Current concepts of prevention and treatment of postpartum reproductive tract disease in dairy cows

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Abstract

This paper briefly reviews recent data and concepts on the development and mitigation of infection and inflammation in the reproductive tract of dairy cows during the first 2 months after calving. The incidence of metritis is typically between 10 and 20 %, of clinical endometritis or purulent vaginal discharge (PVD) approximately 15%, and of subclinical or cytological endometritis a further 15%. Worse postpartum negative energy balance is associated with more severe or prolonged uterine inflammation. Changes in feed intake, expression of genes for pro-inflammatory cytokines (notably interleukin (IL) 1, IL6 and IL8, circulating concentrations of BHBA or NEFA, and innate immune function precede both metritis and endometritis by several weeks. Infections with E. coli and A. pyogenes are associated with both metritis and PVD. There are new data to suggest that specific virulence factors in E. coli associated with adherence may be important in metritis and PVD. Cytological endometritis and PVD are overlapping but largely distinct conditions, and there are emerging data that cervicitis exists both concurrent with and separate from endometritis. Much remains to be learned about what initiates and sustains harmful inflammation of the reproductive tract. Such information is necessary to develop effective treatments for the various forms of disease and more importantly to develop means to prevent endometritis and cervicitis. In particular, vaccination against specific uterine pathogens and interventions to modulate innate immune response appear to be important avenues for investigation. Presently, commonly recommended best management practices for cows in the transition period are likely to be helpful to mitigate the risk of reproductive disease.

1. Introduction

Essentially all peripartum dairy cattle experience bacterial contamination of the uterus for 2 to 3 weeks after calving, and a substantial minority have at least one form of pathology of the reproductive tract, ranging from overt systemic disease to subtle but important chronic inflammation. This high risk of disease is attributable in part to reduced immune function from approximately two weeks before to three weeks after calving. The severity of concurrent insulin resistance, reduced feed intake, negative energy balance, and weight loss contribute to the
degree and duration of reduced immune defence. 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 (RP) [1], metritis, and endometritis [2]. While metabolic (e.g. ketosis and fatty liver) and uterine disease are excessively common, the determinants of disease risk between herds or even within a herd in which cows apparently have similar nutritional and management experiences are unclear. This paper briefly reviews recent data and concepts on the development and mitigation of infection and inflammation in the reproductive tract of dairy cows during the first 2 months after calving.

2. Origins of reproductive tract disease

There is abundant evidence that the vast majority of cows have bacterial contamination of the uterus in the 2 to 3 weeks after calving [3], including bacteria associated with uterine disease. Nevertheless, the lactational incidence of metritis is typically between 10 and 20 %, of clinical endometritis or purulent vaginal discharge (PVD) approximately 15%, and of subclinical or cytological endometritis a further 15%. In a recent large field study [4], among almost 1600 cows from 3 farms, 37% had at least one of metritis, PVD, or cytological endometritis. While these incidence risks are undesirably high, they represent less than half the proportion of cows that have bacterial contamination of the uterus soon after calving. What then determines whether an infected cow will develop systemic or more subtle disease or progress through normal involution and not experience conditions that may impair fertility? Attention has focussed on the role of the immune system in clearing uterine contamination. It is clear that many or most dairy cows experience several weeks of substantial reduction of immune function around calving, typically reaching a nadir approximately one week postpartum [3, 5], although recent data have suggested that ‘phagocytic overall power’ (the product of neutrophil activity, function, and circulating numbers) may not be as impaired as commonly thought [6]. The precise causes of impaired immune function in transition cows are unclear, although the peripartum drop in energy, vitamin and mineral intake, negative energy balance and mobilization of body fat and protein, dramatic changes in progesterone and oestrogen levels in late gestation, and the massive transient increase in cortisol level at calving appear to contribute
The hormonal and energy effects of lactation appear to exert an immunosuppressive effect beyond those associated with parturition itself [9]. Cows in greater negative energy balance have more pronounced impairment of at least some immune functions [2]. Cows with RP, metritis, or endometritis have earlier and more profound impairments of innate immunity, starting several weeks before disease occurs [2, 10-12].

