Postpartum uterine diseases in dairy cows

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Abstract

Uterine diseases in dairy cows can be classified as puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis. These diseases are highly prevalent (affect between 20 and 30% of dairy cows) in high producing dairy cows and have been associated with decreased pregnancy per artificial insemination (AI), extended interval to pregnancy, increased culling, and economic losses. 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 (>39.5^{o}C) within 21 days in milk (DIM). Animals without systemic signs but with an enlarged uterus and a fetid uterine discharge within 21 DIM may be classified as having clinical metritis. Clinical endometritis is characterized by the presence of purulent (>50%) uterine discharge after 21 DIM or mucopurulent (50% pus, 50% mucus) after 26 DIM. In the absence of clinical endometritis, subclinical endometritis is defined by the presence of >18% neutrophils (PMN) in uterine cytology samples collected between 21 and 33 DIM or >10% PMN between 34 and 47 DIM. The main risk factors are dystocia, twins, retained placenta, stillbirth, abortion, prolapsed uterus, and ketosis. Metritis can be successfully treated either by systemic or intrauterine antibiotic treatment. Ceftiofur hydrochloride (Excenel®) intramuscularly or ceftiofur crystalline-free acid (Excede®) subcutaneously are effective in treating metritis, and oxytetracycline intrauterine is effective in abrogating the negative effects of metritis on milk yield and fertility. Intrauterine administration of cephapirin benzathine (Metricure®) is an effective treatment for clinical and subclinical endometritis, although not approved in the United States. Administration of PGF2α does not seem effective for the treatment of clinical or subclinical endometritis.

Keywords: dairy cows, endometritis, metritis, uterine diseases.

Introduction

Uterine diseases in dairy cows can be classified as puerperal metritis, clinical metritis, clinical endometritis, and subclinical endometritis (Sheldon *et al.*, 2006). These diseases are highly prevalent in high producing dairy cows and have been associated with decreased pregnancy per artificial insemination (AI), extended interval to pregnancy, increased culling, and economic losses (Bartlett *et al.*, 1986; Sheldon and Dobson, 2004; Gilbert *et al.*, 2005). Metritis affects about 20% of lactating dairy cows, with the incidence ranging from 8 to >40% in some farms (Curtis *et al.*, 1985; Goshen and Shpigel, 2006; Hammon *et al.*, 2006; Huzzey *et al.*, 2007; Galvão *et al.*, 2009a). Clinical endometritis also affects about 20% of lactating dairy cows, with the prevalence ranging from 5 to >30% in some herds (LeBlanc *et al.*, 2002; McDougall *et al.*, 2007; Galvão *et al.*, 2009b). Subclinical endometritis is the most prevalent of all uterine diseases; it affects ~30% of lactating dairy cows, with the prevalence ranging from 11 to >70% in some herds (Kasimanickam *et al.*, 2004; Gilbert *et al.*, 2005; Hammon *et al.*, 2006; Barlund *et al.*, 2008; Galvão *et al.*, 2009a).

Retention of fetal membranes is a condition in which the cow fails to release the placenta 12 to 24 h after calving. Although retention of fetal membranes is not a disease per se, many researchers have tried to treat (systemically or intrauterine) this condition because it is a major risk factor for metritis (Risco and Hernandez, 2003; Drillich *et al.*, 2006; Goshen and Shpigel, 2006). Although treatment has been found to prevent metritis (Risco and Hernandez, 2003), it has not been found to improve fertility or milk yield (Risco and Hernandez, 2003; Drillich *et al.*, 2006; Goshen and Shpigel, 2006); therefore, it will not be emphasized in this paper. Pyometra is characterized by a pus-filled uterus in the presence of a corpus luteum (CL) and a closed cervix (Sheldon *et al.*, 2006). Pyometra can be considered a subset of endometritis in which cows ovulate in the presence of a contaminated uterus. Common treatment is administration of prostaglandin F2α (PGF2α).

