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Infections of the Uterus on Postpartum Cows: A Review

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Abstract: *Postpartum* uterine infections result from uterine contamination with bacteria during parturition. Uterine infection implies adherence of pathogenic organisms to the mucosa, colonization or penetration of the epithelium and/or release of bacterial toxins that lead to establishment of uterine disease. The development of uterine disease depends on the immune response of the cow as well as the species and number (load or challenge) of bacteria. A variety of species of bacteria, both Gram-positive and Gram-negative aerobes and anaerobes, can be isolated from the early postpartum uterus. Most of these are environmental contaminants that are gradually eliminated during the first 6 weeks of postpartum. The diagnostic criteria for subclinical endometritis should identify cows at risk of pregnancy failure within an appropriate time. A variety of methods such as uterine palpation, ultrasonographic features of the uterus, vaginoscopy, endometrial cytology, uterine culture and uterine biopsy has been reported to identify endometritis in postpartum cows. Prostaglandin F2α and its analogues have been used for the treatment of pyometra, as this hormone induces luteolysis in cows with luteal tissue and also increases uterine contractility. Evacuation of the uterus contributes to the success of further antibiotic therapy. Evacuation can be done by repeated palpations of the uterus by a veterinarian and/or the use of drugs to expel the fluid or hasten the onset of estrus. Estrus is usually the best way of stimulating uterine contractions and expelling the fluids. Once fluids are expelled, the effectiveness of antibiotics in clearing the remaining infection is improved.

Key words: Uterine infections · *Postpartum* period · Cows · Diagnosis · Treatment

INTRODUCTION

The uterus and in particular the endometrium lining the uterus have important roles in normal reproductive cycles, implantation and placentation and supporting a healthy foetus until parturition. However, microbial infections of the uterus are common in man and animals and uterine infections are important because they cause infertility, abortion, pre-term labor and clinical disease [1].

Parturition is a period of high risk for mother and off-spring in all species and cattle are not exceptional. The risks of physical damage during the birth process or failure to release the placenta after parturition and there is often an upsurge of microbial infections in the cow. Some animals acquire infections of the uterus or mammary gland during late gestation, which may lead to premature parturition, or compromise fetal or calf health. However, the greatest impact on health and productivity is associated with microbial contamination of the uterine lumen after parturition. Indeed 80-100% of animals have bacteria in their uterine lumen within the first 2 weeks after calving. Although immune responses progressively eliminate the microbes, up to 40% of animals still have a bacterial infection 3 weeks after calving [2]. Parturition is a significant cause of trauma to the endometrium leading to the loss of surface epithelium, which exposes the underlying stromal cells to bacteria and additional trauma, such as difficult parturition or retained placenta, thereby increasing the risk of uterine infection [3].

The normal uterus is a sterile environment compared to vagina which hosts many microorganisms. Opportunistic pathogens from the normal vaginal flora may invade the uterus at different times especially, at mating and parturition [4]. During the Postpartum period reduction in the size of uterus to its normal non-gravid size is extremely important in order to decrease the susceptibility to the multiplication of infectious agents; this reduction is mostly managed by PGF2α and oestrogen [5]. If the uterus is not return to normal non-gravid size at proposed period of time and the cleanness of the calving area is not satisfactory there is the like hood development of uterine infections [6].

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Uterine diseases can be classified as puerperal metritis, clinical metritis, clinical endometritis and subclinical endometritis. These diseases are highly prevalent in high producing dairy cows and have been associated with decreased pregnancy per AI, extended interval to pregnancy, increased culling and economic losses. Endometritis and metritis are both inflammations of the uterus. Endometritis only involves the endometrium and the under laying glandular tissues. Metritis involves the endometrium (lining of the uterus), the under laying glandular tissues and the muscular layers [7].

