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International Symposium on Ruminant Physiology: The immunometabolism of transition dairy cows from dry-off to early lactation: lights and shadows

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ABSTRACT

The mismatch between the nutrient intake from the diet and the output by the mammary gland causes a negative energy balance in transition dairy cows, that, if excessive, can promote several metabolic disorders. Other relevant phenomena occur during transition, such as inflammation at calving and changes in immunocompetence, redox balance, and mineral metabolism. Despite the efforts, some aspects of the adaptive mechanisms observed in the transition period still need to be clarified. For instance, alterations of physiological responses even before the dry-off or during the dry period can affect the success of the whole transition period in certain cows. In this context, the mechanism regulating the inflammatory response around calving may play a pivotal role, as suggested by the variety of factors influencing it and its consequences, particularly feed intake depression, that can amplify and anticipate the negative energy balance. When this mechanism derails is still unclear, but detecting the triggers of diverted or abnormal physiological responses and where they stem (e.g., liver, rumen and gut epithelia, uterus, or mammary gland) will help to discover the weak points in the immune system and the possible ways of restoring it. Furthermore, the postpartum healthy cow appears to have an acute phase response at the liver level, despite a decrease in circulating proinflammatory cytokines. What is physiological and what is pathological in this context? To understand the latter, finding markers of an unsuccessful transition period that go beyond the energy deficit would be advisable. Future efforts should be dedicated to clarifying the causes of the acute phase response at calving, exploiting the potential of the system biology. Moreover, it would be helpful, for both basic and applied research, to define biomarkers associated with pathological responses (i.e., cytokines and acute phase proteins) and to introduce in the genetic selection phenotypes related to the ability of cows to adapt to the immunometabolic stress typical of the transition period.
Keywords: Inflammation, mammary gland, liver, acute phase response

INTRODUCTION

The complexity of the periparturient period has been recognized for a long time. Dairy cows experience a variety of challenges from late gestation to the onset of lactation: some of them are unpredictable and depend mainly on the environment, but others are strictly related to the physiological adaptation to this complex phase. These include the coordinated endocrine regulation of the conceptus growth (fetus and fetal membranes), the renewal process of the mammary gland (active involution, resting state, and redevelopment), and the preparation for calving. These physiological processes alter the nutrient partitioning among the different body functions in the periparturient cow (Bauman and Currie, 1980). Three challenging phases are particularly critical from the endocrine, immune, and nutritional points of view: the dry-off, with the milking cessation and the mammary involution process; the last month of pregnancy, accompanied by the quick growth of fetus; and the delivery, with the resumption of the galactopoietic activity. Up to 4 diet changes may be applied between late and early lactation to adapt the cows at different phases. Each dietary change requires the adaptation of the gastrointestinal tract, affecting the microbiota, altering the digestive processes, and promoting the remodeling of epithelia and organs. To make these diet changes possible, cows are frequently moved to different pens and often exposed to a distinct hierarchical structure within the group, which leads to additional stress related to the establishment of

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new social interactions, with possible interference with the welfare of the cows.

The adaptation to the peripartum phase: the onset of ketosis beyond the simple energy deficit

