopurulent vulvar discharge are observed [25]. The method most commonly used in the diagnosis of clinical endometritis is vaginal examination. This method is based on an evaluation of the colour and purulency of the discharge [5,14]. Clean vaginal discharge containing transparent mucus is scored as 0, while discharge with a greater amount of transparent mucus but a low level of pus is scored as 1. Discharges containing white or yellow pus at a level lower than 50% are scored as 2, while discharges containing yellow and white pus at a level greater than 50%, and which may sometimes also contain blood, are scored as 3 [9,14]. The scoring of the vaginal discharge according to its odour is as follows: odourless=0, malodorous or putrified=1 [5].

The ultrasonographic measurement of the diameter of the cornu uteri and cervix uteri, the quantity of fluid in the lumen of the uterus, and the thickness of the endometrium provide insight on the presence of endometritis and its severity [26,28]. Animals with a cervix uteri diameter greater than 7.5 cm on postpartum day 20 are considered to suffer from clinical endometritis [9]. Bacterial isolation can be performed with endometrial swabs and endometrial biopsy samples taken during the postpartum period [25,29]. If no colony growth is observed the score is considered as 0, while the growth of less than 10 colonies is scored as 1. The growth of 10 to 100 colonies is scored as 2, the growth of 101 to 500 colonies as 3, and the score of the growth of more than 500 colonies is 4 [5]. Another method used for the diagnosis of endometritis is biopsy sampling [30]. Endometrial cytology (the lavage and cytobrushing of the uterus) has a very important place in the diagnosis of endometritis [14,31]. Subclinical cases of endometritis are best diagnosed based on the evaluation of neutrophil leukocyte infiltration by cytological examination [14,25,28]. The percentage of neutrophil leukocytes and the neutrophil leukocyte/lymphocyte ratio increase in postpartum uterine infections [32]. Cows determined to have a neutrophil leukocyte percentage >18% between postpartum days 20-33 [26], >10% between postpartum days 34-47 [14], and >5% between postpartum days 40-60 are considered to have developed subclinical endometritis [10]. In recent years, it has become possible to diagnose uterine infections by the polymerase chain reaction (PCR) technique [33], and based on the levels of acute-phase proteins (creatine kinase, haptoglobulin, α_1-acid glycoprotein, non-esterified fatty acid (NEFA), β-hydroxy butyric acid (BHBA) and nitric oxide levels) [34-37]. The reduction of dry matter intake by pregnant animals during advanced gestation results in the mobilisation of NEFA from fat tissue. The body levels of NEFA and BHBA increase [38]. Decreased levels of dry matter intake and increased NEFA levels inhibit the immune system and cows become prone to infection [38,39]. Hammon et al.[39] determined that serum NEFA and BHBA levels increased significantly in animals with subclinical endometritis and puerperal metritis, when compared to healthy animals. Although BHBA levels have been reported not to be correlated with the bactericidal capacity of polymorphonuclear leukocytes [39], literature reports are available, which suggest ketone bodies to induce adverse effects on the migration of polymorphonuclear leukocytes (PMN) and to damage the structure of PMN prior to their migration [40]. It has been indicated that the risk of the development of metritis is three-fold higher in animals with high BHBA levels during the first two weeks after parturition [41]. Walsh et al.[42] indicated that animals with increased BHBA levels presented with reduced conception rates after the first postpartum insemination, and suggested that increased BHBA levels led to a lower

number of conceived cows by postpartum day 140, and to a prolongation of the parturition-conception interval. In the past few years, the reagent test strips have also found use in the diagnosis of subclinical endometritis [37]. These test strips provide information on the hydrogen ion (pH level), leukocyte esterase, and protein levels of the uterine lavage fluid [43]. The level of leukocyte esterase is an indicator of the presence of white blood cells in the lavage fluid. The principle of measurement is based on the formation of a purple colour as a result of the reaction of indoxyl carbonic acid with neutrophil esterase. The intensity of the colour varies with the number of leukocytes involved in the reaction [44]. Animals with endometritis have been determined to present with increased pH values and increased leukocyte esterase and protein levels. It has been ascertained that the correlation of endometritis with the pH value is stronger than that with the leukocyte esterase level or protein level [43]. The diagnosis of pyometra is based on the ultrasonographic detection of a CL on the ovarian surface, fluid accumulation of heterogenic ecogenicity in the uterus lumen and uterine enlargement. Furthermore, although the cervix uteri is physiologically closed, in some cases the accumulation of pus may be observed in the vagina [14].

