Managing Transition Period Health for Reproduction

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Summary
Essentially all dairy cattle experience a period of insulin resistance, reduced feed intake, negative energy balance, hypocalcemia, reduced immune function, and bacterial contamination of the uterus soon before, or in the weeks after calving. Half of dairy cows have some form of metabolic or reproductive disorder in early lactation. High NEFA (> 0.4 mmol/L) in the last 10 days before expected calving is associated with increased risk of retained placenta, culling before 60 days in milk, and less milk production in the first 4 months of lactation. Subclinical ketosis (serum BHB > 1.2 mmol /L) in the first or second week after calving is associated with increased risk of metritis, endometritis, prolonged postpartum anovulation, and lower milk production in early lactation. Recent data also indicate that serum calcium < 2.15 mmol/L in the first week postpartum is associated with increased risk of DA, lower milk production in early lactation, and reduced probability of pregnancy at first insemination.

There are few management practices or interventions that specifically prevent reproductive disease or support fertility. The general objective is to support innate immune function and so reduce the risk that the inevitable inflammation and bacterial contamination after calving progress to reproductive tract disease. 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 metabolic health and reproductive performance in dairy cattle, management practices generally recommended for peripartum dairy cows are likely to contribute to reducing the incidence of reproductive disease in the early postpartum period.

Introduction
After parturition the uterine lining is sloughed and must be regenerated, and essentially all dairy cows have bacterial contamination of the uterus in the 2 to 3 weeks after calving (Sheldon, 2009a). Therefore, inflammation is a necessary component of involution. However, pathogenic bacteria may overcome innate immune defences (insufficient response) or the severity or duration of inflammation may impair rather than enhance fertility (excessive response). It is not clear if excessive or persistent inflammation is provoked by the type or quantity of bacterial infection, by metabolic influences on immune function and regulation, or both. It appears that systemic inflammation due to metabolism outside the reproductive tract (i.e. adipose mobilisation and hepatic function) may be at least as important as the local interactions of bacteria and inflammatory response in the reproductive tract. This fits the observation that endometritis is often present in the absence of concurrent bacterial infection. However, the contribution of metabolic inflammation remains to be quantified.

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Between 5 and 20% of cows experience metritis (overt systemic illness due to uterine infection) in early lactation, and if examined systematically, 15 to 25% have purulent vaginal discharge (PVD) at 4 to 5 weeks postpartum, and 30 to 50% of cows have subclinical inflammation of the uterus (endometritis) and/or cervix at 4 to 8 weeks postpartum that is associated with reduced reproductive performance. Collectively, these conditions may be termed reproductive tract inflammatory disease (RTID). While distinct, they are interrelated such that approximately one-third of cows experience at least one element of RTID in early lactation. These conditions are costly for the affected cows (Overton and Fetrow, 2008) and their high incidence risks make them costly for herds and the industry. The high incidence of disease is attributable in part to reduced innate immune function (which is common and well-documented; Hammon et al., 2006) and impaired regulation of inflammation from approximately two weeks before to three weeks after calving. Insulin resistance (IR) and adaptation to negative energy balance (NEB) contribute to the degree and duration of reduced immune defence. Essentially all cows experience NEB in early lactation with IR as part of the adaptions in support of lactation (DeKoster and Opsomer, 2013). The points at which NEB, IR, or reproductive tract inflammation shift from physiologic to undesirable and the determinants of these processes are only partially understood and likely include numerous interactions. However, there are emerging lines of investigation that promise to improve understanding of, and the ability to treat and ultimately prevent these conditions.

Metabolic health and inflammation

On average, dairy cows experience a 30% decrease in feed intake in the last week before calving. There have been recent advances in knowledge of the interactions among metabolism (specifically related to insulin and fat), inflammation, and immune function in humans and laboratory animals (Osborn and Olefsky, 2012; McArdle et al., 2013) and in dairy cows (Ingvartsen and Moyes, 2013; Sordillo and Raphael, 2013). The inflammatory response in the liver is stimulated by interleukin (IL)-1, Il-6 and tumour necrosis factor (TNF)-α and is measurable by changes in serum concentrations of acute phase proteins including increased haptoglobin (Hp) and serum amyloid A and decreased albumin (Bertoni et al., 2008; Trevisi et al., 2012). One source of these pro-inflammatory mediators is mobilization of fat, and another is the inflammatory process in the postpartum uterus (LeBlanc, 2012). Mobilization of adipose, especially visceral fat, produces TNFα and IL-6 (Tilg and Moschen, 2008) which block the intracellular signalling of insulin and so contribute to IR, which in turn exacerbates release of non-esterified fatty acids (NEFA) from adipose. Additionally, NEFA may contribute directly to greater inflammation by binding Toll-like receptor (TLR)-4 (the sensor for lipopolysaccharide (LPS); Hotamisligil and Erbay, 2008). Both obese human patients, particularly with non-alcoholic fatty liver disease (Osborn and Olefsky, 2012), and high producing dairy cows in early lactation are characterized by elevated plasma NEFA, insulin resistance, and inflammation. This is consistent with the strong associations of over-conditioning in dairy cows (e.g. body condition score > 3.5 out of 5 at calving) with increased risk of fatty liver and metabolic disease. The same mechanism seems likely to contribute to metabolic and infectious disease risk in cattle that are not visibly over-conditioned, but may have metabolic inflammation associated with reduced feed intake due, for example, to inadequate feeding space (competition and social stress), to prior clinical disease, or to endocrine signals for feed intake which remain poorly understood.