The first critical phenomenon hindering peripartum cow's health that captured the interest of the scientific community was the mismatch between energy and nutrients provided by feed intake and those required to satisfy metabolic demands, primarily those driven by mammary gland uptake (Shaw, 1956). It became clear that the shortage of energy and nutrients around calving was accompanied by a massive mobilization of body reserves (mainly from adipose and muscle tissues) to sustain energy requirements and liver gluconeogenesis (Drackley, 1999). This condition predisposes the cow to develop the so-called primary ketosis (Bell, 1995), the most typical metabolic disease of the postpartum phase, supported by insufficient availability of glucose substrates in the liver, incomplete oxidation of nonesterified fatty acids (**NEFA**) and the release of ketone bodies (as BHB). Classically, the approach used to investigate the pathogenesis of ketosis was based on the paradigm that, especially in high-yielding cows, the nutrient flux cannot match the primary metabolic pathways related to the nutrients in the peripartum. Thus, the NEB around parturition has been related quite exclusively to the insufficient feed intake, and the most probable factor for ketosis etiology has mainly been retained the unbalanced energy and nitrogen metabolism. As the imbalance between these 2 processes begins a few days before calving and reaches the peak during the first weeks of lactation, management strategies aimed to increase the energy intake (i.e., promoting feed intake or increasing the energy density of the diet during the dry period) were investigated. The latter would, in turn, minimize lipid mobilization, the risk of insufficient NEFA oxidation by the liver, and the consequent ketone release. However, the establishment of ketosis was often cow-specific, and mostly independent of management practices and diet formulation adopted in the herd (Grummer, 1993), suggesting that ketosis onset would not be related only to the amount of energy provided by the diet. Moreover, Shaw (1956) first hypothesized an involvement of the liver in the ketosis progression, with the organ that resulted "enlarged and exhibited fatty degeneration." Now it is clear that when the hepatic uptake of NEFA exceeds the oxidation and secretion capacity by the liver, fat infiltrates the liver tissue, with excess lipids being stored as triacylglycerol (Drackley, 1999) and, although most high-yielding cows are in severe NEB condition at the onset of lactation (Carvalho et al., 2014), only up to the 50% of them develops this pathological condition, named fatty liver (Grummer, 1993; Bobe et al., 2004). Later, Baird (1982) realized that other organs besides the liver were involved in ketosis etiology, such as the central nervous system and the hypothalamus-pituitary-adrenal axis, and concluded that any research aimed at understanding disease etiology "should be directed toward understanding mechanisms conferring priority on milk production and regulating appetite" (Baird, 1982). This view has been confirmed in the last 2 decades only, when it became clear that the partitioning of nutrients across the multiple metabolic pathways of peripartum cows was not only driven by the nutrient availability but also by other programmed alterations of the enzymatic activities occurring in several organs, with a major role in determining the severity of the NEB experienced by the cow. Feed-restricted mid-lactating cows have reduced mobilization of body reserves compared with early lactating ones with a similar energy balance and have different liver gene expression, suggesting a different endocrine regulation in the 2 phases of lactation in this organ (Gross et al., 2011). Moreover, adipose tissue and skeletal muscle, which also have relevant endocrine roles, undergo their deepest changes during the transition period (Contreras et al., 2018; Sadri et al., 2023), confirming the role of dysregulations in multiple metabolic pathways in the onset of metabolic diseases in the post-calving period.

The updated view on transition cow metabolism: the role of the immune system

The interactions between metabolism and the immune system (i.e., immunometabolism) have been investigated for decades in peripartum cow biology, with recent studies continuing to refine our understanding of these complex processes. The most investigated response to assess the immunocompetence of the organism is inflammation, a general defense provided by the innate immune system against a wide range of threatening agents (both biotic and abiotic) (Medzhitov, 2008). Once established, inflammation diverts nutrients from tissues to support the immune cell activity. In peripartum cows, several phenomena concur in establishing inflammatory conditions in multiple organs and, almost physiologically, even at a systemic level, as inflammation is necessary for several processes occurring in this physiological phase (i.e., placental expulsion, uterine involution, and adipose tissue remodeling). Despite that, exaggerated inflammation could have detrimental effects on several metabolic functions, and the magnitude and duration of inflammatory conditions experienced by peripartum cows are well related to the health status, performance, and metabolism of several organs (Trevisi and Minuti, 2018). Of interest, experimental administration of proinflammatory cytokines (**PIC** – as tumor necrosis factor α – **TNFα**) to

dairy cows through subcutaneous injections promoted triglycerides storage in the liver (Bradford et al., 2009), confirming the role of inflammation in the liver impairment that occurs in peripartum. Furthermore, oxidant species, including reactive oxygen and nitrogen species, released not only by activated leukocytes during inflammation but also by cells in organs with high respiratory rates and energy demands (such as the mammary gland and skeletal muscle), could promote the establishment of an oxidative stress condition in periparturient cows. This condition may impair cell communication and induce cellular damage. Thus, is not surprising that the immune system exerts a documented role in the etiology of several metabolic diseases, including ketosis (Horst et al., 2021), and clues supporting this view have been provided decades ago. Since the '50s, Shaw (1956) underlined that "ketosis is frequently accompanied by various complications, such as metritis, retained placenta, nephritis, and other abnormalities." Of interest, a recent clinical trial highlighted that bacteremia or endotoxemia and systemic inflammation are potentially involved in the development of clinical ketosis and that targeting lipolysis and inflammation with the treatment protocol can improve recovery (Chirivi et al., 2023). In this context, a probable role of pathological diseases independent of the energy balance of the cow could be considered as having a pivotal role in ketosis etiology.

