INFLAMMATION, METRITIS, AND REPRODUCTION

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INTRODUCTION

It seems that most, if not all, dairy cattle have bacterial contamination of the uterus for 2 to 3 wk after calving (Sheldon, 2004), with 10 to 20% developing metritis (systemic illness with fetid vulvar discharge and fever, mostly between 3 and 9 d after calving), 5 to 15% having purulent vaginal discharge (PVD; Dubuc et al., 2010a), 15 to 40% having cervicitis (inflammation within the cervix based on > 5% neutrophils (polymorphonuclear leukocytes; PMN) on cytology; Deguillaume et al. 2012) approximately 1 mo after calving, and 10 to 30% having cytological endometritis between 1 and 2 mo after calving (summarized in LeBlanc et al. 2011). Each condition has been demonstrated in the large field studies cited previously to be associated with significant increases in the median time to pregnancy among affected cows. Although a formal economic impact analysis has only been published for metritis (Overton and Fetrow, 2008), based on the prolonged time to pregnancy and increased risk of non-pregnancy, it is reasonable to assume that PVD, endometritis, and cervicitis also incur financial losses. Although these reproductive tract diseases are distinct and may occur alone, affected cows commonly have more than one of these problems during the postpartum period, such that when studied together in the same cows, 37% had at least one of metritis, PVD, or endometritis (Dubuc et al. 2011) and 56% had endometritis or cervicitis (Deguillaume et al. 2012) by 5 wk postpartum.

These large incidence risks are attributed in part to reduced immune function and impaired regulation of inflammation from approximately 2 wk before to 3 wk after calving.Severity of concurrent insulin resistance (IR), reduced feed intake, negative energy balance (NEB), and weight loss contribute to the degree and duration of reduced immune defense. There is a necessary immune and inflammatory response to inevitable bacterial contamination of the uterus and the need for substantial tissue repair as part of involution. What determines whether these processes will be timely and effective versus allowing disease to develop or unhelpful inflammation to persist is not clear. This paper reviews reproductive tract inflammatory disease in dairy cows during the first 2 mo after calving.

CURRENT CONCEPTS IN METABOLIC HEALTH AND INFLAMMATION

There is emerging understanding that energy and fat metabolism are linked with inflammation (Sordillo and Raphael, 2013). There is a growing body of information about interactions among metabolism (specifically related to insulin and fat), inflammation, and immune function in humans and lab animals (Osborn and Olefsky, 2012). These phenomena of “metabolic inflammation” are being investigated in dairy cows in which they seem to be central to health in the transition period (Bertoni et al., 2009; Bradford, 2011; Trevisi et al., 2012). This may be useful to understanding the determinants of effective inflammatory response in the reproductive tract of postpartum dairy cows. Fat is metabolically active and contributes proinflammatory signals (TNFα and IL-6; Tilg and Moschen, 2008). Many inflammatory mediators block the intracellular signalling of insulin (Hotamisligil, 2006) and so contribute to IR. Oxidative stress is also known to contribute to this process. Insulin resistance in dairy cows has recently been reviewed (DeKoster and Opsomer, 2013).

The mechanisms of pathogen detection, immune response, and to a lesser degree, regulation of inflammation in the uterus of dairy cows in the postpartum have been described (Sheldon et al., 2009a; Singh et al., 2008; LeBlanc, 2012), and temporal associations with metabolic changes of the transition period exist (Goff and Horst, 1997). It is hypothesized that reproductive tract disease represents a failure of the immune sys-
tem to switch fast enough or far enough from the down-regulated state necessary for maintenance of pregnancy to a heightened state of function for postpartum clearance of bacteria and tissue debris, and then to a “quiet” state 3 to 4 wk later. A desirable response seems to be a prompt, substantial (and presumably effective) immune and inflammatory response in the uterus after calving (Gilbert et al., 2007). An excessive proinflammatory state early during the postpartum period seems to be a key feature of cows with endometritis about 1 mo later (Herath et al., 2009; Sheldon et al., 2009a,b; LeBlanc, 2012). Generally, worse postpartum NEB is associated with more severe or prolonged uterine inflammation.