Risk factors

Traditionally, risk factors associated with metritis include primiparity, dystocia, twins, retained placenta (RP), stillbirth, abortion, prolapsed uterus, and ketosis (Erb *et al.*, 1981a, b; Dohoo and Martin 1984; Markusfeld, 1984, 1985, 1987; Curtis *et al.*, 1985; Gröhn *et al.*, 1990; Correa *et al.*, 1993; Kaneene and Miller, 1995; Goshen and Shpigel, 2006; Dubuc *et al.*, 2010). Risk factors for endometritis include dystocia, twins, RP, stillbirth, abortion, metritis, problems with vulval conformation, male offspring, and ketosis (Gröhn *et al.*, 1990; Galvão *et al.*, 2009a, b; Dubuc *et al.*, 2010;

Potter *et al.*, 2010; Cheong *et al.*, 2011). While metritis is more prevalent in primiparous cows (Markusfeld, 1985, 1987; Goshen and Shpigel, 2006), incidence of endometritis has been found to increase, to decrease, or to be conditional with the level of milk yield in primiparous compared to multiparous cows (Galvão *et al.*, 2009b; Potter *et al.*, 2010; Cheong *et al.*, 2011). Interestingly, multiparous cows have increased bacterial contamination ~50 days after calving compared to primiparous cows (Galvão *et al.*, 2009b). Milk production has a detrimental effect on leukocyte function (Kimura *et al.*, 1999; Nonnecke *et al.*, 2003); therefore, leukocytes from multiparous cows are expected to be more severely affected because of greater milk yields. In fact, phagocytic activity of neutrophils in older cows is more markedly reduced after calving compared to younger cows (Kehrli *et al.*, 1989; Gilbert *et al.*, 1993). Therefore, increased levels of pro-inflammatory cytokine production in the uterine endometrium might help to prevent metritis; however, because multiparous cows have greater demands for milk yield, they might be less able to clear an infection completely and therefore might be more likely to have endometritis. Another important factor that might be involved in the susceptibility to metritis is the circulating levels of immunoglobulins. Immunoglobulins work as opsonins, which greatly enhance phagocytic capacity. Primiparous cows have lower immunoglobulin content in colostrum, which indicate lower circulating immunoglobulin levels (Muller and Ellinger, 1981); therefore, phagocytosis might not be optimal in early lactation in primiparous cows.

Recent studies have focused on the effect of dry matter intake, indicators of energy balance such as nonesterified fatty acids (NEFA) and betahydroxybutyrate (BHBA), haptoglobin, glycogen stores in neutrophils, and calcium on uterine disease (Hammon *et al.*, 2006; Huzzey *et al.*, 2007; Duffield *et al.*, 2009; Dubuc *et al.*, 2010; Galvão *et al.*, 2010; Ospina *et al.*, 2010; Martinez-Patino *et al.*, 2012). Dry matter intake has been recognized as an important risk factor for the development of uterine disease. Recent observations show that cows that developed metritis and endometritis had a decrease in dry matter intake up to 2 weeks before calving (Hammon *et al.*, 2006; Huzzey *et al.*, 2007). This decrease in dry matter intake was accompanied by an increase in NEFA and BHBA in blood, indicating a greater degree of negative energy balance and immunosuppression in those cows (Hammon *et al.*, 2006; Galvão *et al.*, 2010). Recent work also showed that cows with subclinical hypocalcemia had greater concentrations of NEFA and BHBA, and increased odds of developing metritis (Martinez-Patino *et al.*, 2012). Furthermore, the odds of developing metritis decreased by 62% for every 1 mg/dl increase in serum Ca in the first 3 days after calving (Martinez-Patino *et al.*, 2012).

Identification

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 (>39.5°C) within 21 days in milk (DIM). Animals without systemic signs but with an enlarged uterus and a fetid uterine discharge within 21 DIM may be classified as having clinical metritis (Sheldon *et al.*, 2006). 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 (Benzaquen *et al.*, 2007) observed that a high proportion (~60%) of cows did not have a fever (>39.5°C) at the time metritis was diagnosed, indicating that this condition is not always accompanied by a fever. This finding suggests that diagnosis and treatment consideration for metritis should include the character of the uterine discharge (fetid or not) and the attitude of the cow, besides measurement of rectal temperature. Cows diagnosed with metritis without a fever were just as likely to later develop clinical endometritis as cows with metritis and a fever. This suggests that metritis without a fever might have the same negative effects on fertility as metritis with a fever (Benzaquen *et al.*, 2007).