Uterine Luminal Environment: The uterine environment has to offer optimal conditions first for the fertilizing spermatozoa and later for the development of the embryo. Uterine fluid consists of hormones, prostaglandins, enzymes, energy substrates, ions, vitamins, amino acids, peptides, serum proteins and uterine proteins [8]. The blood uterine lumen barrier determines the rate at which matter is exchanged between the vascular and extravascular fluids of the uterus. Extracellular uterine fluid can be divided into the following compartments: vascular, endometrial extracellular and luminal extracellular [9].

Introduction of Micro-Organisms into the Uterus: The genitalia of domestic animals have a microflora that consists of harmless species and opportunistic organisms that can be pathogenic in susceptible animals. Bacterial numbers decrease from the vagina to the uterus and the few organisms found in the cervix and uterus are probably transient [10]. Micro-organisms enter the uterus by ascending from the vagina. Breeding and giving birth are the most important ways of introducing bacteria into the uterus. Some procedures performed by veterinarians may also carry bacteria into the uterus, e.g. insemination, embryo transfer, uterine biopsies or swabs and infusion of medication [11]. Conformational abnormalities, such as pneumovagina, urovagina and cervical lesions, facilitate the entrance of bacteria into the uterus but properly functioning vulvovaginal fold serves as barrier to ascending bacterial contamination.

Postpartum Physiology: Postpartum period is the time required for the completion of uterine involution that is the interval from parturition to complete uterine involution. The events that must be completed after parturition before a cow is likely to conceive again are uterine involution, regeneration of the endometrium, elimination of bacterial contamination of the uterus and the return of ovarian cyclical activity. The initial stimulus for these changes to occur is the expulsion of the fetus along with the associated membranes and fluids at calving [12].

Uterine Involution: Uterine involution is a complex process that begins immediately after calving and involves uterine contractions, physical shrinking, necrosis, sloughing of caruncular material and regeneration of the endometrium. Following the loss of the allantochorion, there is necrosis of the uterine caruncles, which are usually sloughed by 12 days after parturition. Sloughing of the uterine caruncles contributes significantly to the rapid reduction in weight of the involuting postpartum uterus from 13 kg at parturition to about 1 kg 3 weeks later, because the caruncles account for over half of the weight of the uterus. The sloughed caruncles form the lochial discharge, along with the remains of fetal fluids and blood from the ruptured umbilicus. There is initially regeneration of the endometrium in the inter-caruncular areas and then by centripetal growth of the cells over the caruncle. Epithelial regeneration is complete by about 25 days after parturition, but the deeper layers of tissues are not fully restored until 6–8 weeks after calving [13]. PP involution in cows is generally complete by 45 to 50 days PP [14].

Ovarian Activity in Postpartum Dairy Cows: During late gestation, the hypothalamic-pituitary axis is under the negative feedback effect of estrogens and progesterone produced by the placenta and ovaries. Therefore, the first ovulation occurs between 14 to 28 days postpartum [15]. The return of ovarian cyclic activity postpartum both influences and depends on the uterine immune response. In fact, high concentrations of progesterone during the luteal phase suppress the immune response of the uterus and make the uterus more susceptible to bacterial infection. Early ovulation and elevation of circulating progesterone concentrations before elimination of uterine bacterial contamination has been linked to the establishment of pyometra in postpartum cows [16]. Intrauterine infusions of A. pyogenes and *E. coli* in postpartum beef cows when progesterone concentrations were basal did not cause uterine infection whereas, all cows developed uterine infections when the bacteria were infused after the onset of luteal function and progesterone concentrations had begun to rise [17].

Contamination and Elimination of Bacteria from the Postpartum Uterus: In pregnant cows the vulva, vestibule, vagina and cervix function as anatomical barriers that protect the uterus from bacterial

contamination during pregnancy. Relaxation of the vulva and cervical dilation during parturition allow for the entrance of bacteria into the uterus [18], therefore, bacterial contamination of the uterus at postpartum period is common. It has been demonstrated that 33% of dairy cows had positive bacterial cultures during the first week after calving and by the second week the number of positive cases had increased to 44% [19]. Some works, perhaps using more sensitive culturing techniques, has shown that the number of dairy cows with a uterine infection during the first 2 weeks postpartum is almost 80 to 100%, despite an uneventful calving [20].