It is generally considered that bacterial infection of the uterus initiates inflammation of the uterus. This inflammation is a normal adaptive response, but it may be inadequate for the task (i.e., the balance tips in favor of bacterial growth, adhesion, inflammation, and tissue damage rather than clearance and healing – insufficient response) or inflammation may be disproportionate in degree or duration (excessive response). It is not clear if excessive or persistent inflammation is provoked by the type (species, strain or virulence factors – see later) or quantity of bacterial infection, by genetic or metabolic influences on immune function and regulation, or both. Presently, more data are available to support the importance of immune response as a critical variable in the development of reproductive tract disease. This underlines the importance of understanding what determines the variation in the effectiveness of the inflammatory response to parturient tissue trauma and postpartum pathogen challenges. Specific variables that link metabolism, inflammation, and reproductive health are reviewed (LeBlanc, 2012).

THE SPECTRUM OF REPRODUCTIVE TRACT DISEASE

The process of normal involution and current concepts of uterine inflammation and defense have been reviewed (LeBlanc, 2008; Sheldon et al., 2009a,b; LeBlanc, 2012). In most cows, this process leads to clearance of bacterial infection and eventual repair of the epithelium, at which point inflammatory response is switched off. Most cows have bacterial infection of the uterus for several weeks after calving, but the relative importance of infection (the stimulus side of the inflammation equation) versus immune response (effectiveness and regulation of inflammation) is in question. Escherichia coli (E. coli) are particularly prevalent during the first postpartum week and are associated with metritis, with increased risk of infection with Trueperella pyogenes during the second and third weeks, and with endometritis (Gilbert et al., 2007; Williams et al., 2005). Metritis and endometritis are commonly associated with mixed bacterial infection of the uterus, often including anaerobes, notably Fusobacterium and Prevotella species. Recent studies have explored the potential for specific virulence factors or strains of bacteria to be associated with uterine disease and these data have recently been summarized (LeBlanc et al., 2011). Briefly, there are strains of E. coli that seem to be adapted uterine pathogens (Bicalho et al., 2010; Sheldon et al., 2010). New data (Bicalho et al., 2012) build the case that specific virulence factors in E. coli, T. pyogenes, and F. necrophorum are associated with metritis and PVD.

With metritis, the balance between bacterial infection and immune defense tips in favor of the pathogens. In cows with more chronic, localized reproductive tract inflammatory disease, the magnitude and/or duration of response overshoot and produce conditions that impair uterine or ovarian function. The distinction between physiologic and pathologic inflammation depends on the severity, timing, and duration of inflammation and whether it contributes to or impairs fertility by the start of the breeding period. Differences in immune function are a key determinant of the risk of metritis (Cai et al., 1994) and endometritis (Hammon et al., 2006).

Metritis

By definition, metritis is an obvious systemic illness that reduces production and cow well-being in the short-term. Limited data exist on the longer term (full lactation) effects of metritis on production, reproductive performance, and culling (Benzaquen et al., 2007; Overton and Fetrow, 2008; Wittrock, 2011; Dubuc et al.,
2011; Giulodori et al., 2013), and these effects might be somewhat less than might be expected by the clinical severity of at least some cases of metritis. Therefore, better criteria and methods are needed to identify cows with meaningful short and long-term impacts and to select cows for treatment to mitigate these problems.

