Postpartum discharges: myths and reality

John Cook BVSc DCHP MRCVS, RCVS-recognised specialist in cattle health and production, Yew Tree House, Cumbria, UK, discusses the presence of postpartum discharges in dairy cows

Reproductive-tract disease is common in the two months post-calving, with up to half of all cows being affected by at least one of metritis, endometritis, purulent vaginal discharge (PVD) or cervicitis (Le Blanc, 2014).

These conditions may appear as a range of clinical effects from severe systemic illness to an unapparent yet substantial impairment of fertility and poorer than expected milk production.

This high incidence of disease is attributable, in part, to both a reduced innate immune function and impaired regulation of the inflammatory response in the period lasting from approximately two weeks pre-calving to three weeks post-calving. The innate immune system is the main defence mechanism of the uterus and an inadequate or reduced immune response resulting in a failure to clear bacteria or a poorly regulated response resulting in persistent inflammation appear to be a potential mechanism by which disease can occur.

Almost all dairy cows experience bacterial contamination of the uterus post-calving (Sheldon et al, 2009) and because of this, as well as the considerable repair and restoration of the endometrium that is required, inflammation is a normal and indeed necessary component of involution.

Postpartum reproductive-tract disease may represent a failure of the required immune shift from the state required for the maintenance of pregnancy to the heightened state needed for the clearance of bacteria and tissue repair post-calving before returning to a 'quiet' state some three to four weeks later (Le Blanc, 2013). The severity and duration of the innate immune response appears critical to the development of disease and whether unhelpful inflammation resulting in impairment of fertility can persist (Le Blanc, 2012).

The factors determining the timeliness and effectiveness of the innate immune response remain unclear, although the role of metabolic influences, such as insulin resistance (IR) and negative energy balance, (NEB) are becoming clearer. The severity of IR and NEB experienced by some cows, may go beyond that needed for the homeorhetic adaption to lactation and become a contributory factor to the reduced

and poorly regulated responses of some cows.

INFLAMMATION AND METABOLIC HEALTH

The term "metabolic inflammation" has been used to describe the growing understanding of the links and interactions between energy and fat metabolism and inflammation (Sordillo & Raphael, 2013). These interactions have been studied in humans and laboratory animals (Osborn & Olefsky, 2012) and are being investigated in the dairy cow as they appear pivotal to transition cow health (Trevisi et al, 2012) and may be useful in understanding the determinants of an effective inflammatory response in postpartum dairy cows.

Fat is not only a storage tissue for energy in the form of glycerol and fatty acids, but is also metabolically active, releasing proinflammatory mediators tumour necrosis factor-α (TNFα) and interleukin 6 (IL6) during fat mobilisation, which block intracellular signalling of insulin and contribute to IR in dairy cows (DeKoster & Opsomer, 2013).

Both dairy cows and obese-human patients share the metabolic similarities of elevated circulating non-esterified fatty acids (NEFA), IR and inflammation, despite the contrasting situations of fat mobilisation and hypoglycaemia versus obesity and hyperglycaemia. These similarities relate to the interactions between the innate immune inflammatory responses and energy and fat metabolism and particularly, the loss of feedback against excessive rates of fat mobilisation mediated by the action of TNFα, which in turn leads to further IR and release of NEFA. This mechanism is consistent with the strong association of over-conditioned dairy cows and the risk of fatty liver and metabolic disease.

Cows that experience reduced feed intake are also likely to increase mobilisation of fat reserves and begin spiralling into a similar metabolic and inflammatory state. Fat mobilisation increases the supply of NEFA, which brings with it, the release of the proinflammatory cytokines TNFα and IL6, which, in turn, suppress intracellular signalling of insulin and contribute to IR, which again, in turn, can contribute to further release of NEFA.

The high-metabolic demands and pathogen challenges in early lactation result in substantial oxidative stress also contributing to a heightened pro-inflamatory state by activating the nuclear factor-κβ (NFκβ) pathway and increasing the production of TNFα by immune cells leading to greater IR (Sordillo et al, 2009). NEFA themselves may contribute directly to the inflammatory cascade by binding to toll-like receptor 4 (TLR4) the main pathogen-associated receptor for lipopolysaccharide (LPS) and initiating further TNFα release (Hotamisligil & Erbay, 2008). Polymorphonuclear (PMN) function may also be impaired by NEFA and particularly, the saturated fatty acids stearate and palmitate which also trigger TLR4 providing a further inflammatory stimulus.

As the concentration of the proinflammatory saturated fatty acids rise in the transition dairy cow, other fatty acids ,such as the polyunsaturated fatty acids (PUFA) decrease (Lock et al, 2009). A number of PUFA and, in particular, the n3 series, such as eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), activate peroxisome proliferator-activated nuclear receptors, which generate antiinflammatory responses.