Specific fatty acids (FA), particularly saturated FA which predominate in cows, appear to trigger TLR (Ingvartsen and Moyes, 2013), which provides an inflammatory stimulus in addition to LPS from gram-negative pathogens in the uterus, and further input into the vortex of hepatic or whole body inflammation. While manipulation of dietary fatty acids has been the subject of considerable research and may help to modulate inflammation (Silvestre et al., 2014), development of methods to maintain the overall magnitude of NEFA flux below the concentrations associated with compromised immune function (Ster et al., 2012) or clinical disease (Dubuc et al., 2010) is a high priority. To do so will require a better understanding of the relationship between feed intake, energy supply, and inflammation.

Inflammatory response and regulation

Uterine involution should include a prompt, robust and effective local inflammatory response but not of a degree or duration that impairs reproductive function. Loss of this regulation appears to be central to the pathogenesis of endometritis. A similar process is better described in the mammary gland (Ballou, 2012). In severe coliform mastitis, the excessive inflammatory response induced by LPS appears to be responsible for most of the clinical signs. The inflammatory response of macrophages and endothelial cells to LPS is heightened in early lactation (Sordillo and Raphael, 2013), the same time at which the pathophysiologic processes of RTID are at play. This heightened inflammatory response the product of local and systemic factors (Ballou, 2012). It is associated with the metabolic milieu of the peripartum period but may be further modulated by diet: cows fed 150% vs. 100% of energy requirement for 45 days prepartum had greater increases in NEFA, Hp and liver fat in response to LPS challenge (Graugnard et al., 2013). Therefore, diet and the effects of energy status may influence the response to LPS from the uterus and in turn, reproductive tract inflammation.

Markers of aspects of adaptation to NEB (e.g. NEFA and β-hydroxybutyrate (BHB)) are associated with the risk of many metabolic and infectious diseases, in part through their associations with suppressed immune function and excessive inflammation (Ingvartsen and Moyes, 2013). Approximately 35% of peripartum cows have NEFA and 45% have BHBA above thresholds associated with metabolic disease or compromised production or reproduction (McArt et al., 2013). In a large dataset, 44% of cows had at least 1 disease condition in early lactation, and of these, 39% had 2 or more separate diseases (Santos et al., 2010. Clinically unapparent inflammation can set off a cycle of inflammation, IR, and elevated NEFA which contributes to the interrelationships among these clinical and subclinical diseases.

Metabolic inflammation

Trevisi et al. (2012) propose that greater inflammatory status, starting before calving, is reflected in a lower “Liver Functionality Index” based on changes in concentrations of albumin, cholesterol and bilirubin in early lactation. Greater inflammation, as estimated by this index, was associated with increased circulating IL-6 and decreased serum lysozyme concentrations through the transition period, and higher Hp after calving. The authors postulate that dysregulated inflammatory response starting approximately 1 month prepartum, apparently associated with IL-6 and serum lysozyme, leads to an unhelpfully heightened acute phase

response postpartum. Elevated serum Hp in the week after calving is associated with subsequent occurrence of metritis, PVD, and endometritis (Dubuc et al., 2010). A mechanism for this is suggested by experimental simulation of pre-partum inflammation with interferon-α or TNF, which was associated with decreased glucose and increased BHBA, NEFA, reactive oxygen species, and liver fat accumulation (Trevisi et al., 2009; Bradford et al., 2009). Elevated Hp in the week after calving was associated with reduced neutrophil (PMN) oxidative burst function at weeks 1 and 2 postpartum (Wittrock, 2012). Bertoni et al. (2009) have also demonstrated an association of Hp and feed dry matter intake (DMI) in the 2 weeks before calving. Experiments with oral anti-inflammatory medication in the first week postpartum resulted in apparently unfavourable responses of lower glucose and higher NEFA and BHBA, but treated cows had greater apparent insulin sensitivity in the short term and produced more milk in the whole lactation (Farney et al., 2013a, b). Therefore, there are likely additional variables that influence the relationship between inflammation as we currently measure it and health. Furthermore, the effects of inflammatory modulation were not assessed in the reproductive tract in the work above by Bertoni, Bradford, and Farney. Given that dysregulation of immunity and inflammation is a central determinant of reproductive tract disease, better understanding of variables associated with insulin resistance and inflammatory regulation will improve both metabolic and reproductive tract health.