**Diagnosis of metritis.** The pathophysiology and diagnostic criteria for metritis have been reviewed elsewhere (LeBlanc, 2008; Sannmann et al., 2012). Briefly, metritis may practically be identified based on at least 2 of fetid discharge, fever, and signs of systemic illness (dullness, inappetance, or decreased milk production). The evidence base for this working definition and the consistency of application of valid diagnostic criteria, however, is weak (Sannmann et al., 2012). Assessment of the odor of discharge was better with an electronic device than by human smell, and agreement of classification among people was only moderate (Sannmann et al., 2013). Daily monitoring of rectal temperature for 7 to 10 d after calving may increase the rate of diagnosis of metritis, and if this practice is implemented it should not be the sole basis for treatment with antibiotics. Routine, systematic screening of fresh cows is likely useful to increase early detection of health problems, especially in large herds, but it is likely most useful if training and experience of personnel and facilities allow for assessment of the cows’ attitude, appetite, ketosis status (once or twice weekly), rumination, and abomasal displacement.

**Treatment of metritis.** Treatment of metritis is presently based on administration of systemic antibiotics, and has been reviewed (LeBlanc, 2008). Although intuitively rational, addition of anti-inflammatory treatment has not been shown to provide an incremental clinical benefit (Drillich et al., 2007). Recent studies report conflicting results on the efficacy of single dose of long-acting ceftiofur (crystalline fee acid) to reduce the incidence of metritis in cows with risk factors at calving (Dubuc et al., 2011; McLaughlin et al., 2013). Data exist to support treatment of cows having metritis with ceftiofur or penicillin (summary in LeBlanc, 2008; McLaughlin et al., 2012), but clinical cure is only around 75 to 80% and impacts on subsequent health and reproductive performance are unclear. Drug concentrations are not maintained consistently above the target levels in the uterus of cows with metritis (vonKrueger et al., 2013). Spontaneous clinical resolution occurs in some cases (Chenault et al., 2004; McLaughlin et al., 2012) and preliminary data (Sannmann et al., 2013) indicate that delay in the onset of treatment of metritis – a “wait-and-see” approach to allow for spontaneous resolution in perhaps 20% of cases – is worth testing in larger randomized clinical trials.

**Purulent Vaginal Discharge and Endometritis**

Each case of PVD and endometritis is associated with substantial reductions in subsequent reproductive performance and their effects are additive (Dubuc et al., 2011; LeBlanc et al., 2011). It was assumed that discharge found in the cranial vagina, or less commonly, observed externally on the vulva or tail, resulted from endometritis. The nature of vaginal content is associated with the density of putative bacterial pathogens in the uterus (Sheldon, 2004), but there is only fair agreement between PVD and endometritis defined by uterine cytology (Dubuc et al., 2010a). This leads to the question of the source of the pus in the vagina if it is not always from the uterus. Cervicitis exists as a distinct condition which is associated with both separate and additive impaired reproductive performance (Deguillaume et al., 2012). Approximately one-half of cows with PVD have cervicitis and vice versa, and 50 to 75% of cows with endometritis have cervicitis and vice versa (Deguillaume et al., 2012; Osawa and LeBlanc, unpublished observations).

**Diagnosis.** Accurate diagnosis of purulent vaginal discharge (clinical endometritis) requires examination of discharge in the vagina after a minimum of 3 wk postpartum (LeBlanc et al, 2002), which may be done with a vaginoscope, clean gloved hand, or a Metricheck device (Pleticha et al., 2009). Subclinical endometritis is common and has substantial impacts on reproductive performance (Gilbert et al., 2005). Subclinical endometritis is diagnosed by endometrial cytology obtained transcervically either by uterine lavage or cytobrush (Barlund et al.,
2008). Neither technique for subclinical endometritis is sufficiently rapid or practical for widespread use in clinical practice, although rapid cow-side tests have been explored (Cheong et al., 2012).