Grummer (1995) was the first to define as transition period the high-risk period to develop multiple metabolic diseases, encompassing the 3 wk before and after calving. He also suggested that "feed intake is usually decreased 30 to 35% during the final 3 wk prepartum," although the energy-protein deficiencies become evident only in early lactation (Grummer, 1995). Thus, the primary cause of the pathological nutrient deficiency accompanying ketosis progress was difficult to identify: did it really depend on the cow's inability to ingest an adequate amount of nutrients, or was the decline in intake caused by other preexistent pathological conditions? In a magistral review, Drackley (1999) highlighted that several knowledge gaps still hindered the comprehension of the transition cow's biology and that the relationship linking the onset of ketosis to the inadequate feed intake, the dysregulated lipid mobilization, and the occurrence of lipid infiltration in the liver still required deeper research efforts to be fully elucidated. A tentative visualization of the crosstalk between the immune system and metabolism of the postpartum cow is provided in Figure 1. Nowadays, most of these gaps remain open, and even in the most recent updates, authors agree on the fact that "factors predisposing cows to metabolic, physiological, and immune dysfunction may precede the negative manifestations of production and health commonly credited to ketosis" (Rico and Barrientos-Blanco, 2024). Based on this evidence, understanding the mechanisms tuning the adaptation of dairy cows to the new lactation requires extending the explored phase behind the peripartum, to consider alterations affecting the immune system and the endocrine-metabolic asset way before calving.

A RE-EVALUATION OF THE CONVENTIONAL TRANSITION PERIOD DEFINITION

The traditional definition of the transition period, encompassing 3 wk before and 3 wk after calving, may need a reevaluation, as factors originating much earlier than 3 wk prepartum have been recognized to affect the cow's ability to overcome this phase, and consequences arising from a poor transition could extend beyond the conventional 3-wk postpartum window. The period that should be considered as the starting point in this re-evaluation has not been defined yet, as depends mainly on the onset of the inflammatory processes acting as potential threats to the metabolic adaptation of the cow to the new lactation.

Recent studies underscored the potential of assessing blood biomarkers in this respect, and those reflecting the inflammatory status seem to be the most promising. Several studies documented prepartum trends of PIC, as those of serum amyloid A, TNFα, IL-1, and IL-6, measured as early as 8 wk before calving to provide a predictive view on the successful adaptation of the cows to the transition period (Dervishi et al., 2016; Zhang et al., 2016). An insightful study conducted by Wisnieski et al. (2019) tested the potential of 3 different models, obtained by combining trends of blood analytes related to inflammation, nutrient metabolism, and oxidative stress measured during the dry period, in identifying cattle at risk of developing transition-related diseases. Notably, the nutrient metabolism model – which included biomarkers such as BHB, NEFA, and Ca – exhibited lower predictive ability compared with the inflammation and oxidative stress models. This finding aligns with Horst's (2021) remarks which challenge the dogma of ketosis as a metabolic disorder, arguing that in healthy cows the changes in circulating NEFA, ketone bodies, and Ca are signs of immune activation rather than physiological metabolic adaptations to the onset of lactation. Another study documented that the subclinical ketosis status in early lactation was preceded by significant alterations in inflammatory parameters, detectable as early as 48 d before calving (Mezzetti et al., 2019). More recently, signs reflecting altered immune system function and impacting the adaptation of the cow to the peripartum phase were detected before dry-off (Cattaneo et al., 2021). In the experiment, cows were classified based on the severity of the acute phase response (**APR**), as reflected by the albumin-to-globulin ratio calculated 7 d before dry-off.