Regulation of innate immunity and inflammation in the uterus

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; LeBlanc 2012). Innate immunity from PMN is the predominant mechanism of early immune defence in both the udder and the uterus. It is clear that there are interactions between energy status and immune function. The fuels used by bovine PMN are not well characterized but glucose appears to crucial (Ingvartsen and Moyes, 2013). PMN glycogen stores were lower in cows that subsequently developed metritis or endometritis (Galvao et al., 2010). An excessive pro-inflammatory state early in the postpartum period is a key feature of cows with endometritis about one month 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.

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 2 and 3, and with endometritis (Dohmen et al., 1995; Gilbert et al., 2007; Williams et a 2005). 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 (LeBlanc et al., 2011). Briefly, there are strains of E. coli that appear to be adapted uterine pathogens, particularly expressing virulence factors related to adhesion (Bicalho et al., 2010; Sheldon et al., 2010). Bicalho et al., (2012) 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, 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 (LeBlanc et al., 2011), by genetic or metabolic influences on immune function and regulation, or both.

Investigation of underlying causes and on-going monitoring programs

Several practical guidance documents for investigation of health problems in transition dairy cows by veterinary practitioners or other advisors have been put forward (summarized in LeBlanc 2011). The critical principles are to investigate and ensure that all cattle have unrestricted access to feed at the time of fresh feed delivery, to clean water, and to a comfortable resting place. Biochemical tests that may be useful as elements of investigation of a problem or as part of a routine monitoring scheme are briefly reviewed below.

NEFA- In a large multi-region field study, NEFA ≥ 0.3 mmol/L was associated with increased incidence of RP (Chapinal et al., 2011). Similarly, as NEFA in the week before calving increased by 0.1 mmol/L, the odds of RP increased by 5% (Quiroz-Rocha et al.,, 2009). Cows with NEFA ≥ 0.3 (0.2 in one study region) mmol/L in the week before calving were more likely to develop metritis (OR = 1.8) (Chapinal et al., 2011). Similar large field studies (Ospina et al., 2010a, b) confirm that NEFA > 0.3 mmol/L in the 1 to 2 weeks before expected calving is associated with increased risk of RP, metritis, or displaced abomasum (DA) , decreased milk production (1.6 kg/day (Chapinal et al., , 2012) or 683 kg 305 d mature equivalent) and increased time to pregnancy. Similarly, in the 2 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 650 kg less milk in multiparous cows (Ospina et al., 2010a, b). Dubuc et al., (2010) 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 purulent vaginal discharge (PVD) or endometritis.

There was substantial and dose-dependent decrease of proliferation of blood mononuclear cells 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 (Ster et al.,, 2012). The effects on monocytes were present as low as 0.013 mmol/L NEFA and started at 0.5 mmol/L for neutrophil oxidative burst.

Ketosis - Cows with milk BHB > 100 µmol/L in the first week postpartum were 1.5 times more likely to be anovular at 9 weeks postpartum (Walsh et al., 2007a). Cows that experienced ketosis in the first two weeks of lactation had reduced probability of pregnancy at the first

insemination. Furthermore, cows that had ketosis in one or both of the first two weeks after calving had a lower pregnancy rate until 140 DIM. The median interval to pregnancy was approximately 108 days in cows without ketosis, was significantly longer (124 days) in cows with ketosis in the first or second week postpartum, and tended to be longer still (130 days) in cows that had subclinical ketosis in both of the first weeks of lactation (Walsh et al., 2007b).

Subclinical ketosis (BHB > 1.2 to 1.4 mmol/L) in the first or second week after calving was associated with 3 times greater risk of metritis (Duffield et al., 2009). Milk yield at first test was reduced by 1.9 kg/d when BHB was > 1.4 mmol/L in week 1 and by 3.3 kg/d when BHB was > 2.0 mmol/L in week 2. Cows with serum BHB > 1.8 mmol/L in week 1 had > 300 kg lower projected production for the whole lactation. A herd prevalence of > 15 % of cows with prepartum NEFA > 0. 3 mmol/L, postpartum NEFA > 0.7 mmol/L, or BHB > 1.15 mmol/L was associated with increased herd risks of DA or clinical ketosis, lower pregnancy rate, and decreased herd average milk production (Ospina et al., 2010c).