**Treatment.** Treatment of reproductive tract disease has been reviewed (LeBlanc, 2008; Lefebvre and Stock, 2012). Consistent evidence exists that cows with PVD have improved reproductive performance when treated with a single intra-uterine (IU) infusion of cephapirin approximately 1 mo before first insemination, relative to receiving no treatment (LeBlanc et al., 2002; Runciman et al., 2009). Intraterine infusion of ceftiofur at approximately 6 wk postpartum between 2 injections of prostaglandin F2α (PGF) 2 wk apart reduced the prevalence of uterine bacterial infection with E. coli from 10 to 2% and with T. pyogenes from 6 to 1% among cows with PVD, but did not improve the probability of pregnancy in a Presynch timed AI protocol (Galvão et al., 2009a). In the same study, only 41% of cows with PVD had any bacteria cultured from the uterus at the time of diagnosis. These data support the lack of association between cytological endometritis and concurrent uterine bacterial infection.

Numerous older studies reported that 1 or 2 injections of PGF improved reproductive performance or produced clinical outcomes similar to IU antibiotics. In studies specifically considering cows, with risk factors for or with endometritis, PGF consistently did not improve reproductive performance, but many of these studies lacked valid case definitions, statistical power, or both (LeBlanc, 2008). In a clinical trial in more than 2,000 cows, including more than 600 cows with PVD, cytological endometritis, or both, cows were assigned randomly to receive PGF at postpartum weeks 5 and 7 (Dubuc et al., 2011). Overall, or among cows with reproductive tract disease, no difference was detected in time to pregnancy between PGF-treated and control cows, which is similar to the findings of Galvão et al. (2009b) for cytological endometritis. Data from Dubuc et al. (2011), however, were re-analyzed to examine cows with PVD specifically and without regard to endometritis status (i.e., to address the clinical question of treatment of cows examined only for PVD (which is practical) but without diagnosis of endometritis by cytology (which is well validated, but impractical for routine clinical application). Among 323 cows with PVD at 5 wk postpartum, clinical resolution (absence of PVD) at 8 wk postpartum was 72% in cows that received PGF at weeks 5 and 7 and 58% in untreated controls (bivariable $\chi^2 P = 0.01$). Among these cows with PVD, 43% had a corpus luteum (CL; serum progesterone > 1 ng/ml) at week 5 and 63% had a CL at week 7; 69% had a CL at least once before either of the PGF injections. Accounting for parity, BCS at calving, occurrence of dystocia, RP or twins, and herd, cows with PVD that received 2 injections of PGF tended ($P = 0.07$) to become pregnant sooner than untreated cases (hazard ratio = 1.2, 95% confidence interval 0.95 to 1.6). There was no interaction of the effect of PGF with the presence of a CL. Therefore, these results join others (LeBlanc et al., 2002) pointing to an equivocal effect of PGF for treatment of PVD. A recent trial showed that among cows with PVD, a program of treating those with a palpable CL with PGF produced equivalent reproductive performance as treating all with IU cephapirin (McDougall et al., 2013). Different strategies for PGF as therapy for reproductive tract inflammatory disease merit further investigation.

Taken together, it seems that IU cephapirin is beneficial for reproductive performance in cases of PVD (which may be associated with cervicitis or endometritis), but the benefit of PGF commonly employed as therapy for PVD is unclear. Although 1 study (Kasimanickam et al., 2005) reported a benefit to reproductive performance of either PGF or IU cephapirin relative to no treatment, further investigation of rapid cow-side diagnostic tests and treatments for cytological endometritis are needed. Development of more effective treatments for reproductive tract inflammatory disease will require a better understanding of the factors that initiate and sustain endometrial inflammation, but investigation of anti-inflammatory approaches to treatment are of interest.
PREVENTION OF REPRODUCTIVE TRACT DISEASE

Only a little is known about how resistance to uterine disease may be enhanced through management. Cows with severe metritis ate less (2 to 6 kg DM per day) than healthy cows in the 2 to 3 wk preceding clinical signs of metritis (Huzzey et al., 2007). Less feed intake is associated with increased circulating concentrations of NEFA, which may directly (Scalia et al., 2006; Ster et al., 2012) or indirectly (Zerbe et al., 2000; Hammon et al., 2006) inhibit neutrophil function. Because of both large metabolic demands and pathogen challenges, cattle also routinely experience substantial oxidative stress in early lactation (Sordillo and Aitken, 2009), which also contributes to a pro-inflammatory state that may not be effective for immune defense.