While scope exists to alter the amounts of PUFA by dietary supplementation, maintaining the overall magnitude of the flux in NEFA below concentrations associated with reduced immune function (Ster et al, 2012) or clinical diseases, remains problematic and further investigation is necessary (Dubuc et al, 2010). An intriguing aspect of metabolic health, is the recent finding in mice regarding the role of intestinal microflora and its interaction with inflammation and metabolism (Henao-Mejia et al, 2012).

Dietary changes around calving may result in the unintended consequence of rumen acidosis altering the bacterial populations in the rumen, potentially increasing the uptake of LPS from gram-negative bacteria and lipoteichoic acid (LTA) from gram-positive bacteria. It is possible that the flux of either these or similar products of a shifting bacterial population may also influence inflammation and metabolism through mechanisms yet to be fully explored in ruminants. LPS, aside from inducing a potent proinflammatory response, also reduces feed intake (Mani et al, 2012) and contributes to sickness behaviour.

REGULATION OF THE INNATE IMMUNE SYSTEM AND INFLAMMATORY RESPONSE

The mechanisms of pathogen detection, immune response and regulation in the uterus of dairy cows have been described (Sheldon et al, 2009; LeBlanc, 2012) and a temporal relationship with the metabolic changes is known to exist (Goff & Horst, 1997). PMN leucocytes or neutrophils are the predominant cells involved in the early response to infection, while macrophages are important for the initial recognition of infection and initiation of the inflammatory response.

Changes in PMN function precede the occurrence of reproductive-tract disease. In cows that develop retained placenta, the ability of PMNs to migrate is decreased two weeks prior to calving (Kimura et al, 2002).

Associations between phagocytosis functions of PMN and endometritis are inconsistent (Kim et al, 2005) and phagocytosis appears to be relatively stable in the transition period (Ster et al, 2012) and that overall phagocytic power (the combined effect of PMN activity, function and circulating numbers) is stable to increased (Sander et al, 2011) during this time. A more consistent association exists with impaired PMN-oxidative burst function and the occurrence of metritis and endometritis (Hammon et al, 2006).

More recently, both increased numbers of PMNs in the uterus and circulation in the first week post-calving have been associated with improvements in reproductive performance (Aungier et al, 2014, Gilbert & Santos, 2016). The goal is to achieve a robust, prompt and effective response that is constrained and beneficial to future reproduction rather than the more severe or prolonged


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response that appears to impair future reproduction. Loss of this regulation appears to be central to the pathogenesis of endometritis.

The risk of many metabolic and infectious diseases is associated with transition and the switch to lactation. Failure to adapt to the demands of lactation and more specifically the degree and duration of NEB provides mechanisms leading to further immune suppression, poor immune regulation and excessive inflammation (Ingvartsen & Moyes, 2013).