2. Immune defences in the uterus

The mechanisms of impairment of immune defence in the mammary gland in the transition period have been described [13] 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. Uterine immunity in dairy cows has recently been reviewed [14, 15] and readers are directed to these papers for details. It is known that cows with severe metritis ate less (2 to 6 kg of dry matter per day) than healthy cows in the 2 to 3 weeks preceding the clinical signs of metritis [16]. Lower feed intake is associated with increased circulating concentrations of non-esterified fatty acids (NEFA) which contributes to the risk of fatty liver which in turn is associated with impaired neutrophil function [17]. Additionally, NEFA have been shown to inhibit neutrophil function in vitro [18]. Because of both high metabolic demands and pathogen challenges, cattle also routinely experience substantial oxidative stress in early lactation [19], which also contributes to a pro-inflammatory state that may not be effective for immune defence [20].

Sheldon et al [15] have demonstrated that Toll-like receptor 4 (TLR4) which binds endotoxin (lipopolysacharide; LPS) is a key player in the inflammatory cascade in the bovine uterus. They have also shown that both prostaglandin type switching (F series (luteolytic) to E series (luteotrophic)) in response to LPS and the presence of TLR4 complexes in ovarian follicles may help to explain links between uterine infection and inflammation and impaired ovulation, steroidogenesis and luteal regression. See [15] for details of their framework of infection and inflammation. There is evidence in other species that fatty acids may bind to TLR4 and induce a
pro-inflammatory cascade [20], which may be an important link between lipid metabolism and innate immune function.

Retained placenta is a disease of immune function, with changes in neutrophil function and interleukin 8 (IL8) levels two weeks before calving [21]. Recruitment and function of an adequate flux of neutrophils to the uterus is also important in the days after calving for clearance of bacteria and lochia and prevention of subsequent endometritis [22]. However, there is evidence that substantially higher, apparently excessive, inflammatory status in the first [23] and perhaps second [24] week postpartum is associated with endometritis. Specifically, there was substantially greater expression of genes for the pro-inflammatory cytokines IL1A and IL1B, their receptor IL1R2, and TLR4, as well as a higher ratio of IL1 to the anti-inflammatory IL10 in week 1 postpartum among 4 cows with cytological endometritis at week 5 postpartum (that failed to become pregnant during that lactation), relative to 4 cows without endometritis that were pregnant at first AI [23]. Additionally, increased expression of pro-inflammatory cytokines IL6, IL8, and interferon γ (IFNγ), nuclear factor κB (NFKB1) which is a transcription factor for the pro-inflammatory cytokines above, and IL12A within two weeks postpartum appears to be associated with greater uterine inflammation at that time [24] and possibly later.

Measurable changes were noted in phagocytosis, TNFα and IL6 prepartum in cows with postpartum endometritis [25], weeks before disease becomes manifest, coincident with the onset of insulin resistance and lipolysis (at least in cows at higher risk of disease). Cows in greater negative energy balance, and in particular those that go on to have metritis or endometritis have more pronounced impairment of at least some immune functions [2]. Cows in a greater degree of negative energy balance prepartum, as evidenced by higher NEFA concentrations were 80% more likely to have RP, and that those with lower circulating vitamin E were at greater risk of RP [26]. This supports the notion that severe negative energy balance may contribute to impairment of immune function. This concept is further supported by recent evidence that worse postpartum negative energy balance is associated with more severe or prolonged uterine inflammation and impaired tissue repair capacity (both measured by gene expression) [27]. Among other genes, those for IL1 receptor and IL8 and its receptor (IL8Rβ),
which are associated with uterine inflammation, were substantially more expressed in cows with severe NEB.

Hammon et al [2] showed that cows with metritis or cytological endometritis had worse neutrophil myeloperoxidase activity (a measure of killing capacity after phagocytosis) 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 myeloperoxidase activity. Additionally, there was an association between lower feed intake in the 3 weeks before calving and lower neutrophil killing capacity from the week before until 3 weeks after calving. Similarly, Galvao et al [28] found associations of higher BHB and NEFA near calving with the risk of metritis and cytological endometritis, and found lower neutrophil glycogen concentrations in cows that later developed metritis or endometritis. Huzzey et al [16] did not measure immune function but similarly showed that cows that developed metritis had lower feed intake 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 [27] 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 3 weeks before calving for reproductive performance [29]. The data on the association of prepartum feed restriction with uterine health are contradictory [16, 29] and based on fairly small numbers of cows per study.