Necrotized caruncles, blood and cell debris provide a perfect media for bacteria to grow during the immediate postpartum period. Under normal circumstances, the process of uterine involution effectively expels debris and encourages endometrial regeneration, so that the percentage of cows in which bacterial infection remains present at 3 weeks postpartum should decline to 40%; however, in approximately 10 to 17% of postpartum cows, conditions favoring bacterial growth persist and eventually cause endometritis. Specific factors that may delay the elimination of bacteria from the postpartum uterus include the level and nature of the bacterial contamination, the degree of uterine involution, retention of fetal membranes and the cow's immune status [21].

Risk Factors and Causes of Uterine Disease

Risk Factors: Like most diseases, uterine diseases are multifactorial; therefore it becomes extremely difficult to discuss all factors affecting its occurrence. Some of the traditional risk factors associated with metritis include primiparity, dystocia, male offspring, twins, stillbirth, abortion, prolapsed uterus, RP, ketosis and hypocalcemia [22]. Risk factors for endometritis include dystocia, male offspring, twins, stillbirth, abortion, RP, metritis, problems with vulval conformation and ketosis [23].

Because of the multifactorial nature of uterine diseases, it is helpful to think of the disease triangle when trying to understand their causes. In that regard, for establishment of disease, it is necessary a susceptible host, a virulent pathogen and an environment favorable for disease development. Starting with the host, the dairy cow undergoes dramatic metabolic and physical challenges during the transition to lactation (3 weeks before to 3 weeks after calving). Regarding the metabolic challenge, the transition period is characterized by a state of negative energy, mineral and vitamin balance in which there is a decrease in dry-matter intake (DMI), leading to a sharp decrease in glucose, minerals (e.g. calcium, selenium) and vitamins (e.g. A and E) right after parturition and an increase in body fat mobilization in the form of non-esterified fatty acids (NEFA) [24]. High mobilization of NEFA results in excessive uptake by the liver; therefore, leading to incomplete oxidation of this fatty acids and the accumulation of ketone bodies such as beta-hydroxybutyrate (BHBA) in the blood [25]. This state of negative energy, mineral and vitamin balance leads to immunosuppression [26] and increased susceptibility to disease [27]. The metabolic challenge is likely a result of preparation for and initiation of lactation [28, 29]; however, dairy cows also have a high incidence of dystocia which help breach the physical barriers such as the vulva and endometrium and probably affect DMI intake postpartum because of discomfort [30]. Stillbirths are also highly correlated with dystocia and hypocalcemia [31].

Causes: The bacterial agents commonly isolated from the uterus of postpartum cows are *Escherichia coli*, *Streptococci spp., Arcanobacterium pyogenes*, *Bacillus licheniformis*, *Prevotella spp.* and *Fusobacterium necrophorum*. The most common pathogenic species are *Escherichia coli*, *Arcanobacterium pyogenes*, *Fusobacterium necrophorum* and *Prevotella species* [32]. Some bacteria, including *A. pyogenes, F. necrophorum* and *Prevotella spp.*, act synergistically to enhance the severity of uterine disease. Each of these species produces substances to enhance bacterial growth. *Fusobacterium necrophorum* actively invades uterine tissues and produces a leucocidal toxin that inhibits phagocytosis. *A. pyogenes*, protected by the leucocidal toxin, in turn provides catalase and a growth factor which supports the proliferation of *F. necrophorum.* It has been reported that persistent infection with *A. pyogenes* after 21 days postpartum will reduce conception rates at the first postpartum service [33]. Studies to evaluate the appearance and odor of vaginal mucus have shown that *A. pyogenes*, *Proteus species* and *F. necrophorum* are associated with purulent or mucopurulent discharge evident in the vaginal mucus while *A. pyogenes*, *E. coli* and non-hemolytic *Streptococci* are associated with foul smelling exudates [34].