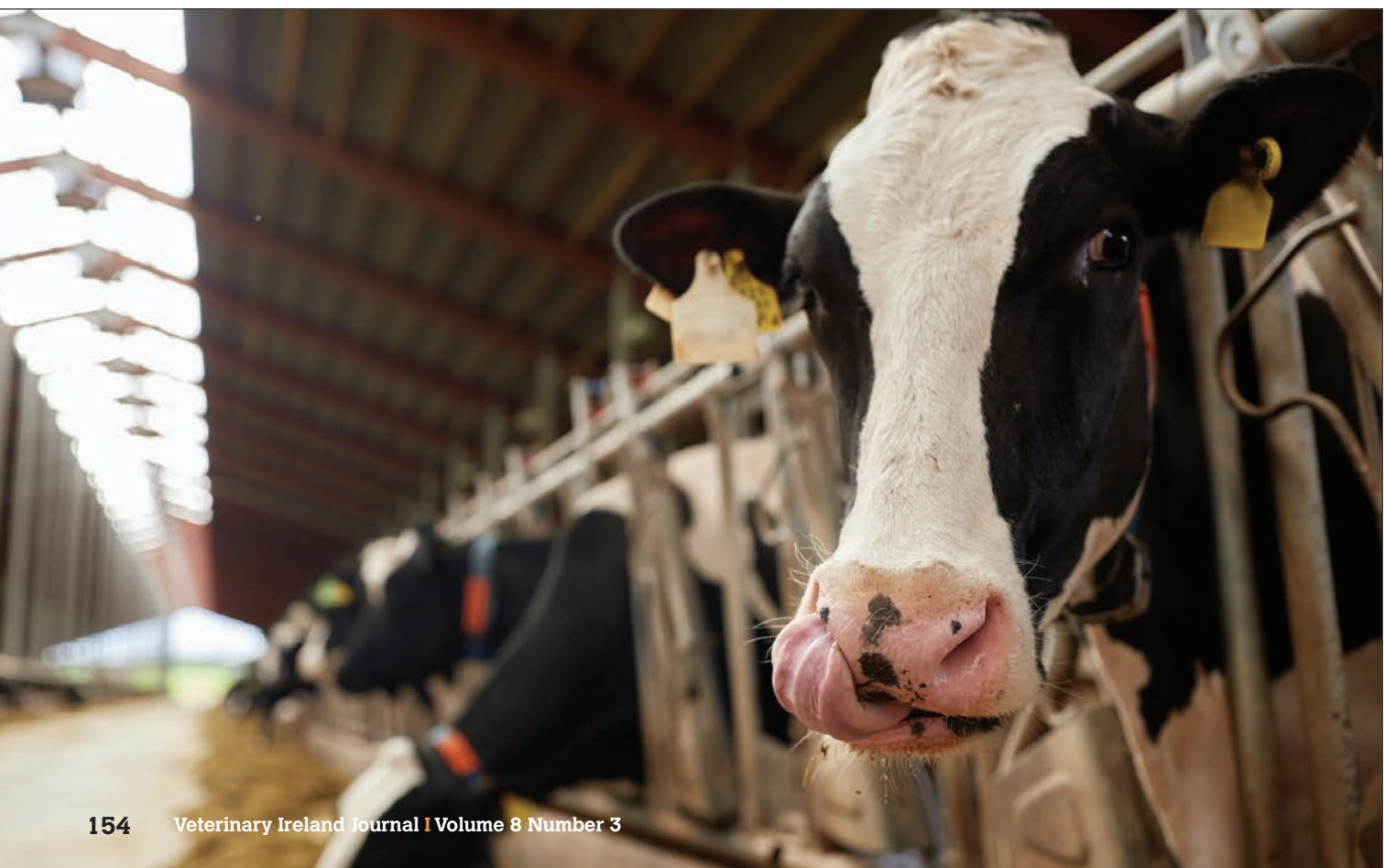
All dairy cows most likely experience IR in early lactation and 30-35% have NEFA and 45% beta-hydroxybutyrate (BHB) above thresholds associated with metabolic disease and compromised performance (McArt et al, 2013) and while it is clear that pathogens can provoke a cycle of systemic inflammation that exacerbates IR and exposing the cow to further metabolic and infectious disease risk post-calving, an increasing probability exists suggesting that non-pathogen-associated inflammation may also be able to initiate a similar, if less severe, cycle. Trevisi et al (2012), hypothesised that a dysregulated immune response resulting in a heightened inflammatory status occurring approximately one-month pre-calving would lead to an unhelpful rise in the acute phase-response postpartum and is consistent with the findings of Dubuc et al (2010), who observed that elevated haptoglobin in the week post-calving was associated with the subsequent occurrence of metritis, PVD and endometritis.

Bacterial infection is considered to initiate inflammation of the uterus itself and that this inflammation is a normal adaptive response. This response may however be inadequate, allowing the balance to tip in favour of the infection or disproportionate and excessive in severity and duration.

Currently, it is not clear if excessive or prolonged inflammation is provoked by the quantity, type or species of bacteria or by genetic and metabolic influences on immune function. *Escherichia coli*, during the first week postpartum, are particularly prevalent and associated with metritis and increased risk of infection with *Trueperella pyogenes* in the second and third weeks, and with endometritis (Gilbert et al, 2007).

Both metritis and endometritis are associated with mixed bacterial infections of the uterus, often including the anaerobes *Fusobacterium necrophorum* and *Prevotella* species. Strains of *E coli* (Bicalho et al, 2010; Sheldon et al, 2010) appear to be adapted as uterine pathogens and specific virulence factors have been associated with *E coli*, *T pyogenes* and *F necrophorum* in both metritis and PVD (Bicalho and others 2012). The presence of α-haemolytic *Streptococcus* species in endometrial samples at seven days postpartum has also been associated with improved reproductive performance (Gilbert & Santos, 2016). Reduced killing ability of PMNs is associated with NEB, but the mechanisms of this effect are not clear although intracellular glycogen stores of PMNs are lower in metritis cows for up to six weeks postpartum (Galvaõ et al, 2010) and killing ability measured as myeloperoxidase activity is lower from one-week pre-calving to four weeks post-calving (Hammon et al, 2006).

Fatty acids also directly affect PMN function (Ster et al, 2012) but, at best, these effects only explain approximately 20% of the variation in killing capacity of PMNs between cows.