Cows diagnosed with metritis (puerperal or clinical) should be evaluated for concurrent metabolic or infectious diseases (ketosis, displaced abomasum, mastitis, pneumonia, etc.) since these conditions are associated (Curtis *et al.*, 1985). Although not performed on a routine basis, vaginal examination 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 (Williams *et al.*, 2005). Dairies should have a clear standard operating procedure on when to evaluate cows for metritis and how to identify them. Metritis can occur at any time after calving, even after 21 DIM; however, most of the cases (~95%; 709/753) occur in the first 14 DIM with a peak around 5-7 DIM (Fig. 1).

Because of this concentration in incidence of metritis in the first 14 DIM, and in an effort to target monitoring of cows, different strategies have been proposed by the pharmaceutical industry and by academia to diagnose and manage metritis: Pfizer (Pfizer Animal Health, New York, NY) devised what is called the 100-day contract for health and reproductive management. Part of that program includes daily monitoring of fresh cows in the first 10 DIM. Although

monitoring cows in the first 10 DIM would be sufficient to diagnose most of the cows, a substantial proportion (~20%; 140/753) would be missed. At the University of Florida Dairy Research Unit, targeted monitoring of all cows at 4, 7, and 12 DIM in combination with physical examination of cows with milk deviation of more than 12% or failure to increase milk yield by at least 4% (primiparous) or 7% (multiparous) per day in the first 20 DIM have proven very efficacious in diagnosing cows with metritis and metabolic diseases (ketosis and displaced abomasum). Although the system has proven effective, it requires individual daily milk weights. Others have targeted the first 13 (Benzaquen *et al.*, 2007) or 14 DIM (Galvão *et al.*, 2009b) for daily monitoring. Regardless of the monitoring regimen adopted, compliance with the protocol and skill of the evaluator are paramount to the success of the monitoring program.
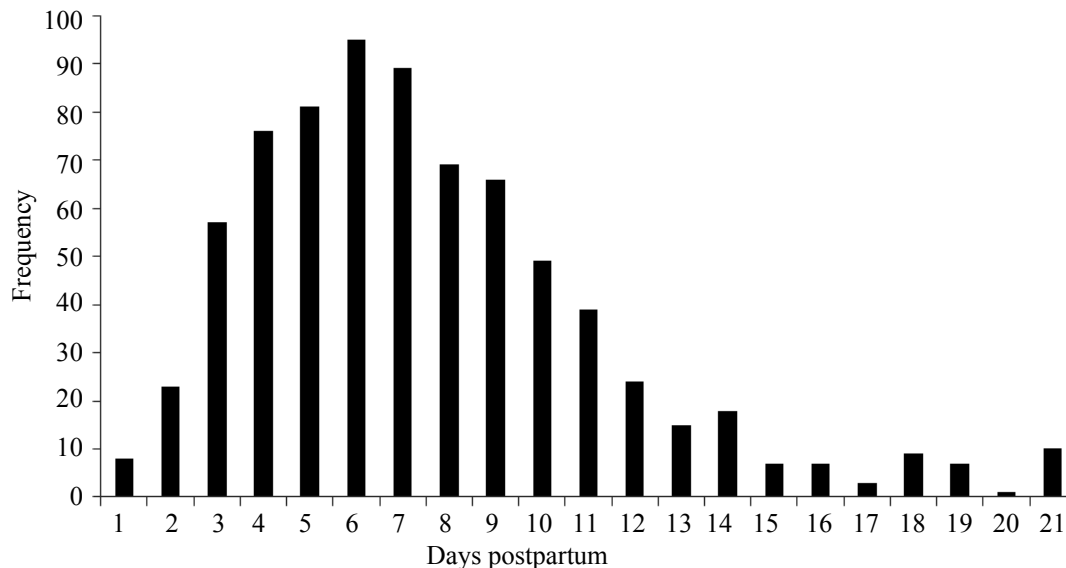

Figure 1. Frequency distribution of metritis incidence by days postpartum in a sample of 753 metritis cases that occurred over a one-year period in dairies in Ohio, New York, and California.