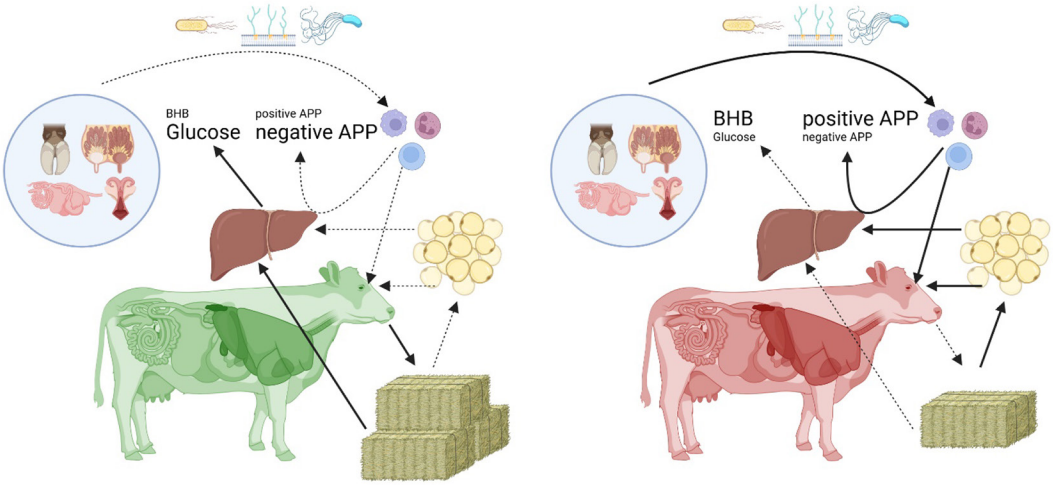

Figure 1. Crosstalk between metabolic functions and the immune system in the postpartum period. The green cow on the left shows a successful adaptation to lactation. Dry matter intake is adequate to provide sufficient energy flux to the liver gluconeogenesis and to lower the lipomobilization process. The immune system does not affect the physiological course of the other processes, and the liver synthetizes primarily negative APP. Conversely, the red cow on the right shows a poor adaptation to lactation. Dry matter intake is lower, enhancing NEB and lipomobilization. Greater NEFA influx to the liver promotes BHB release and fat infiltration in the hepatocytes. Activated immune system increases the energy demand. The immune system can be activated by several stressors (LPS, bacteria, abiotic causes, arising, for instance, from the hooves, the mammary gland, the gastrointestinal tract, or the uterus), releasing PIC that further depresses feed intake and boost the acute phase response in the liver through increasing positive APP synthesis. Solid lines indicate positive impact, dashed lines negative impact.

Cows having the lowest ratio had the worst performances during late pregnancy and the subsequent lactation. These studies open new perspectives on identifying the cows at risk of facing a bad transition to the next lactation before dry-off, encouraging the implementation of interventions aimed at improving their health status and performances over a long-term period (Cattaneo et al., 2023).

THE IMMUNE AND INFLAMMATORY RESPONSES IN PERIPARTURIENT COWS

Immune cell function: is immune suppression a real condition?

Alterations affecting the immune system of transition cows interested the scientific community for a long time. Goff and Horst (1997) reported that the immune system activities were impaired during the week before and after calving, inferring that, at least partially, this could explain the high frequency of infectious diseases (e.g., mastitis) documented at the beginning of lactation in most dairy herds. These data have been used to support the presence of an immunosuppression state in cows during the transition period, that overlaps with the energy and protein deficiency status. In the last 2 decades, a wide body of literature has been developed by exploring the immune functions of dairy cows during the periparturient phase (Bertoni and Trevisi, 2013; Horst et al., 2021). Of interest, divergent effects of the peripheral and systemic innate immune responses approaching calving have been reported (Jahan et al., 2015). Compared with prepartum, postpartum cows seem to have milder immune responses in peripheral tissues (as reflected by a reduced ability of leukocytes to mount a local inflammation following subcutaneous stimulation), but a greater susceptibility to develop systemic inflammation (i.e., heightened release of PIC by circulating leukocytes). Similarly, early-lactating cows can have a more intense inflammatory response than mid-lactating ones following both intravenous and intramammary LPS administration, as suggested by the greater rectal temperature increase and PIC release (Opgenorth et al., 2024a; b). In addition, the transcriptomic changes in circulating leukocytes around calving indicate mainly an increase in their activities and functions and not a reduction (Minuti et al., 2020). Most of the genes related to the immune system begin to be upregulated a few days before calving, playing a major role immediately after calving. Interestingly, BHB (negatively) and IL-1β (positively) were the plasma variables most correlated with the gene expression in circulating leukocytes (Minuti et al., 2020).

These data mined the assumption of an immunosuppressive state affecting all the cows during the transition period, suggesting that the reactivity of the immune system is, at least, maintained during this phase and that the immune status of peripartum cows could depend on a different regulation of the immune system as compared with those at play during the mid and late lactation phases.

The regulation of the immune system during pregnancy

The immunological variations and cytokine alterations during pregnancy in dairy cows have not been as extensively studied as in humans. However, significant insights can be derived from human studies, that identify 3 distinct immunological phases during pregnancy (Mor and Cardenas, 2010). The first phase, implantation, is characterized by a marked inflammatory response necessary for tissue remodeling. The second phase, fetal growth, shifts toward an immunotolerant and anti-inflammatory state, allowing for the fetus's growth within the mother. The third phase, prepartum, involves a return to an inflammatory state, preparing reproductive tissues for labor and the expulsion of the fetus and placenta. Throughout these phases, variations in leukocyte populations and hormonal changes play crucial roles.

