Antimicrobial therapy in bovine reproduction

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Abstract

The use of antimicrobials, both systemically and locally, has been a hallmark of therapy in the treatment of uterine infections in cattle. However, the use of antimicrobials has not been without controversy regarding their efficacy, effects on future fertility, risk for bacterial resistance and potential residues. This article reviews the immunology and pathophysiology of postpartum uterine infections in cattle and evaluates research regarding the use and efficacy of local and systemic antimicrobial therapies.

Keywords: Bovine uterus, metritis, endometritis, antibiotic therapy

Introduction

The use of antimicrobials has been a conventional therapy in treatment of uterine infections in cattle although the use of antibiotics has not been without controversy. Debate continues regarding antimicrobial efficacy, effects on future fertility, risk for bacterial resistance and residues. The proper use of antimicrobials to treat uterine infections must first begin with an appropriate diagnosis and thorough understanding of the immunology of the uterus, the pathophysiology of uterine infections, and the properties of the various antimicrobial agents that may be used therapeutically.

Normal uterine involution

Understanding normal uterine involution is vital to understanding and defining postpartum disease in cattle. Lochia are normally present until 14 to 23 days

postpartum.[1] After placental detachment, uterine involution is complete in an average of 39 days in normal cows. By day 6 postpartum, caruncle septa are disorganized, and by day 15 caruncles are completely sloughed due to necrosis. By day 26 to 30 postpartum, the surface of the endometrium is covered by new endometrium.[2] Cervical involution is slower than uterine involution and by day 15 postpartum, the diameter of the cervix normally exceeds that of the uterine horns. Reported times for gross involution of the uterus and cervix vary from 25 to 47 days. Complete histologic uterine involution takes longer than palpable involution and occurs at 42 to 50 days.[1]

Infection of the bovine uterus

The majority of cattle experience bacterial contamination of the uterus at the time of parturition. In the normal cow, the uterus is cleared of this bacterial contamination by four weeks postpartum and when these bacteria are not cleared by the cow's defense mechanisms, a uterine infection ensues. Numerous bacteria have been isolated from the cow's postpartum uterus, some of which may be incidental and not cause problems. Uterine infections are most commonly due to *Arcanobacterium pyogenes*. The gram negative anaerobes *Fusobacterium necrophorum* and *Bacteroides melaninogenicus* are frequently associated with *A. pyogenes*. Other organisms that may be associated with uterine disease in the cow include *Pseudomonas aeruginosa*, staphylococci, hemolytic streptococci, coliforms, etc. *Clostridium sp.* may occasionally infect the uterus and cause a severe gangrenous metritis or tetanus. Uterine infections in the cow are associated with retained fetal membranes, dystocia, and delivery of twins.[3]

Metritis is the result of severe inflammation involving all layers of the uterus – endometrial mucosa and submucosa, muscularis, and serosa. Metritis usually develops

during the first week after calving and is associated with dystocia, retained fetal membranes, and calving trauma. These cattle may be septic and present with fever, depression, and anorexia. A copious amount of fetid vaginal discharge may also be present. Endometritis is characterized by inflammation of the endometrium extending no deeper than the stratum spongiosum. Cows with endometritis are usually not systemically ill, and bacteria are usually eliminated after a few estrous cycles. Endometritis is characterized by a mucopurulent or purulent uterine discharge associated with a chronic uterine infection, usually later than 3 weeks postpartum.[1]

Diagnosing uterine infections

Uterine infections are most commonly diagnosed at routine examination of postpartum cows, at breeding, or upon examination of a sick cow. A diagnosis of metritis or endometritis may be made by a variety of methods which include: clinical signs, rectal palpation, vaginoscopy, ultrasonography, uterine culture, uterine cytology, and uterine biopsy. Clinical signs of uterine infection vary with the virulence of the causative organism and the severity of predisposing conditions of the cow. Uterine discharge may vary considerably in color; however, discharges are not usually considered abnormal unless the uterine fluid is fetid or if the cow has clinical signs of sepsis. Palpation per rectum is the most commonly used technique for evaluating the degree of uterine involution prior to breeding and is used to diagnose endometritis through evaluation of the size and consistency of the uterus and cervix and the presence of fluid within the lumen of the uterus. If involution is normal, fluid should not be palpable within the uterine lumen by 14 to 18 days postpartum. In cases of severe metritis, the uterus will be enlarged and friable with occasional adhesions between the uterus and other organs or the

