Reproductive tract infection and inflammation in dairy cows

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Abstract

Approximately 30 to 50% of cows are affected by at least one form of reproductive tract disease in the postpartum period. Most cows experience a period of insulin resistance, fat mobilization, inflammation, and reduced effectiveness of immune function in early lactation. The mechanisms which influence the severity of these challenges and consequently the risk of retained placenta, metritis, and endometritis are increasingly understood, but it is not clear how to prevent these diseases through management. Numerous links exist between fat metabolism, inflammation, immune function, and probably feed intake regulation. An excessive pro-inflammatory state early in the postpartum period appears to be a key feature of cows with endometritis about one month later. Aspects of innate immune function are commonly impaired in the transition period, particularly in association with elevated non-esterified fatty acid concentrations and to a lesser degree by ketosis. Changes in metabolism and immune function precede reproductive tract disease by several weeks. Implementation of nutritional and management best practices are likely to favor metabolic and reproductive health. The effect of purulent vaginal discharge at four to five weeks postpartum on pregnancy rate is mitigated by intrauterine treatment with cephapirin, whereas the effect of treatment with injection(s) of prostaglandin is equivocal. Practical cow-side diagnostic tools for endometritis are needed and effective treatment of endometritis remains unclear.

Key words: dairy cattle, reproduction, retained placenta, metritis, nutrition

Résumé

Environ de 30 à 50 % des vaches souffrent d'au moins une forme de maladie du système reproducteur au cours de la période post-partum. La plupart des vaches connaissent une période de résistance à l'insuline, de mobilisation adipeuse, d'inflammation et de réduction de l'efficacité de la fonction immunitaire au début de la lactation. Les mécanismes qui influencent l'intensité de ces problèmes, et donc le risque de rétention placentaire, de métrite et d'endométrite, sont de mieux en mieux compris, mais on ne sait pas précisément comment prévenir ces maladies en ayant recours à la gestion. Il existe de nombreux liens entre le métabolisme des graisses, l'inflammation, la fonction immunitaire et, probablement, la régulation de l'alimentation. Un état proinflammatoire excessif au début de la période postpartum semble être une caractéristique clé des vaches souffrant d'endométrite environ un mois plus tard. Les aspects de la fonction immunitaire innée sont souvent affaiblis au cours de la période de transition, particulièrement en association avec des concentrations élevées d'acides gras insaturés et, dans une moindre mesure, par la cétose. Les changements du métabolisme et de la fonction immunitaire précèdent de plusieurs semaines la maladie du système reproducteur. La mise en œuvre de pratiques nutritionnelles et de gestion exemplaires favorisent vraisemblablement la santé métabolique et reproductive. L'effet des écoulements vaginaux purulents à quatre ou cinq semaines post-partum est atténué par un traitement intra-utérin à la céphapirine, tandis que les effets du traitement par injection(s) de prostaglandine est équivoque. Dans les cas d'endométrite, les outils pratiques de diagnostic sur place sont nécessaires et le traitement efficace de l'endométrite demeure nébuleux.

Introduction

This paper provides an overview of the key elements of metabolism and inflammatory response in peripartum dairy cows, and discusses aspects of these specific to endometritis, as well as approaches to treatment and prevention of reproductive tract inflammatory disease.

It appears that essentially all dairy cattle experience bacterial contamination of the uterus for two to three weeks after calving,[53] with 10 to 20% developing metritis (systemic illness with fetid vulvar discharge and fever, mostly between three and nine days after calving), 5 to 15% having purulent vaginal discharge[15] (PVD), 15 to 40% having cervicitis (inflammation within the cervix based on > 5% neutrophils on cytology[13]) approximately one month after calving, and 10 to 30% having endometritis (> 5 to 8% neutrophils in an endometrial smear at four to six weeks postpartum) between one and two months after calving.[36] Each condition has been demonstrated in the large field studies cited above to be associated with significant and substantial increases in the median time to pregnancy among affected cows. While

an economic impact analysis has only been published for metritis,[46] 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 may occur alone, affected cows commonly have more than one of these problems in the postpartum period, such that when studied in the same cows, 37% had at least one of either metritis, PVD, or endometritis[17] and 56% had endometritis or cervicitis[13] by five weeks postpartum.