Endometritis

Clinical endometritis is characterized by the presence of purulent (>50%) uterine discharge after 21 DIM or mucopurulent (50% pus, 50% mucus) after 26 DIM (Sheldon *et al.*, 2006). Clinical endometritis is usually diagnosed by evaluation of uterine discharge detected in the vagina with the aid of a speculum (LeBlanc *et al.*, 2002), the Metricheck tool (McDougall *et al.*, 2007), or a gloved hand (Williams *et al.*, 2005). When using either one of these methods, care should be taken to clean the vulva, to avoid introduction of contaminants into the vagina, and to use lubrication. When using vaginoscopy, the speculum should be introduced into the vagina up to the level of the external os of the cervix, and inspection of the discharge is performed with the aid of a flashlight. When using the Metricheck tool (Metricheck, Simcro, New Zealand), the device should be introduced into the vagina up to the level of the external os of the cervix and the discharge should be scooped for evaluation after exteriorization of the device. When using a gloved hand, the hand should be introduced into the vagina up to the level of the external os of the cervix and the discharge should be scooped for evaluation after exteriorization of the hand.

In the absence of clinical endometritis, subclinical endometritis is defined by the presence of >18% neutrophils (PMN) in uterine cytology samples collected between 21 and 33 DIM or >10% PMN between 34 and 47 DIM (Sheldon *et al.*, 2006). Uterine cytology samples can be collected using the cytobrush (Kasimanickam *et al.*, 2004) or the low-volume uterine lavage (Gilbert *et al.*, 2005) technique. For the cytobrush, a Pap smear cytology brush is attached to a metal rod that is fitted through a metal pipe similar in diameter to an insemination pipette (Fig. 2). The tool is protected with a plastic sheath protector during insertion into the vagina, and then is exposed for passing through the cervix. At the uterine body, the cytobrush is exposed and the uterine body wall is pressed slightly against the cytobrush while the cytobrush is rolled two or three times. After that, the tool is exteriorized, and the cytobrush is smeared onto a glass slide and air dried before staining using Diff-Quick stain.

For the low-volume uterine lavage, an infusion pipette is protected with a sanitary chemise, which is punctured before the pipette is passed though the cervix. At any place in the uterus, 10-20 ml of sterile saline

solution is infused, the uterus is then massaged and a portion (≥5 ml) is harvested. A folley catheter can also be used to perform a low volume lavage in a manner similar to embryo flushing (Galvão *et al*., 2009a). After collection, the sample needs to be centrifuged in a conventional or cytospin centrifuge. If using a conventional centrifuge, most of the supernatant needs to be discarded and one drop of the remaining fluid is smeared onto a glass slide. For the cytospin, ~150 µl of the collected sample is loaded in the cytospin container and centrifuged at 700 g for 5 min. Then, slides are air dried and stained using Diff-Quick. After staining, all cells, including epithelial cells, but excluding erythrocytes, are counted under the microscope, and the proportion of PMN out of a total of 200 cells is calculated.

Endometritis has been diagnosed by detection of fluid in the uterus using ultrasonography (Kasimanickam *et al*., 2004); nonetheless, this method was found to be less sensitive than endometrial cytology (Barlund *et al*., 2008).

Figure 2. Cytology tool with cytobrush attached.

Treatment

Metritis

The most common method of treatment is either intrauterine (Thurmond *et al*., 1993; LeBlanc *et al*., 2002; Kasimanickam *et al*., 2005; Goshen and Shpigel, 2006; Galvão *et al*., 2009b); or systemic (Chenault *et al*., 2004) antibiotic administration. Currently, in the United States, there is no approved antibiotic for intrauterine administration in dairy cows. There are only three approved antibiotics for systemic administration for treatment of metritis in dairy cows: ceftiofur hydrochloride (Excenel®, Pfizer Animal Health, Madison, NJ), and ceftiofur crystalline-free acid (Excede®, Pfizer Animal Health, Madison, NJ), which are broad-spectrum third-generation cephalosporins, and Liquamycin LA-200 (Pfizer Animal Health) a long-acting oxytetracycline. Because of the long withhold time for milk (4 days) and meat (28 days) for Liquamycin, Excenel (no withhold for milk and 3 days for meat) is the treatment of choice. Excede also has no withhold for milk but has a 13 day withhold for meat; therefore, its adoption is still uncertain. The advantage of Excede would be the treatment regimen; two doses 72 h apart compared to daily injection for 5 days for Excenel. The recommended dose for treatment of metritis in postpartum dairy cows using Excenel is 2.2 mg/kg (2 ml/100 lbs) of body weight intramuscularly, and for Excede is 6.6 mg/kg (1.5 ml/100 lbs) of body weight given subcutaneously in the middle third or the base of the ear (https://animalhealth.pfizer.com/sites/PAHWeb/US/EN/products/Pages/Excede_Dairy_OLD.aspx). It is recommended to alternate ears.