3. Role of pathogens versus immune defence in reproductive disease

Metritis and endometritis are commonly associated with mixed bacterial infection of the uterus, often including anaerobes, notably *Fusobacterium* and *Prevotella* species. Current evidence shows that *Escherichia coli* (E. coli) is particularly prevalent in the first week postpartum and is associated with metritis and with increased risk of infection with *Arcanobacterium pyogenes* in weeks 2 and 3 [30, 31]. Infection with A. pyogenes that persists beyond three weeks postpartum has been associated with endometritis. Conversely, uterine
inflammation may be present in cows with no bacteria isolated concurrent with the diagnosis of inflammation [15, 22]. 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.

3.1 E. coli

Silva et al [32] examined 72 E. coli isolates from 12 healthy cows and 18 with metritis and found no associations of phylogenetic group or specific virulence factors with disease, although strain associations with herd were noted. Sheldon et al [33] used 114 E coli isolates from 64 cows from one herd sampled between weeks 1 and 4 after calving. They found an association of phylogenetic group B2 with metritis. E. coli from cows with metritis were more adherent and invasive to endometrial epithelial and stromal cells despite a lack of 16 genes typically associated with these factors in other pathogenic E coli. These strains, coined “EnPEC” (endometrial pathogenic E coli), or their LPS could reproduce inflammation through TLR-4 in a mouse model. In cell culture, LPS from EnPEC induced a greater inflammatory response than LPS from strains not associated with metritis. These results pointed for the first time to possible variables in the type of E. coli that may contribute to the risk of metritis. Soon after, Bicalho et al [34] reported on the same question with a larger sample (611 E. coli isolates from 374 cows in 4 herds sampled in the first week after calving) that considered 32 potential virulence genes. They found 6 genes (fimH, astA, cdt, kpsMII, ibeA and hlyA; most associated with adhesion or invasion) associated with both metritis and clinical endometritis. The fimH adherence gene was present in 87% of uterine E. coli and in 29% of cows and was strongly associated with increased risk of disease: metritis was 6 times more likely in cows with E. coli with fimH relative to culture-negative cows, and the risk of metritis was highest when this gene was present with one of the other five virulence genes identified. The presence of E. coli with fimH, cdt or astA genes at week 1 postpartum was associated with 3 to 5 times greater odds of purulent vaginal discharge at 4 weeks postpartum.
3.2 A. pyogenes

*Arcanobacterium pyogenes* is consistently, though not always, associated with purulent vaginal discharge [31, 35]. The susceptibility of A. pyogenes to antimicrobial drugs is variable [36-38] but the clinical relevance of this is unclear. The presence of the adherence gene fimA has been associated with A. pyogenes from cows with metritis [37]. *Arcanobacterium pyogenes* secrete the exotoxin pyolysin [39] and based on in vitro and in vivo inoculation with killed bacteria or bacteria-free filtrate, it appears that a heat-labile component rather than A. pyogenes itself may be responsible for inducing uterine inflammation, but that intact endometrium in healthy animals may be protective [40]. Therefore, while A. pyogenes are commonly found in cows with metritis and especially with endometritis, it is not clear that there are specific strains or virulence factors of A. pyogenes associated with uterine disease [32].

4. Emerging understanding of infection and inflammation in the reproductive tract

It is clear that PVD is associated with substantial reductions in subsequent reproductive performance [4, 41-43]. It was assumed that this discharge found in the cranial vagina, or less commonly, observed externally on the vulva or tail, resulted from endometritis. There are data that demonstrate the association between the nature of vaginal content and the density of putative bacterial pathogens in the uterus [3]. However, we have recently shown [44] only fair agreement between PVD and endometritis defined by uterine cytology. This leads to the question of the source of the pus in the vagina if it is not always from the uterus. There are emerging data to indicate that cervicitis exists as a distinct condition, though sometimes concurrent with endometritis, which is associated with both separate and additive impaired reproductive performance [45-47].