Both PVD and endometritis are associated with substantial reductions in subsequent reproductive performance, and their effects are additive.[17,36] 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,[53] but only 42% of cows with PVD had endometritis at the same time.[15] 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.[13] Approximately half of cows with PVD have cervicitis and vice versa, and 50 to 75% of cows with endometritis have cervicitis and vice versa.[13] (Osawa and LeBlanc, unpublished observations).

Essentially all peripartum dairy cattle experience a period of insulin resistance (IR), reduced feed intake, negative energy balance, lipolysis and weight loss in early lactation, and bacterial contamination of the uterus for two to three weeks after calving. These factors, as well as dramatic changes in circulating progesterone, estrogen, and cortisol concentrations, are associated with substantial reduction of immune function, in particular of neutrophils, for one to two weeks before, and two to three weeks after calving.[23,30] Innate immunity from neutrophils is a primary means of immune response in the uterus, and neutrophil migration and phagocytic and oxidative activity are associated with the risk of retained placenta[32] (RP), metritis, and endometritis.[24] Yet, while metabolic and uterine disease are common, only a minority of cows experience these problems, even with a herd in which cows apparently have similar nutritional and management experiences.

Peripartum cattle go through a period of substantial IR that has elements in common with Type 1 and Type 2 diabetes,[39] with the important difference that cows have low blood glucose. Dairy cattle also go through substantial lipolysis and a high flux of fatty acids to the liver. High circulating non-esterified fatty acid (NEFA) concentrations are a risk factor for fatty liver, and may also have direct effects on neutrophil function.[50,59] In peripartum dairy cattle in the absence of obesity, the determinants of whether the degree of IR is an adaptive response or pathological is not clear.

Inflammation in the Reproductive Tract

Recruitment and function of an adequate flux of neutrophils to the uterus is important in the days after calving for clearance of bacteria and lochia and prevention of subsequent endometritis.[22] An excessive pro-inflammatory state appears to be a key feature of cows with endometritis. Pro-inflammatory cytokines (IL-1, IL-6, and IL-10 mRNA) are more expressed in cows that have endometritis,[7,25,54] particularly in the first and second weeks after calving in cows diagnosed with endometritis at week four or five.[37] What is not known is what sets up this excessive inflammatory status.

An important concept is that reproductive tract disease represents a failure of the local (and perhaps systemic) immune system 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 back away from active inflammation three to four weeks later. A desirable response appears to be a prompt, substantial (and presumably effective) flux of neutrophils into the uterus after calving.[22]

Associations of Bacteria with Reproductive Tract Disease

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 in the first week postpartum and are associated with metritis, with increased risk of infection with *Arcanobacterium pyogenes* in weeks two and three, and with endometritis.[14,22,61] Metritis and endometritis are commonly associated with mixed bacterial infection of the uterus, often including anaerobes, notably *Fusobacterium* and *Prevotella* species. Until recently, these pathogens have been assumed to be 'generic' or not specifically adapted to or associated with metritis or endometritis. 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.[36] Briefly, some strains of *E. coli* appear to be adapted uterine pathogens, particularly expressing virulence factors related to adhesion.[3,55] New data[4] build the case that specific virulence factors in *E. coli*, *A. pyogenes*, and *F. necrophorum* are associated with metritis and PVD. It is generally considered that bacterial infection of the uterus initiates inflammation of the endometrium and

perhaps deeper layers 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 and 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) or quantity of bacterial infection,[36] by genetic or metabolic influences on immune function and regulation, or both.