EFFECT OF UTERINE INFECTIONS ON OVARIAN FUNCTIONS AND FERTILITY

Uterine infections may cause the formation of chronic endometrial scars, the narrowing of the oviduct, and the adherence of the bursa to the ovaries (at an approximate rate of 2%). Although uterine infections have been suggested not to have any impact on the formation of a new follicular wave or on the peripheral level of the follicle stimulating hormone (FSH) [6], they have been detected to have effect on the hypothalamus and the hypophysis [45]. Once absorbed from the uterus lumen, endotoxins released from the wall of Gram negative bacteria pass into the peripheral blood circulation, and prevent the secretion of the gonadotropin releasing hormone (GnRH) from the hypothalamus and the luteal hormone (LH) from the hypophysis. These toxins also reduce the susceptibility of the hypophysis to the secretion of endocrine and exocrine GnRH [1,46,47]. Lipopolysaccharides secreted by Gram negative bacteria, inhibit the transcription of steroidogenic enzymes, including 17β-hydroxylase/17,20-lyase and P450 aromatase, and thereby, inhibit follicular activity [48]. In this context, it has been determined that, in animals in which the uterus is exposed to a high level of bacterial contamination following parturition, the growth of the first dominant follicle slows down, the oestrogen level decreases, when compared to healthy animals a smaller CL develops following ovulation, the production of the progesterone hormone decreases and the development of ovarian cysts increases [49,50]. On the contrary, Strüve et al.[51] suggested that the effect of metritis on luteal activity was temporary and limited to the first oestrus cycle after parturition, and indicated that no alteration occurred either in the size of the CL or in the progesterone levels in the following postpartum oestrus cycles.

Another disorder caused by uterine infections in endocrine functions is the impairment of the luteolytic mechanism. It has been determined that, in uterine infections, due to the damage of the endometrium, inadequacy of the secretion of PGF_2 alpha is observed [17,20], while the secretion of luteotropic PGE_2 from the uterine epithelial cells and stromal cells is induced as a result of the direct effect of bacterial toxins on these cells. It has been detected that PGE_2 leads to the persistence of the CL, and thus to the continued production of progesterone from the CL. In this event, the onset of oestrus is prolonged and the uterine immune mechanism, known to be susceptible to oestrogenic effect, proves to be insufficient [36,39].

Since genital infections cause the disorder of ovarian and uterine functions, the parturition-first insemination and parturition -conception intervals are prolonged [49,52]. Sandals et al.[53] indicated that clinical endometritis prolonged the parturition-conception interval for a period of 9 days. In another study, it was determined that the parturition-conception interval was 14 days longer in animals diagnosed with endometritis, in comparison to healthy animals [7]. Barlund et al.[28] suggested that the parturition-conception interval was 24 days longer in animals suffering from endometritis, when compared to healthy animals. Similarly, Goshen et al.[54] determined this interval to be 25 days longer in animals with endometritis. In a research conducted by Kasimanickam et al.[26], it was ascertained that the rate of pregnancy of animals with endometritis (41%) was lower than that of healthy animals (51%). Similarly, Goshen et al.[54] determined that the preganancy rate of animals with clinical metritis was 20% lower than that of healthy animals. Furthermore, LeBlanc et al.[9] detected a reduction of 27% in the total pregnancy rate due to chronic endometritis.

TREATMENT OPTIONS FOR UTERINE INFECTIONS

Postpartum uterine infections are treated with antibiotics, hormones or their combinations [55]. The use of these therapeutic agents is aimed at the elimination of pathogens from the uterus, the induction of the uterine immune system, and the elimination of the adverse effects of inflammation products on fertility [8,12].

Antibiotics and PGF_2 alpha analogues are used for the parenteral treatment of uterine infections [8]. The most commonly used antibiotic is ceftiofur hydrochloride, which is a member of the family of third-generation broad

spectrum cephalosporins [56]. In severe cases of uterine infection, the antibiotic treatment is combined with intravenous fluid treatment and the administration of anti-inflammatory agents [55].

Prostaglandin F_2 alpha has been shown to be effective in the treatment of uterine infections [57]. The administration of exogenous PGF_2 alpha both induces the secretion of endogenous PGF_2 alpha from the uterus and enables the development of the immune functions [27]. It has been reported that the use of PGF_2 alpha and its analogues during dioestrus resulted in approximately 90% of the cows showing oestrus [58]. Each oestrus enables the self-cleansing of the uterus by means of physiological leukocytosis and increased uterine motility [59]. Oestrogen increases uterine contractions, mucus production and leukocyte infiltration to the uterus [2,22]. Another systemic method used for the treatment of uterine infections is homeopathy. Homeopathic substances enable the organism to stimulate itself and trigger the onset of the treatment process [60]. The administration of *Tarantula cubensis* extract, a homeopathic substance, to animals in the early postpartum period, has been demonstrated to both decrease the incidence of retentio secundinarum and accelerate uterine involution [61]. Similarly, the last period of pregnancy levamisole treatment (2.5 mg/kg) has been reported to accelerate uterine involution [62]. Some reports indicate that trace elements have effect on the immune functions [63,64]. On the other hand, Machado et al.[65] reported that some trace elements (zinc, copper, manganese and selenium) did not alter leukocyte activity.