Although systemic administration of ceftiofur hydrochloride improves clinical signs of metritis (Chenault *et al*., 2004), the effects on fertility have not been evaluated. On the other hand, intrauterine treatment with 5 g chlortetracycline twice weekly for 2 weeks prevented the negative effects of metritis on fertility and milk yields in multiparous cows (Goshen and Shpigel, 2006); however, this treatment is not approved in the United States and would lead to long milk withdrawals. Assuming that the treatment would cost $10 and milk would be discarded for 21 days, the overall cost would be $199 (60 lbs x 21 x $15.00 per 100 lbs cwt = 189 + 10 = $199). Cows that received this treatment regimen produced 1,438 lbs more milk and conceived 29 days sooner; therefore, the return would be $273.70 [(1438/100 x 15) + (29 x $2 per additional day open)], and the net profit would be $74.70.

Endometritis

A formulation containing 500 mg of cephapirin

benzathine in 19 g emulsifier (Metricure®, Intervet, Boxmeer, The Netherlands) is approved for treatment of clinical endometritis by intrauterine administration in Canada, Europe, New Zealand, Australia, and other countries around the world. Intrauterine infusion of Metricure® improved reproductive performance of cows with clinical endometritis (LeBlanc *et al.*, 2002). In the same study, treatment with prostaglandin F2α (PGF2α) was found to be intermediate. A large clinical trial found that PGF2α did not improve fertility in cows with clinical endometritis (Dubuc *et al.*, 2011). Treatment with Metricure® was also found to improve fertility in cows with a history of retained fetal membranes, stillbirths, or a vulval discharge after 13 DIM (McDougall, 2001). In another study, a formulation containing 125 mg of ceftiofur hydrochloride in 10 ml oil-based sterile suspension (Spectramast LC, Pfizer Animal Health, New York, NY) labeled for treatment of clinical mastitis was shown to reduce the bacterial contamination of dairy cows with clinical endometritis; however, it did not improve fertility (Galvão *et al.*, 2009b).

Although there is no approved treatment for subclinical endometritis, Metricure® was found to improve reproductive performance of cows with subclinical endometritis (Kasimanickam *et al.*, 2005). Interestingly, in that study, PGF2α had a similar beneficial effect (Kasimanickam *et al.*, 2005). The benefit from PGF2α administration is believed to arise from induction of estrus in cows having a PGF2α-responsive corpus luteum; the estrus leads to physical expulsion of bacterial contaminants and inflammatory products as well as a possible improvement in the uterine defenses under low progesterone (Kasimanickam *et al.*, 2005). It is generally agreed that a high-progesterone environment suppresses cervical mucus production, myometrial contractility, uterine-gland secretion, and the phagocytic activity of uterine neutrophils (Frank *et al.*, 1983; Hussain, 1989; BonDurant, 1999), and is therefore permissive to uterine infection. PGF2α is not only luteolytic but also appears to have pro-inflammatory actions that might enhance neutrophil function (Lewis, 2004). Because there is increased concern about bacterial acquisition of antibiotic resistance, PGF2α would provide an efficacious method of treatment of endometritis. Nonetheless, later studies found no beneficial effect of PGF2α for treatment of subclinical endometritis (Galvão *et al.*, 2009a; Dubuc *et al.*, 2011); therefore, the combined literature suggests that PGF2α is not an efficacious method to treat subclinical endometritis.

Conclusions

Uterine diseases are prevalent in high producing dairy cows and require prompt diagnosis and treatment. Main risk factors are dystocia, twins, RP, stillbirth, abortion, prolapsed uterus, and ketosis.

Metritis can be successfully treated either by systemic or intrauterine antibiotic treatment. Ceftiofur hydrochloride (Excenel®) intramuscularly or ceftiofur crystalline-free acid (Excede®) subcutaneously are effective in treating metritis, and oxytetracycline intrauterine is effective in abrogating the negative effects of metritis on milk yield and fertility. Intrauterine administration of cephapirin benzathine (Metricure®) is an effective treatment for clinical and subclinical endometritis, although not approved in the United States. Administration of PGF2α does not seem effective for the treatment of clinical or subclinical endometritis.

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