Immune Function

The mechanisms of impairment of immune defense in the mammary gland in the transition period have been described[51] and may be a useful reference for the uterus, which also depends heavily on innate immunity, largely from neutrophils. Less is known about the determinants of uterine health or how resistance to uterine disease may be enhanced through animal management. The mechanisms of initiation of inflammation of immune function in the uterus of dairy cows have been reviewed.[36,37,54]

Lower feed intake is associated with increased circulating concentrations of non-esterified fatty acids (NEFA) which may directly[50,59] or indirectly[24,62] inhibit neutrophil function. Because of both high metabolic demands and pathogen challenges, cattle also routinely experience substantial oxidative stress in early lactation,[57] which also contributes to a pro-inflammatory state that may not be effective for immune defense.[26]

Retained placenta is a disease of immune function, with changes in neutrophil function and IL-8 levels two weeks before calving.[32] Similarly, measurable changes were noted in phagocytosis, TNFα, and IL-6 prepartum in cows with postpartum endometritis,[31] weeks before disease becomes manifest, coincident with the onset of insulin resistance and lipolysis (at least in cows at higher risk of disease). Worse postpartum negative energy balance is associated with more severe or prolonged uterine inflammation and impaired tissue repair capacity (both measured by gene expression).[60] Among other genes, those for IL-1 receptor and IL-8 and its receptor, which are associated with uterine inflammation, were substantially more expressed in cows with severe negative energy balance.

Hammon *et al*[24] showed that cows with metritis or endometritis had worse neutrophil killing capacity than did unaffected cows, and these changes preceded disease by several weeks. They also reported associations between increasing NEFA concentration, especially in the last week before calving, and lower neutrophil oxidative burst. Additionally, there was an association between lower feed intake in the three weeks before calving and lower neutrophil killing capacity from the week before until three weeks after calving.

Neutrophils rely primarily on glucose uptake or glycolysis for chemotaxis. However, glycogen stores are necessary for phagocytosis and oxidative burst, even in the presence of glucose.[21] Intra-neutrophil glycogen was lower at calving in cows that had metritis than in healthy cows, and lower at weeks 1, 4, and 6 postpartum in cows with endometritis than in healthy cows.[21] Neutrophil killing ability was lower from at least one week before until four weeks after calving in cows that developed metritis or endometritis and to a greater degree, but only in the week of calving, neutrophil cytochrome c reduction was diminished in cows with subsequent metritis.[24] Huzzey *et al*[27]did not measure immune function, but showed that cows that developed metritis had lower feed intake (4.4 to 13.2 lb (2 to 6 kg) dry matter) than unaffected cows from two weeks before calving (three weeks before clinical signs of metritis). Taken together, these studies support the evidence from mechanistic studies[60] of important interactions between energy and lipid metabolism and immune function in peripartum dairy cows, and point to the importance of unrestricted access to feed (though not excessive energy consumption) in the three weeks before calving for reproductive performance.[9]

Links between Metabolism, Immune Function, and Inflammation

There is a rapidly growing body of information in human medicine, based on studies in rodents and in people, on interactions among metabolism (specifically related to insulin and fat), inflammation, and immune function, such as the publication by Osborn and Olefsky.[43] Close behind, these phenomena are being investigated in dairy cows, where they appear to be central to health in the transition period.[2] The interactions among metabolism, inflammation, and reproductive health in dairy cows have recently been reviewed,[37] and further details are available there. Both obese people and high-producing dairy cows are characterized by elevated circulating plasma NEFA, insulin resistance, and a pro-inflammatory state. Fat releases NEFA, but also glycerol and pro-inflammatory cytokines. Therefore, anti-inflammatory treatments may represent an 'upstream' approach to prevent or control IR[1] and undesirable inflammation.

Non-esterified fatty acids

In a large multi-region field study, NEFA ≥ 0.3 mmol/L was associated with increased incidence of RP.[5] Similarly, as NEFA in the week before calving increased by 0.1 mmol/L, the odds of RP increased by 5%.[47] Cows with NEFA ≥ 0.3 (0.2 in one study region) mmol/L in the

week before calving were more likely to develop metritis (odds ratio (OR) = 1.8).[5] Similar large field studies[44,45] confirm that NEFA > 0.3 mmol/L in the one to two weeks before expected calving is associated with increased risk of RP, metritis, or displaced abomasum (DA), decreased milk production (3.5 lb (1.6 kg)/day[6] or 1503 lb (683 kg) 305 day mature equivalent) and increased time to pregnancy. Similarly, in the two weeks after calving NEFA > 0.6 mmol/L was associated with increased risk of metritis or DA, and NEFA > 0.7 mmol/l was associated with longer time to pregnancy and with 1430 lb (650 kg) less milk in multiparous cows.[44,45] Dubuc *et al*[16] found that NEFA ≥ 0.6 mmol/L in the week before calving was associated with increased odds of metritis (OR = 1.6) but not with PVD or endometritis.