In cases of chronic endometritis and subclinical endometritis, intrauterine treatment is preferred. Intra-uterine treatment methods aim to maintain a high concentration of therapeutics in the endometrium. Thereby, it is ensured that the therapeutics penetrate at a limited level to the deeper layers of the uterus or to the other genitalia [8]. Mostly, the antibiotics of choice for intrauterine treatment are chlortetracycline [54] and ceftiofur hydrochloride [66]. Furthermore, peroxyacetic acid [67,68], meta-cresol sulfonic acid (Lotagen®) [69], ozone [70], mixtures prepared from healing herbs (AV/RMI/45) [71] and *Pelargonium sidoides* extract (EPs 7630) [72], proteolytic enzymes including chemotrypsin, trypsin and papain [73], liquid paraffin [74], hyperimmune sera produced against *Arcanobacter pyogenes* and *Escherichia coli* [75], formo-sulphathiazole, the phytotherapeutic EucaComp® [76] and hypertonic dextrose solution (50%) are also used for intrauterine treatment [77].

It has been demonstrated that treatment (peroxyacetic acid, Lotagen® and PGF_2 alpha) do not alter the levels of C-reactive protein, plasma lipopolysaccharides, and serum creatinase aspartate aminotransferase, which are all important criteria used in the diagnosis of endometritis [67,78,79].

Apart from intrauterine treatment methods, in recent years, intravaginal bacteriophages have also found use against *Esherichia coli*, which is a major cause of uterine infections, with an aim to prevent the development of infections in the postpartum period. Although having been demonstrated to show beneficial effects when used *in vitro* [80], the use of intravaginal bacteriophages has been proven to be ineffective *in vivo* [81].

ECONOMIC IMPLICATIONS OF UTERINE INFECTIONS

Uterine infections not only reduce fertility and cause infertility, but also adversely affect animal welfare [45]. Uterine infections cause major economic losses as a result of several reasons, including among others, reduced reproductive performance, decreased milk yield, the culling of infected animals from the herd, and the prolongation of the parturition-first insemination and parturition-conception intervals [49,52,82]. Taking into consideration all possible reproductive disorders, Overton and Fetrow [83] calculated the loss arising from the culling of animals from the herd up to day 60 postpartum, due to metritis, as 85 USD per case, the loss for reduced milk yield as 83 USD, and the total loss as 109 USD. It is estimated that the annual cost of metritis ranges between 329 USD and 386 USD per case. The economic implications of endometritis have not been investigated in detail in Turkey before. In a previous study conducted in the United Kingdom between the years 1998 and 1999, the daily cost of the prolongation of the parturition-conception interval, due to endometritis, to the holding was reported as 3 Euros [84]. In another research conducted in the United Kingdom, the direct and indirect costs of uterine infections at country level were reported to exceed 16 million Euros per year [85].

RECOMMENDATIONS

The peripartum period is of critical importance for uterine health and fertility. The increased growth rate of the foetus in the advanced stages of gestation and the start of milk production after parturition increase the energy requirements of the animal. This requires the feeding of animals with good quality roughage and high-energy feed. The ideal body condition score for bovine animals in this period is indicated as 3.00-3.75. A negative energy balance triggered by poor nutrition after parturition affects the restart of the oestrus cycle, the normalisation of the sexual cycle, and the success achieved with artificial insemination. The cleanliness of the calving paddocks, and compliance with disinfection and hygiene rules during interventions to aid birth and dystocia, have an important role in reducing the risk of uterine infections. Regular controls during the postpartum period enable the monitoring of the uterine involution process, the differentiation of

physiological and pathological changes and the treatment of infected animals without delay. Furthermore, the access of the veterinary practitioner to the records kept on the farm and to information on the previous parturition of the cow, the diseases the animal has undergone, whether the animal has given brith to twins, the oestrus intervals, findings of the last gestational examination and previous inseminations of the animal, as well as the oestrus and ovulation synchronization protocols and treatments the animal has been subjected to, all shed light on the reproductive problems of the animal and aid in diagnosis and treatment.

CONCLUSION

This review provides an assessment of the current approaches to uterine infections. Light uterine inflammation is generally eliminated by means of the immune system of the organism. Uterine infections not able to be eliminated by the defence system of the body affect the reproductive performance. Although the economic implications of uterine infections are not able to be precisely measured, these infections are well known to have negative effects of varying degree on both the reproductive performance and yields. With the acquisition of increased information on the correlation between uterine infections, the immune system and reproductive performance, it has become ever more important to develop protection and treatment strategies for infertility caused by uterine infections.

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