In a large field study in NY (778 cows in 38 herds) Cheong et al., (2011) reported that producer-recorded clinical ketosis (incidence = 5%) was a risk factor for endometritis (OR = 3.8), particularly in multiparous cows. However in an even larger study, Chapinal et al., (2011) found no association of producer-reported clinical ketosis or serum BHB measured systematically in week 1 postpartum with metritis. In a study with 1295 cows Dubuc et al., (2010) found that ketosis (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 that developed metritis, and similar to Dubuc et al., (2010), higher at week 1 postpartum in cows that later had endometritis (Galvao et al., 2010). Likewise, cows with metritis or endometritis had higher BHB from 1 until 4 weeks after calving, although there was no association of BHB with neutrophil killing ability (Hammon et al., 2006). In vitro titration of BHB did not affect proliferation of blood mononuclear cells or their production of IFN , or oxidative burst activity of neutrophils (Ster et al., 2012). Therefore the effect of ketones per se on immune function is at best inconsistent. It is not clear if the mechanism of fatty liver/ketosis association with diminished neutrophil function is direct (and if so whether it is on mature PMN in circulation, or whether NEFA, ketones or other signals or metabolites affect PMN in the bone marrow), or through effects on mononuclear cells that are responsible for antigen presentation and initial chemokine signalling/stimulation of neutrophils (Zerbe et al., 2000).

Administration of propylene glycol, insulin, or corticosteroids might be beneficial, but further research is needed on treatment regimes that might be effective at reducing the risk of disease or reduced performance among cows identified at high risk of these problems. Based on currently available data (Nielsen and Ingvartsen, 2004; McArt et al., 2011 and 2012), drenching with 300-500 ml of propylene glycol once a day for 5 days may be a reasonable treatment for cows with elevated NEFA or BHB.

Hypocalcaemia - Essentially all cows experience some degree of hypocalcaemia at calving and for 1 – 3 days after. There are conflicting data about thresholds of circulating calcium concentrations that may be associated with undesirable outcomes. Recently we have shown

that serum calcium concentrations < approximately 2.2 mmol/L in the week after calving, despite being within the range for healthy cows, was associated with increased odds of displaced abomasum, approximately 3 kg/d lower milk yield in early lactation, and slightly decreased odds of pregnancy at first insemination (Chapinal et al., 2011; Chapinal et al., 2012).

In large field studies no association of milk fever was found with metritis, PVD, or endometritis (Dubuc et al., 2010; Cheong et al., 2011). Chapinal et al., (2011) also found no association of serum calcium measured in week 1 (but before disease diagnosis) with the odds of metritis. Similarly, in pastured cows, Burke et al., (2010) found no association of plasma calcium through the peripartum period with endometritis at week 6, but did find that plasma magnesium was significantly lower (at 2 and 4 weeks postpartum) in cows with endometritis. However, Martinez et al., (2012) studied 110 cows in one herd in Florida, USA. Cows with Ca < 2.14 mmol/L at least once between 0 and 3 DIM had 4. 5 fold increased odds of metritis; the attributable risk of metritis for hypocalcemia was 75%. Hypocalcemia associated with decreased neutrophil oxidative burst and decreased circulating neutrophil counts at 1 and 3 DIM.

Haptoglobin - Haptoglobin (Hp) is an acute phase protein produced by the liver and associated with several inflammatory and disease conditions in cattle. Huzzey et al., (2009) found that Hp > 1.0 g/L at 3 DIM was preceded and increased the incidence of metritis (OR = 7). Haptoglobin ≥ 0.8 g/L in week 1 postpartum was associated with increased risk of metritis (OR = 2.2), PVD (OR = 2), and endometritis (OR = 1.6) (Dubuc et al., 2010). Consistent with that, Galvao et al., (2010) also found slightly higher Hp at week 1 postpartum in cows that later had endometritis.

Presently, there are few management practices or interventions that can be supported specifically to prevent RTID. 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. 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.

Table 1. Checklist of management practices to support metabolic and reproductive health in transition dairy cows.

Management

- Feed daily for 3-5% left over
- ≥ 75 cm (30”) bunk space per cow or no more than 4 cows per 5 headlocks
- ≤ 85% stall stocking density
- > 11m2 (120 ft^2)of bedded pack/cow
- Build for 130-140% of the average number of monthly calvings

- < 24 h in calving pen
- House heifers in pens separate from mature cows if possible
- Minimize group changes
- Heat abatement (sprinklers and fans) when THI > 68
- BCS = 3.0 - 3.5 at calving

Transition diet
- 3-4 weeks on close-up diet or 6 weeks as 1 dry group
- Meet but do not exceed E requirement 8 to 3 weeks prepartum
- Water ad lib; 10 cm linear per cow; 2 sources per pen
- 1000 IU vitamin E/d; up to 2000 IU/d for RP; 0.3 ppm selenium (Ideally ~ 6mg/d)

Monitoring
- Serum total calcium > 2.15 mmol/L from 1 DIM
- NEFA <0.4 mEq/L in last week prepartum; <0.7 in week 1
- BHB < 1.1 mmol/L in week 1
- BHB < 1.2 mmol/L weeks 2 - 3

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