body wall. Although rectal palpation is commonly used for diagnosis of endometritis, it is neither sensitive nor specific. The evaluation of purulent exudate with the aid of a vaginal speculum may also be a useful tool for diagnosing endometritis. Ultrasonography has also been used to evaluate and characterize intralumenal fluid within the uterus as well as the thickness of the uterine wall. Uterine infections are associated with intrauterine fluid with echogenic particles and a variably thickened uterine wall. Bacterial culture is rarely used as treatment must be initiated before culture results are available. Culture is more commonly used in cases where cows fail to respond to treatment. Endometrial biopsy is not used as commonly in cattle as it is in mares and is reported to have a detrimental effect on future fertility.[3,4]

Acute-phase proteins have been evaluated as markers for endometritis in the postpartum cow. These acute-phase proteins include peripheral blood haptoglobin and α_1-acid glycoprotein. Haptoglobin is synthesized in the liver in response to tissue damage and binds free hemoglobin to protect from the oxidative activity of hemoglobin. Serum haptoglobin concentrations increase in dairy cows with acute metritis, but do not increase significantly in cases of chronic metritis.[5,6] Another study showed that cows with acute postpartum metritis generally have low concentrations of plasma haptoglobin while cows with severe metritis had consistently higher levels.[7] A more recent study indicated that cows with $\geq$1 g/L of haptoglobin on day 3 postpartum are 6.7 times more likely to develop severe or mild metritis. This study also suggests that an acute phase inflammatory response precedes clinical metritis and that haptoglobin screening may assist in the early detection of metritis.[8] The α_1-acid glycoprotein has been evaluated as well, but the results were less diagnostic than those for haptoglobin.[7] The significance of

these acute phase proteins in the diagnosis of endometritis or metritis is not fully understood. Intrauterine oxygen reductase potential (Eh) and pH have also been explored as a means to assess the level of bacterial contamination within the lumen of the uterus. The Eh values fell in the presence of infection which created an anaerobic environment within the uterus. It is thought that the drop in Eh is due to either bacterial metabolism or increased oxygen consumption by polymorphonuclear (PMN) cells.[9] The pH of uterine discharge collected from cases of endometritis varied from 6.9 to 7.3 which favors the growth of *A. pyogenes*.[10,11]

Immunology of the bovine uterus

The uterine defense mechanisms against contaminating bacteria are maintained in many ways which include 1) anatomically by the simple or pseudostratified columnar epithelium covering the endometrium, 2) chemically by the mucoid secretions from the endometrial glands, and 3) immunologically by PMNs and humoral antibodies.[10] Disruption of these natural defense mechanisms allows for invasion and colonization of the endometrium by opportunistic pathogens. Inflammation of the bovine endometrium can occur following coitus, artificial insemination, or more commonly postpartum.

Uterine cellular immunity

In the uterus, the cellular defense against bacterial invaders is provided by uterine leukocytes. The PMN population within the uterine lumen increased after experimentally-induced uterine infections.[12,13] At approximately 48 hours postpartum in unassisted calvings, leukocytes begin to accumulate in the uterine lumen along with bacterial contaminants.[14] This is the beginning of the normal process of uterine involution. In cases of metritis there is an initial decrease in the phagocytic activity of

uterine PMNs.[10,15] Two to three weeks later when clinical recovery has occurred, the phagocytic activity increases which also coincides with lower numbers of bacteria in the uterine lumen.[10,16]

The cellular immune response of the uterus may be negatively affected by some treatments commonly used to treatment postpartum disorders in the cows.[10,17] It has been found that manual removal of fetal membranes, intrauterine antiseptics and disinfectants, and intrauterine antibiotics may inhibit or suppress uterine leukocyte phagocytic activity for several days.[10,18] It has also been noted that Lugol's iodine and polyvinyl-pyrrolidone both cause necrosis of the endometrial epithelium and stimulate uterine defense mechanisms and release of prostaglandin $F_{2\alpha}$.[19-22] Although these agents stimulate uterine defense mechanisms, they also cause endometrial fibrosis and thus should not be used as an intrauterine therapy.[10,19,23]