Metritis and endometritis are associated with uterine infection with E. coli in the first week after calving, and PVD is associated with A. pyogenes infection that persists beyond 2 to 3 weeks postpartum [30, 31, 35, 48]. Conversely, there are conflicting data [22, Osawa and LeBlanc, unpublished data] about the association of bacterial infection with cytological endometritis. Preventive antibiotic treatment at calving reduced the prevalence of PVD but not
of cytological endometritis at five weeks postpartum [4]. In the same study, the prevalence of PVD was three times higher (15 vs. 5%) in cows with RP, dystocia or twins, but the prevalence of cytological endometritis was the same (13%) in both groups. The relationship between bacteria in the uterus and endometrial inflammation has been described [22, 49] but is not well understood, and it has been suggested that endometrial inflammation may persist after bacterial pathogens are no longer detected [15].

Endometrial inflammation appears to be an inevitable and necessary part of involution but down-regulation of the immune response within a few weeks after calving appears to be important, and apparently excessive inflammation even in the first week postpartum is associated with persistent and deleterious inflammation one month later [23]. It is not clear if excessive or persistent inflammation is provoked by the type (species, strain or virulence factors) or quantity of bacterial infection, by genetic or metabolic influences on immune function and regulation, or both. While the risk factors and pathophysiology of PVD and cytological endometritis are at least partly shared, uterine and cervical tissue trauma and bacterial infection appear to have a greater role in PVD, while regulation of the immune response appears to have a greater role in cytological endometritis. These hypotheses require further investigation both mechanistically and under field conditions.

Endometritis diagnosed based on uterine cytology is common and consistently associated with substantial impairment of reproductive performance. There is good agreement that > 5 to 8 % neutrophils in an endometrial smear at 4 to 5 weeks postpartum identifies cows with an undesirable level of inflammation (Table 1 and Figure 1). However, it may be that not all uterine inflammation after 3 to 5 weeks postpartum (coincident with the expected completion of gross uterine involution [50] is undesirable. Interestingly, in an observational study of 201 cows in 1 herd in which cytobrush cytology was conducted 4 hours after first AI (median = 78 d postpartum), cows with no neutrophils had significantly lower probability of pregnancy to that AI (39%) than cows with 1 to 15% neutrophils (58%); cows with > 15% neutrophils were not statistically different (30%) from those with 0 PMN [51]. These data suggest that perhaps in cows, as in mares, insemination provokes an inflammatory reaction that may be physiologic rather than pathologic [52 LeBlanc MM].
5. Treatment of reproductive tract disease

Treatment of reproductive tract disease has been reviewed elsewhere [50 LeBlanc 2008]. There is consistent evidence that cows with PVD have improved reproductive performance when treated with a single intra-uterine (IU) infusion of cephapirin approximately one month before first insemination, relative to receiving no treatment [41, 43, 53]. Intrauterine infusion of ceftiofur at approximately 6 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 AI protocol [54]. 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 that there is weak or no association between cytological endometritis and uterine bacterial infection [47].

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 [49]. We recently conducted a clinical trial in over 2000 cows, including over 600 with PVD, cytological endometritis or both, in which cows were randomly assigned to receive PGF at weeks 5 and 7 postpartum, or not [4]. 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 [55] for cytological endometritis.

Taken together, it appears that IU cephapirin is beneficial in cases of PVD (which may be associated with cervicitis or endometritis) but that PGF as commonly employed is not. While there is one study [56] that 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 such treatments 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.
6. Prevention of reproductive tract disease

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 negative energy balance and circulating free fatty acid concentrations, and excessive insulin resistance contribute to a state of (metabolic) ‘meta-inflammation’ that may actually impair neutrophil function [20]. 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 2 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.

To explore the hypothesis that reduction of the load of potential bacterial pathogens in the uterus immediately after calving could reduce the risk of uterine disease we recently conducted a large clinical trial in which over 1000 cows at high risk of uterine disease (having had retained placenta, dystocia or twins) were randomly assigned to receive a long-acting antibiotic (1 injection of ceftiofur crystalline free acid) that was expected to be effective against E. coli, A. pyogenes, and relevant anaerobic bacteria, or to be untreated [4]. There were conditional and modest reductions in the incidence of metritis, and a significant overall reduction in the prevalence of PVD at five weeks postpartum from 28 to 20%. Further studies are needed to better identify selection criteria for metaphylactic treatment of cows for uterine disease and to identify treatments that result in greater reductions in the incidence of metritis or endometritis. While we do not recommend preventive treatment with antibiotics, our results nevertheless support the notion that the degree or nature of bacterial contamination soon after calving is one component of both metritis and purulent vaginal discharge one month later. On the other hand, on balance it appears that innate immune function may be an even more important determinant of reproductive tract health [15].
7. Conclusion