BoHV-4 is a member of the herpes virus causing reproductive disease in cattle in the form of endometritis, vulvovaginitis and associated abortion. Most of the time BoHv-4 cause's subclinical endometritis and also cause retained fetal membrane which intern leads to endometritis. *Bovine herpesvirus 4* (*BoHV-4*) is the only virus consistently associated with uterine disease after parturition in cattle and BoHV-4 infection is widespread in the endometrium [35, 36].

Classification of Uterine Infection

Metritis: Puerperal metritis is characterized by the presence of an abnormally enlarged uterus, a fetid watery red-brownish uterine discharge associated with signs of systemic illness and fever (>103^0 F) within 21 days in milk (DIM). Animals without systemic signs but with an enlarged uterus and a purulent uterine discharge within 21 DIM may be classified as having clinical metritis (Sheldon et al. 2006a). There may be predisposing factors such as retained placentas, fetal maceration, or difficult calving [37].

Impact: The impact of metritis on milk production remains unclear as some studies found a detrimental impact and others did not. When reported as detrimental, the effect of metritis is influenced by parity and stage of lactation. The magnitude of this impact in multiparous cows is between 2 and 13 kg of milk per day during a period that can vary from 2 to 20 weeks [38]. The impact of metritis on reproduction and culling is unclear. Some data suggest that metritis decreases subsequent reproductive performance and increases culling [39].

Clinical Endometritis: It has been defined as a purulent or mucopurulent discharge or a cervical diameter >7.5 cm after 20 days in milk (DIM), or mucopurulent discharge after 26 DIM [40]. Whether these cows have deeper uterine tissue involvement, higher degrees of bacterial contamination, a different or more extensive inflammation, or are cows that are experiencing a shortened inter-estrus interval which would facilitate an evacuation of uterine content has not been determined [41].

Purulent vaginal discharge was formerly known as clinical endometritis and reflects the presence of mucopurulent or purulent material in the vagina. It affects 20% of dairy cows between 21 and 40 DIM [42]. Diagnosis of this condition can be performed by using a vaginoscope, a gloved hand, or a metricheck device. A metricheck device is a stainless steel probe with a semi-spherical rubber cup at the end. This device was invented in New Zealand and its use is becoming more popular in North America. Although there are some subtle differences, practically the use of any of these 3 techniques for diagnosing PVD provides similar results. Most commonly, vaginoscope and metricheck are used. Risk factors for PVD include RP, metritis, parity and metabolic disorders such as ketosis, displaced abomasum and hypocalcemia [43].

Impact: By definition, diagnostic criteria for PVD are established based on detrimental impact on subsequent reproduction. Therefore, PVD increases time to pregnancy by reducing pregnancy rate. The magnitude of this effect is similar between studies and is reported to be around 30 days. In other words, cows affected by PVD need on average 30 days longer to become pregnant than unaffected cows. This detrimental impact is important economically considering the cost of additional days open (roughly $3 per day per cow in USA). Overall, it may represent a loss of $1,800 per year on an average 100-cow dairy farm in USA. Because PVD is a localized chronic disease, it causes no direct loss of milk production or mortality [44].

Subclinical Endometritis: Subclinical endometritis is defined as an inflammation of the internal lining of uterus (endometrium) in the absence of clinical signs of endometritis seen externally such as vaginal discharge. Or in the absence of clinical endometritis, subclinical endometritis is defined by the presence of >18% PMN in uterine cytology samples collected between 22 and 33 days postpartum and >10% PMN between 34 and 47 days postpartum. Subclinical endometritis have negative impact on subsequent reproductive performance of dairy cattle [45]. Subclinical endometritis usually occurs over a long period of time rather than progressing quickly. The pus discharge from the vagina may be present under speculum examination. The prevalence of subclinical endometritis varies depending on the parity of the cows; of 82 cross breed dairy cows the prevalence of subclinical endometritis were 100%, 52.4% and 71.4% in pregnant heifer, primiparous and multiparous cows respectively [46].