Hammon *et al*[24] reported a negative correlation of prepartum (weeks -1 and -2) and week 1 postpartum NEFA with neutrophil killing ability, and that cows in the lowest quartile of feed intake (DMI) in the two weeks before calving had substantial (> 50%) and sustained (> 4 weeks) decreases in neutrophil killing ability. Cows with metritis or endometritis had higher NEFA from two weeks before until four weeks after calving. Chronic elevated NEFA may harm pancreatic B cells, leading to decreased insulin secretion to compound insulin resistance.[28] There was substantial and dose-dependent decrease of proliferation of monocytes and their production of IFNγ in vitro, as well as decreased neutrophil oxidative burst activity with addition of NEFA to reflect levels in the first week postpartum.[59] Effects on monocytes were present as low as 0.013 mmol/L NEFA and starting at 0.5 mmol/L for neutrophil oxidative burst.

Ketosis

The association of ketosis with reproductive disease and immune function is inconsistent. Subclinical ketosis (BHB > 1.2 to 1.4 mmol/L) in the first or second week after calving was associated with three times greater odds of metritis.[18] In a large field study in NY (778 cows in 38 herds)[8] producer-recorded clinical ketosis (incidence = 5%) was a risk factor for endometritis (OR) = 3.8), particularly in multiparous cows. However, an even larger study[5] found no association of producer-reported clinical ketosis or serum BHB measured systematically in week 1 postpartum with metritis. A study with 1295 cows[16] found that BHB > 1.1 mmol/L in week 1 postpartum was a risk factor for endometritis (OR = 1.4), but not for PVD or for metritis. Plasma BHB was higher at calving in cows that developed metritis, and similar to[16] higher at week 1 postpartum in cows that later had endometritis.[21] Likewise, cows with metritis or endometritis had higher BHB from one until four weeks after calving, although there was no association of BHB with neutrophil killing ability.[24] In vitro titration of BHB did not affect proliferation of monocytes or their production of IFNγ , or oxidative burst activity of neutrophils.[59] It is not clear if the mechanism of the fatty liver/ketosis association with diminished neutrophil function is direct (and if so whether it is on mature neutrophils, or whether NEFA, ketones or other signals or metabolites affect cells in the bone marrow), or through effects on mononuclear cells that are responsible for antigen presentation and initial chemokine signaling of neutrophils.[62]

Oxidative stress

Phagocytosis and intracellular digestion inherently produce reactive oxygen species and create a burden of oxidative stress, which in turn increases pro-inflammatory output from these cells. Endotoxin interacts with TLR4 to result in increased production of pro-inflammatory cytokines TNFα, IL-1, and IL-8 which form part of the response to gram-negative bacteria. However, at least the TNFα response is heightened when antioxidant status is lower or oxidative stress is greater, e.g. in the peripartum period.[58] It is not clear if this results in a more effective response or just the possibility of increased bystander tissue injury or unintended consequences, such as increased IR. Optimization of antioxidant status (e.g. supplementation with selenium, vitamin E, retinol, or polyunsaturated fatty acids) may help to keep immune responses effective and prevent excessive inflammation or its side effects.[58]

Hypocalcaemia

In large field studies, no association of milk fever was found with metritis, PVD, or endometritis.[8,16] Chapinal *et al*[5] also found no association of serum calcium measured in week 1 (but before disease diagnosis) with the odds of metritis. However, Martinez *et al*[40] studied 110 cows in one herd in Florida and found that cows with calcium < 2.14 mmol/L at least once between 0 and 3 days-in-milk (DIM) had 4.5-fold increased odds of metritis. Hypocalcemia was associated with decreased neutrophil oxidative burst and decreased circulating neutrophil counts at 1 and 3 DIM.