Presently, there are few management practices or interventions that can be supported specifically to prevent metritis or endometritis. Based on current understanding of these diseases, the general objective is to support and maintain innate immune function and so reduce the risk that the inevitable inflammation and bacterial contamination after calving progress to metritis, endometritis, or cervicitis. Excessive NEB and circulating free fatty acid concentrations, and excessive insulin resistance contribute to a state of metabolic inflammation that may actually impair neutrophil function. Although much needs to be learned, Table 1 proposes management practices generally recommended for peripartum dairy cows that are likely to help reduce incidence of reproductive disease during the early postpartum period.

CONCLUSIONS

A balance between bacterial infection of the uterus during the weeks after calving and the effectiveness of the immune and inflammatory response to it will determine the incidence of reproductive tract diseases. New understanding

Table 1. Summary of management practices and monitoring targets to reduce the risks of reproductive tract disease in dairy cows (updated from LeBlanc et al., 2011)

<table>
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<tr>
<th>Recommendation</th>
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<tr>
<td>Prevent consumption of dietary energy above requirement in the “far-off” dry period (weeks 8 to 3 before calving)</td>
<td>Dann et al. (2006) Janovik et al. (2011)</td>
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<td>Provide for unrestricted feed bunk access (i.e., all cows able to eat at the time of fresh feed delivery)</td>
<td>Cook and Nordlund, (2004)</td>
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<td>Provide 30 inches of linear bunk space per cow or no more than 8 cows per 10 headlocks</td>
<td>Nordlund (2010)</td>
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<tr>
<td>Provide space to allow for lying 11 to 12 h per day</td>
<td>Nordlund (2010)</td>
</tr>
<tr>
<td>≥ 1 free stall per cow or 100 ft² of bedded pack per cow</td>
<td>Cook (2007)</td>
</tr>
<tr>
<td>Minimize pen moves and social group changes</td>
<td>Nordlund (2010)</td>
</tr>
<tr>
<td>Build dry cow and fresh pens for approximately 130 to 140% of the expected average number of calvings per month</td>
<td>Nordlund (2010)</td>
</tr>
<tr>
<td>Provide heat abatement (fans and sprinklers) when the THI exceeds 68</td>
<td>Smith (2012)</td>
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<tr>
<td>Manage nutrition so cows calve at BCS of 3.0 or 3.25 (on the 5 point scale), and maintain a minimum BCS of 2.5</td>
<td>Roche et al. (2009)</td>
</tr>
<tr>
<td>Monitoring methods and targets (Serum or plasma tests)</td>
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<td>NEFA &lt; 0.4 mmol/L during the week before expected calving</td>
<td>Dubuc et al. (2010b)</td>
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<td>BHB &lt; 1.1 mmol/L during week 1 and &lt; 1.4 during week 2 after calving</td>
<td>Ospina et al. (2010)</td>
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<tr>
<td>Haptoglobin &lt; 0.8 g/L during week 1 after calving</td>
<td>Chapinal et al. (2011)</td>
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<td>Dubuc et al. (2010b)</td>
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of links between metabolism and inflammation may lead to better approaches to prevent reproductive disease by nutrition and management, and more targeted therapies. Thoughtful selectivity is warranted for development of treatment protocols for metritis, while more widespread diagnosis of PVD at approximately 1 mo postpartum is likely an area of opportunity in many herds.

ACKNOWLEDGMENTS

My research in this area has been supported in part by Pfizer Animal Health and Merck Animal Health, among others. I have no financial links that produce a conflict of interest.

REFERENCES