Infection and inflammation of the uterus and cervix affect approximately one dairy cow in three, with substantial impacts on the probability and timing of pregnancy. While there are well-validated diagnostic tools and criteria for both PVD and cytological endometritis, and an efficacious treatment for PVD, much remains to be learned about what initiates and sustains harmful inflammation of the reproductive tract. Such information is necessary to develop effective treatments for the various forms of disease and more importantly to develop means, from genetic to pharmacologic to nutritional and management, to prevent endometritis and cervicitis. In particular, vaccination against specific uterine pathogens and interventions to modulate innate immune response appear to be important avenues for investigation. Presently, commonly recommended best management practices for cows in the transition period are likely to be helpful to mitigate the risk of reproductive disease.
Table 1. Summary of studies of the prevalence of endometritis diagnosed by cytology in postpartum dairy cows.

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>DIM</th>
<th>Diagnostic method</th>
<th>Prevalence</th>
<th>Impact¹</th>
<th>Median DTP²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasimanickam et al 2004 [57]</td>
<td>228</td>
<td>20 to 33</td>
<td>Cytobrush &gt; 18% neutrophils</td>
<td>35% (excluding cows with PVD³ the day before)</td>
<td></td>
<td>112</td>
</tr>
<tr>
<td>Galvao et al 2009 [54]</td>
<td>202</td>
<td>48 to 54</td>
<td>Flush ≥ 5% neutrophils</td>
<td>29% (not excluding cows with PVD)</td>
<td></td>
<td>112</td>
</tr>
<tr>
<td>Galvao et al 2009 [55]</td>
<td>406</td>
<td>32 to 38</td>
<td>Flush ≥ 7% neutrophils</td>
<td>38% (not excluding cows with PVD)</td>
<td></td>
<td>121</td>
</tr>
<tr>
<td>Dubuc et al 2010 [44]</td>
<td>2072</td>
<td>32 to 38</td>
<td>Cytobrush ≥ 6% neutrophils</td>
<td>20% (13% cytological only and 7% both cytological and PVD)</td>
<td></td>
<td>132</td>
</tr>
</tbody>
</table>

¹ All differences in DTP P < 0.05 in survival analysis
² DTP = Days from calving to pregnancy, accounting for censored animals (those that did not become pregnant)
³ PVD = Purulent Vaginal Discharge
Table 2. Summary of management practices and monitoring targets to reduce the risks of metritis, purulent vaginal discharge and cytological endometritis in dairy cows.

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Prevent consumption of dietary energy above requirement in the ‘far-off’ dry period (weeks 8 to 3 before calving)</td>
<td>60, 61</td>
</tr>
<tr>
<td>Provide for unrestricted feed bunk access (i.e. all animals able to eat at the time of fresh feed delivery) i.e. 75 cm of linear bunk space per cow or no more than 4 cows per 5 headlocks</td>
<td>62, 63</td>
</tr>
<tr>
<td>Provide space to allow for lying approximately 12 h per day ≥ 1 free stall per cow or 10 m² of bedded pack per cow</td>
<td>63, 64</td>
</tr>
<tr>
<td>Minimize pen moves and social group changes</td>
<td>63</td>
</tr>
<tr>
<td>Build dry cow and fresh pens for approximately 140% of the expected average number of calvings per month</td>
<td>63</td>
</tr>
<tr>
<td>Provide heat abatement (fans and sprinklers) when the Temperature-Humidity Index exceeds 72</td>
<td>65</td>
</tr>
<tr>
<td>Manage nutrition so that 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>66</td>
</tr>
</tbody>
</table>

**Monitoring methods and targets (Serum or plasma tests)**

<table>
<thead>
<tr>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA &lt; 0.5 mmol/L in the week before expected calving</td>
<td>67</td>
</tr>
<tr>
<td>BHB &lt; 1.1 mmol/L in week 1 and &lt; 1.4 in week 2 after calving</td>
<td>67</td>
</tr>
<tr>
<td>Haptoglobin &lt; 0.8 g/L in week 1 after calving</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 1. Distribution of cytobrush endometrial cytology in cows from six herds examined at week 5 (n = 2072) and week 8 (n = 2022) postpartum. Data are from Dubuc et al 2011 [4].
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