Feed-restriction models, where NEFA concentrations are raised to levels associated with NEB, show minimal differences in PMN function in response to intramammary challenge with *Streptococcus uberis*, suggesting there may be other component causes of immune suppression in early lactation, while more recently, McDougall et al (2017), have demonstrated that substantial energy deficiency in the month before calving did not blunt the response to pegbovigrastim treatment and that responses in circulating PMN number and function, were similar to cows in energy balance.

In most cows, inflammation leads to bacterial clearance and repair of the endothelium with a concurrent cessation of the inflammatory response.

For most cows undergoing normal involution, the balance between the level of infectious challenge versus the effectiveness of the immune response is unknown. A more complete assessment of the cows' attitude and feed intake in conjunction with monitoring of rumen and abomasal function and ketosis status, should be carried out.

Treatment of metritis is currently based on the use of systemic antibiotics (LeBlanc, 2008) while the addition of anti-inflammatory treatment, although appearing rational has not been shown to provide any additional benefit (Drillich et al, 2007).

Cure rates are reported in the region of 75-80% versus 55-60% in control cases (Chenault et al, 2004; McLaughlin et al, 2012). Spontaneous clinical resolution is reported and a delay in the onset of treatment adopting a 'wait-and-see' approach may be justified in some cows (Sannmann et al, 2013).

PURULENT VAGINAL DISCHARGE (PVD) AND ENDOMETRITIS

PVD, or the presence of purulent discharge in the cranial vagina, typically affects around 5-20% of cows during the fourth and fifth weeks post-calving and is associated with impaired reproductive performance (Lima et al, 2013). PVD can occur in the absence of concurrent endometritis. In cases of PVD without concurrent endometritis, the cervix has been shown to be the source of pus, with about 15-40% of cows being affected by cervicitis at round one-month post-calving based on cytobrush cytology >5% PMN. Cervicitis exists as a distinct condition associated with an impaired reproductive performance separate to that of endometritis, however, where both conditions are concurrent, the detrimental effects on reproduction are additive (Deguillaume et al, 2012). Approximately 50% of cows with PVD have cervicitis and vice versa, while 50-75% of cows with endometritis have cervicitis and vice versa.

Endometritis is often associated with the presence of a purulent discharge in the cranial vagina but, it is also apparent that endometritis often also occurs without clinical signs and requires assessment by endometrial cytology or uterine lavage, which may be impractical for rapid cow-side diagnosis. Between 10 and 30% of cows have been shown to have subclinical endometritis at one to two months post-calving, resulting in estimates that over 50% cows have some form of subclinical inflammation of the uterus and/or cervix between four to eight weeks post-calving that is associated with reduced fertility.

The ultimate goal of treating cows with PVD and endometritis is to significantly improve their reproductive performance and restore milk production in the current lactation with the consequent reduction in economic losses. The value and efficacy of treatment for PVD and endometritis remains equivocal and controversial (Lefebvre & Stock, 2012). The scientific merit of many studies is compromised by lack of precise case definitions and outcomes, lack of negative controls and low statistical power to detect true treatment differences. High-spontaneous cure rates, of the order of 60-70%, also reflect the natural-resolution processes during uterine involution and may minimise the need for treatment (LeBlanc et al, 2002; Dubuc et al, 2011). The rationale behind intrauterine antibiotics is the reduction of the number of pathogens within the uterine lumen and hence, mitigate against the associated inflammation, enhancing immune defence and facilitating repair of the endometrium for a faster return to normal health. Logically, antibiotics effective against *T pyogenes* should be chosen as the most prevalent pathogen present in case of endometritis. Intrauterine administration of antibiotics is expected to reach higher levels in the endometrium than when compared to systemically administered (Bretzlaff, 1987).

Clinically, intrauterine antibiotics may reduce bacterial numbers present at the cervical orifice and in the uterus and the prevalence of discharges. Resolution and absence of vaginal discharges however, does not imply return to normal fertility. PGF2α is an endogenous hormone, which causes lysis of the corpus luteum and muscular contractions of the uterine tract, thereby, eliminating the corpus luteum and the immunosuppressive effect of progesterone.

An ensuing oestrus stimulates uterine contractility and triggering neutrophils and immunoglobulins to enter the reproductive tract. In the absence of a corpus luteum, PGF2α increases leukotriene B4 secretion by the uterus, which supports a number of immune functions including chemotaxis, cell-mediated cytotoxicity, phagocyte and lymphocyte function potentially stimulating involution and reducing the risk of prolonged infection and inflammation.