Elevated blood progesterone concentrations have been found to inhibit both uterine and peripheral blood neutrophil phagocytic activities. The numbers of peripheral blood neutrophils increase slowly from about six weeks prior to parturition and reach a peak on the day of calving.[16,24,25] However, maternal and fetal cortisol at the time of calving may suppress neutrophil function.[26-28] Immediately postpartum, the phagocytic activity of blood neutrophils declines within the uterine lumen.[10,29] The cellular defense mechanisms are preserved by an increase in the number of PMN cells.[10,24] During the first three weeks postpartum, the number of peripheral blood PMNs declines and is likely due to the migration of these cells into the mammary gland and uterine lumen.[24,30,31] In addition, the phagocytic activity of the PMNs declines which is more marked in older versus younger cows.[10,30,31] There is some disagreement as to whether there is a decrease

in the phagocytic activity of neutrophils in the uterine lumen versus the peripheral blood.[32-34] Other leukocytes are present in the endometrium of all animals. Lymphocytes are found within the endometrial epithelium in both cycling and non-cycling ewes and heifers with little variation in numbers at different stages of the estrous cycle.[35,36]

Uterine humoral immunity

Protective immunoglobulins have been found in the bovine uterine secretions.[37-41] Immunoglobulin A (IgA) is produced locally from the mucosa of the bovine uterus whereas IgG is produced from two sites. A portion of IgG_1 is produced locally in the endometrium while the remaining IgG_1 and all of IgG_2 is obtained from peripheral circulation.[10,37,42] Experimental uterine infections with pathogenic bacteria have demonstrated immunoglobulins in cervical and vaginal secretions that appear in the order IgM, IgA, and IgG and disappear in the order IgM, IgG, and IgA.[10,43] The concentrations of each immunoglobulin depends upon the site of sampling with IgG predominately found in uterine lumen and IgA in the vagina.[10,13,43,44] Both IgG and IgM concentrations in lochia from healthy cows fall after calving.[45] In cows with postpartum disease, both IgA and IgG concentrations in uterine fluids increase quite rapidly as endometritis develops. However, IgM remains low in cattle with endometritis.[46]

Intrauterine therapy

A variety of antibiotics and antiseptics have been infused into the uterus of cows to treat postpartum infections. Intrauterine antimicrobials are used in order to achieve high concentrations at the site of infection but are usually unable to penetrate any deeper than the endometrium.[1] The intrauterine use of antimicrobial agents is controversial as some have found intrauterine treatment to be beneficial while others have found these

agents to have no effect or a detrimental effect. The bovine uterus is an anaerobic environment. Thus, antibiotics that are chosen for intrauterine infusion must be active in the absence of oxygen. Additionally, most antibiotics depress the activity of uterine neutrophils and interfere with uterine defense mechanisms.[3] Thus, one must carefully evaluate the evidence regarding intrauterine antimicrobial use and carefully consider both the advantages and disadvantages associated with therapy.

Historically, intrauterine use of antimicrobials has been a common therapy for treatment of uterine infections. Antimicrobials reportedly used for uterine infections include tetracycline, penicillin, cephapirin, chloramphenicol, Lugol's iodine, gentamycin, spectinomycin, sulfonamides, nitrofurasone, povidone iodine solution, urea, and chlorhexidine.[1] Most of these compounds are not approved for intrauterine use and have no published withdrawal times. There are also reports that intrauterine infusion of antibiotics cause drug residues in milk.[47,48] Additionally, regulatory guidelines must be adhered to for extralabel use of antimicrobials in food animals. Intrauterine therapy is considered an extralabel use, and thus may be prohibited for many antibiotics, particularly in the United States.

The organisms that cause most postpartum infections are usually sensitive to penicillin. However, bacterial contaminants present within the uterus during the first several weeks postpartum produce penicillinase which makes penicillin useless if used locally in the early (less than 30 days) postpartum period. By 30 days postpartum, the contaminating bacteria are usually eliminated and intrauterine treatment with penicillin is more likely to be effective.[3] Other factors may also affect the efficacy of intrauterine

antibiotic therapy. Uterine lochia present during uterine infections contain organic fluids and debris that can render certain antibiotics, such as sulfonamides, ineffective.