Impact: Inflammation of the uterus due to SCE has an effect on reproductive performance. Irritation of the endometrium may cause premature release of prostaglandin and lysis of the corpus luteum resulting in a short inter estrus interval. Subclinical endometritis is inflammation of the uterine endometrium without external indicators of apparent clinical signs; such as vaginal discharge. Subclinical endometritis affected cows have prolonged days to first service and days to pregnancy. Subclinical endometritis causes significant economic losses because of decreased reproductive performance, increased feed intake per lactation, reduced milk yield and increased culling rate [47]. The economic cost of

subclinical endometritis depends on the detrimental effect on fertility, increased culling rate and, to a lesser extent to the cost of treatment [48].

Up to 50% of cows 40-60 days after calving had neutrophils in the uterine lumen or endometrium, concomitant with inflammation of the tissues and subclinical endometritis reduces conception rates. Cows with genital tract microbial infections have slower growth of dominant follicles, lower peripheral plasma oestradiol or progesterone concentrations and are less likely to ovulate [49].

A mean of 2.4 services per conception was required for cows that were positive for SCE at the 4th week postpartum whereas 1.7 services per conception was required for cows negative for SCE. For those cows that were positive for SCE at the 8th week PP a mean of 2.7 services per conception was required compared to 1.7 NSPC for normal cows. At both 4th and around 8th weeks postpartum, the presence of SCE has a significant effect on the number of services on conception indicating that cows with subclinical endometritis required more services per conception. The median calving to first service interval for cows with subclinical endometritis at the 4th and 8th weeks postpartum were 96 and 100 days, compared to 80 and 82 days for cows without SCE respectively [50].

Pyometra: It is characterized by a collection of purulent exudate of variable amount within the uterine lumen. This condition is most likely to develop in cows that have their first postpartum ovulation before bacterial contamination of the uterus has been eliminated. Although there is functional closure of the cervix, the lumen is not always completely closed and some pus may discharge through the cervix into the vaginal lumen. Ultrasonographically pyometra is characterized by the presence of a CL on an ovary, an accumulation of fluid of mixed echodensity in the uterine lumen and distention of the uterus [51].

Diagnosis of Uterine Diseases

Diagnosis of Metritis: Metritis is diagnosed by a complete physical examination of the cow including attitude, hydration status, rectal temperature and palpation of the uterus per rectum to evaluate uterine discharge. Evaluation of rectal temperature should be performed before palpation per rectum. A Florida study observed that a high proportion (~ 60%) of cows did not have fever (> 103.0^{0}F) at the time puerperal metritis was diagnosed, indicating that this condition is not always accompanied by a fever. This finding suggests that diagnosis and treatment consideration for puerperal metritis should include the character of the uterine discharge (fetid or not) and the attitude of the cow, besides measurement of rectal temperature [52].

Cows diagnosed with metritis without a fever were just as likely to later develop clinical endometritis as cows with metritis and a fever. This indicates that metritis without a fever might have the same negative effects on fertility as metritis without a fever. Cows diagnosed with metritis (puerperal or clinical) should be evaluated for concurrent metabolic or infectious disease (ketosis, displaced abomasum, mastitis, pneumonia, etc.) since this conditions are associated. Vaginal examination is not performed on a routine basis but can be performed to aid in diagnosis if a cow has a fever of unknown origin and no uterine discharge can be produced after palpation of the uterus per rectum. Care should be taken to wash the vulva with antiseptic solution (e.g. iodine scrub) and to use a clean well lubricated palpation sleeve [53].

Diagnosis of Clinical and Subclinical Endometritis: The diagnostic criteria for subclinical endometritis should identify cows at risk of pregnancy failure within an appropriate time. A variety of methods such as uterine palpation, ultrasonographic features of the uterus, vaginoscopy, endometrial cytology, uterine culture and uterine biopsy has been reported to identify endometritis in postpartum cows [54].

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