For over two decades, prostaglandin F2α (PGF2α) has been used in the treatment of uterine disease. It is inexpensive, easy to administer and avoids the problems associated with damage to the reproductive tract from intrauterine infusion and potential milk-residue issues.

Many studies have tried to assess the effect on reproductive performance of routine treatment with PGF2α on cows with a spectrum of postpartum conditions, often with conflicting results (Lefebvre & Stock, 2012; Haimerl et al, 2013).

RISK FACTORS ASSOCIATED WITH REPRODUCTIVE-TRACT DISEASE

A number of risk factors are associated with metritis, endometritis and PVD, however, no single study has, to date, included all known risk factors and many studies suffer from issues with sufficient statistical power to detect possible effects when disease incidences are low, making it difficult to rank or quantify known risk factors.

Calving difficulties, retained placenta, NEB and reductions in neutrophil function are all consistently associated with reproductive- tract disease post-calving, while the effect of parity, season of calving and milk yield are inconsistent and, at times, contradictory. Differences in gene expression may be related to the occurrence of reproductive-tract disease (Le Blanc, 2012).

- Dry cows off to ensure a target dry period length of 40-60 days for at least 85% of the herd.
- Prevent overconsumption of energy above requirement during the 'far off' period from dry-off to three weeks pre-calving.
- Provide unrestricted feed access at all times for all transition groups, far off, close-up and fresh.
- Ensure feed is provided *ad lib* and all cows can eat at the same time when fresh feed is delivered.
- Provide a minimum of 75cm of utilisable trough space per cow.
- Provide at least 10cm of water-trough space per cow sited at a least two points in the pen.
- Provide adequate cow comfort with cubicles or open yards to ensure at least 11-12 hours lying time per cow per day. At least one cubicle per cow and for open-bedded areas at least 10m^2 per cow plus an additional 1m^2 per additional ,000kg of milk production over 10,000kg.
- Minimise group and social changes. Dry-off and fill pens at no less than seven-day intervals.
- Ensure each cow spends a minimum of 10 days in the close-up group.
- Dry cow and fresh pens should have sufficient capacity to cope with variations in calving pattern. Build to 130% of average monthly capacity.
- Ensure excellent ventilation of dry and fresh cow buildings. If necessary provide heat abatement, fans and sprinklers for when Temperature Humidity Index (THI) exceeds 68.
- Manage overall nutrition for target body condition (BCS) at calving of 3-3.25 (on a five-point scale) and maintain a minimum of 2.5 with no change from dry-off to calving.

Table 1: Suggested management practices to reduce the risk of reproductive-tract disease.

Although, it is possible that each reproductive-tract disease may occur alone, it is common for cows to be affected by more than one of metritis, cervicitis, PVD or endometritis, either at the same time, consecutively or overlapping.

PREVENTION OF REPRODUCTIVE-TRACT DISEASE

Little is known about how the incidence of reproductive-tract disease may be prevented by changes in management and nutrition. It has been shown that cows with metritis ate less (2-6kg of dry matter per day) than healthy cows in the two to three weeks preceding the occurrence of disease (Huzzey et al, 2007) and that this coincides with reduced myeloperoxidase activity of neutrophils around this time (Hammon et al, 2006). Increased circulating concentrations of NEFA are associated with lower feed intake, which can directly (Ster et al, 2012) or indirectly impair neutrophil function (Hammon et al, 2006). Early lactation also precipitates high-metabolic demands and pathogen challenges creating increased oxidative stress (Sordillo et al, 2009) also adding to a pro-inflammatory state unhelpful for immune defence.

Currently, very few management practices can be supported specifically to prevent reproductive-tract disease and the general objective is to support and maintain the function of the innate immune system (Ingvartsen & Moyes, 2013) and so, attempt to reduce the risk of the inevitable bacterial challenge and inflammation post-calving proceeding to metritis, cervicitis, PVD or endometritis. Excess NEB and IR or failure to adapt to the energy demands of early lactation also contribute to impaired immune defence.

While there is much to be learned regarding the impact of management practices on post-natal reproductive-tract disease, those generally recommended for periparturient cows are likely to assist in reducing the risk of disease post-calving and are suggested in Table 1.

SUMMARY

The incidence of reproductive-tract disease is determined by the balance between bacterial infection of the uterus in the weeks following calving, and the effectiveness of innate immune response in providing an adequate, yet appropriately restrained inflammatory response. A better understanding of the links between metabolism and inflammation may lead to better approaches to prevention through nutrition and management strategies, as well as allowing a more targeted approach to therapy. Vaccination against specific uterine pathogens and therapies aimed at modulating the innate immune response, appear to be avenues that merit further investigation.