More recently, oxytetracycline has been the antimicrobial commonly used for intrauterine therapy.[3] However, one study indicated that most isolates of *A. pyogenes* are resistant to oxytetracycline. This study also showed that large doses of intrauterine oxytetracycline did not affect the frequency of isolation of *A. pyogenes*.[3,49] In addition, oxytetracycline and Lugol's iodine are quite irritating and are reported to cause coagulation necrosis of the endometrium.[3,50] Although some studies indicate improved reproductive performance with the use of intrauterine oxytetracycline, it has been speculated that this improvement may be due to local prostaglandin production due to chemical irritation of the endometrium.[51]

In general, intrauterine infusion of antimicrobials has failed to show any increase in reproductive performance.[1] Two large field studies evaluated the use of cephapirin benzoate in cows with clinical endometritis and demonstrated an improvement in reproductive performance.[52-54] But other studies have shown no improvement in reproductive performance when evaluating intrauterine administration of cephapirin benzoate.[53] The appropriate use of intrauterine antibiotics to treat uterine infections still remains controversial as only a limited number of studies indicate the efficacious use of intrauterine antibiotics.

Numerous antiseptics have been used to lavage the postpartum bovine uterus with iodine and chlorhexidine solutions being most commonly used. Many of these solutions are quite irritating to the endometrium and are thought to stimulate endogenous prostaglandin release. One study showed that the incidence of retained fetal membranes

and endometritis was reduced in cows that received 500 mL of 2% Lugol's iodine immediately after calving and again 6 hours later. However, this study did not evaluate the future reproductive performance of these treated cows.[3,55] Another study evaluated the use of 50 to 100 mL of 2% povidone iodine solution in the uterus one month postpartum and found that the reproductive performance of normal cows was not improved and that the treatment was detrimental to the fertility of cows with endometritis.[3,23]

Systemic antibiotic therapy

Cattle with metritis often suffer moderate to severe illness. These cattle are often septic with fever, depression, and anorexia. A variety of antibiotics have been recommended for parenteral use in cattle suffering from uterine infections. Penicillin or one of the synthetic penicillin analogs and ceftiofur are among the more common antibiotics used systemically in cattle suffering from metritis. Systemic use of oxytetracyline may not be efficacious because of the difficulty in achieving the minimal inhibitory concentration (MIC) required for *A. pyogenes* in the uterine lumen.[3] However, one study found clinical improvement of cattle suffering from metritis with the use of tetracycline at 10mg/kg.[56]

Ceftiofur is a third-generation cephalosporin that has broad-spectrum activity against gram negative and gram positive bacteria.[57] Ceftiofur is reported to reach all layers of the uterus without causing violative residues in milk. Ceftiofur is approved in the United States for systemic administration to lactating cows affected with metritis.[3] A subcutaneous dose of ceftiofur at 1mg/kg in postpartum cows results in a concentration of ceftiofur and its active metabolites in plasma, uterine tissues, and lochia higher than the

MIC for most of the common pathogens involved in metritis.[58] One study demonstrated that ceftiofur administered at 2.2mg/kg daily for five days was effective in treating cows with metritis.[57] Another study supported these findings and showed ceftiofur administered at 2.2 mg/kg once daily for five days is as effective for treating metritis as procaine penicillin G or procaine penicillin G with intrauterine infusion of oxytetracycline.[59]

Because of the reported lack of efficacy and potential detrimental effects of future fertility, intrauterine infusion of antibiotics is not a favored treatment for most cases of metritis. Certain systemic antibiotics have demonstrated their effectiveness at treating uterine infections in cattle. Thus, most cases of metritis, especially cows that are toxic, should be treated with systemic antibiotics such as penicillin or ceftiofur.

Conclusion

There are no antibiotics currently approved for intrauterine administration in the United States. Intrauterine infusion of antibiotics leads to contamination in milk and tissues for which appropriate withdrawal times have not been ascertained. In addition, the assays used on farms to detect antibiotics in milk may not be accurate. Although some studies indicate a positive response to therapy with the use of intrauterine antimicrobials, most studies do not show an improvement in reproductive performance or clinical signs of disease when comparing intrauterine antimicrobial therapy and systemic antibiotic therapy. This information, in conjunction with concerns regarding uterine or endometrial damage and withdrawal times following the use intrauterine antimicrobials, suggests systemic antibiotic therapy as the best treatment for many cases of cows with uterine infections.

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