Treatment of Reproductive Tract Disease

Treatment of reproductive tract disease has been reviewed.[35,38] Consistent evidence exists that cows with PVD have improved reproductive performance when treated with a single intrauterine (IU) infusion of cephapirin approximately one month before first insemination, relative to receiving no treatment.[34,41,49] Intrauterine infusion of ceftiofur at approximately six weeks postpartum between two injections of PGF two weeks apart reduced the prevalence of uterine bacterial infection with *E. coli* from 10 to 2% and with *A. pyogenes* from 6 to 1% among cows with PVD, but did not improve the probability of pregnancy in a 'Presynch' timed artificial

insemination protocol.[19] In the same study, it is notable that 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 one or two injections of prostaglandin $F_{2\alpha}$ (PGF) improved reproductive performance or produced clinical outcomes similar to IU antibiotics. However, in studies of 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.[35] In a clinical trial in over 2000 cows, including over 600 with PVD, cytological endometritis or both, cows were randomly assigned to receive PGF at weeks 5 and 7 postpartum, or not.[17] Overall, or among cows with reproductive tract disease, there was no difference in time to pregnancy between PGF-treated and control cows, which is similar to the findings of Galvao *et al*[20] for cytological endometritis. However, the data from Dubuc *et al*[17] 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 five weeks postpartum, clinical resolution (absence of PVD) at eight weeks postpartum was 72% in cows that received PGF at weeks 5 and 7, and 58% in untreated controls (bivariable χ^2 P = 0.01). Among these cows with PVD, 43% had a corpus luteum (CL) with serum progesterone > 1 ng/mL at week 5 and 63% had a CL week 7; 69% had a CL at least one of the times of administration of PGF. Accounting for parity, body condition score (BCS) at calving, occurrence of dystocia, RP or twins, and herd, cows with PVD that received two 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[34] pointing to an equivocal effect of PGF for treatment of PVD. Different strategies for PGF as therapy for reproductive tract inflammatory disease merit further investigation.

Taken together, it appears 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 as commonly employed as therapy for PVD is unclear. While one study[29] 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 treatment 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

Presently, few management practices or interventions 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, PVD, endometritis, or cervicitis. Excessive negative energy balance and circulating free fatty acid concentrations, and excessive insulin resistance contribute to a state of metabolic inflammation that may actually impair neutrophil function. While there is a great deal still to be learned about the determinants of immune function in dairy cattle in the transition period, and in particular about specific means to prevent uterine disease, Table 1 proposes management practices generally recommended for peripartum dairy cows that are likely to contribute to reducing the incidence of reproductive disease in the early postpartum period.

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Table 1. Summary of management practices and monitoring targets to reduce the risks of reproductive tract disease in dairy cows.

Recommendation	Reference
Prevent consumption of dietary energy above requirement in the 'far-off' dry period (weeks 8 to 3 before calving)	12, 33
Provide for unrestricted feed bunk access (i.e. all animals able to eat at the time of fresh feed delivery) i.e. 30 in (75 cm) of linear bunk space per cow, or no more than four cows per five headlocks	10, 42
Provide space to allow for lying 11 to 12 h per day ≥ 1 free stall per cow or 100 sq. ft. (10 m^2) of bedded pack per cow	11, 42
Minimize pen moves and social group changes	42
Build dry cow and fresh pens for approximately 130-140% of the expected average number of calvings per month	42
Provide heat abatement (fans and sprinklers) when the Temperature-Humidity Index exceeds 68	56
Manage nutrition so that cows calve at BCS of 3.0 or 3.25 and maintain a minimum BCS of 2.5	48
Monitoring methods and targets (serum or plasma tests)	
NEFA < 0.4 mmol/L in the week before expected calving	5, 16, 44
BHB < 1.1 mmol/L in week 1 and < 1.4 in week 2 after calving	16
Haptoglobin < 0.8 g/L in week 1 after calving	16

Updated from LeBlanc SJ, Osawa T, Dubuc J. Reproductive tract defence and disease in postpartum dairy cows. *Therio* 2011;76:1610-1618.

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