Since 1993, the recognition of fetal alloantigen by a dichotomy of T helper leukocyte populations (i.e., Th1 and Th2) has been proposed to account for maternal tolerance against the conceptus in mammals (Wegmann et al., 1993). In the peri-implantation period, the Th1 population, characterized by a pro-inflammatory polarization, prevails and favors the activity of trophoblasts, which provide nutrients to the embryo. After placental implantation, the Th1 immune type shifts to Th2, promoting an anti-inflammatory response to protect the fetus and allow placental development. Pregnancy losses in women have been attributed to the impairment of the Th1/Th2 ratio, particularly due to reduced levels of Th2 implicated in autoantibody production and enhanced autoimmunity. However, other T helper populations, such as Th17 and regulatory T cells, are also involved in this process (Wang et al., 2020). In advanced pregnancy, a lowered Th1/Th2 ratio has been documented in mammals, suggesting an increased anti-inflammatory regulation of the immune system. This also occurs in cows many weeks before calving (Maeda et al., 2013) and the increased synthesis of ACTH and nitric oxide from lymphocytes could cause a shift toward Th2 cytokines concurrently decreasing the levels of Th1 cytokines (Dixit and Parvizi, 2001). Nevertheless, in the dry period, the levels of PIC, such as IL-1β and IL-6, are elevated in comparison to lactation and show a dramatic drop only after calving (Trevisi et al., 2015). This supports the observation by Fair (2015), who considered the Th1/Th2 ratio unable to explain the complexity of immune changes occurring during cow pregnancy.

The systemic inflammatory state and the central role of the liver

The liver is a central metabolic organ, regulating the energy and protein metabolisms and maintaining homeostasis in ruminants. The liver undergoes severe metabolic stress in the transition from pregnancy to lactation. In this context, its major roles are the oxidation of mobilized NEFA, the gluconeogenesis to support the glucose demand by the mammary gland, and the production of very low-density lipoproteins to prevent the excessive infiltration of triglycerides in the hepatocytes. Detoxification and clearance of endotoxins and other harmful metabolites are additional functions of the liver that often occur in the peripartum period. The Kupffer cells of the liver, that are specialized macrophages, are responsible for the phagocytosis of LPS and the mitigation of their systemic effects. The liver's ability to detoxify ammonia is also crucial in this phase. Skeletal muscle tissue is mobilized to provide AA precursors for gluconeogenesis and, during inflammation, for acute phase protein (**APP**) synthesis (Opgenorth et al., 2024c). Furthermore, the liver modulates both innate and adaptive immune responses. Hepatic synthesis of complement proteins, which enhances the ability of antibodies and phagocytic cells to clear pathogens, is critical for an effective immune defense. The liver also affects the systemic levels of various hormones, such as IGF-1, which have important metabolic and immunomodulatory effects (Heemskerk et al., 1999). The interplay between these hormones and the immune response is complex and essential for maintaining the balance between effective pathogen clearance and the prevention of excessive inflammatory damage.

Immediately after calving, several pro-inflammatory stimuli concur in establishing an APR condition by the liver. Under this scenario, plasma concentrations of positive APP (i.e., haptoglobin, ceruloplasmin, serum amyloid A) have a sharp peak immediately after calving, but normal concentrations are recovered within the first week of lactation in healthy subjects (Gabay and Kushner, 1999; Ceciliani et al., 2012). They play an important role in modulating the immune response, preventing microbial growth, and facilitating tissue repair (Ceciliani et al., 2012). Conversely, the plasma concentrations of the so-called negative APP (i.e., albumin, paraoxonase, retinol-binding protein) and other liver function indicators (i.e., cholesterol as an index of lipoprotein synthesis, bilirubin as an index of the synthesis of the enzymes necessary for its clearance, Zn and Fe as an index of increased liver metallothionein) are reduced during the transition period (Bertoni and Trevisi, 2013; Trevisi and Minuti, 2018). Classically, APR is induced by pathogen-associated molecular patterns (i.e., LPS, lipoteichoic acid) or PIC (Ringseis et al., 2015), that enter the blood-

stream of peripartum cows due to infections or injuries occurring during labor, placental expulsion and uterine involution (Medzhitov, 2008). A sterile APR has been also documented in peripartum cows, as reactive oxygen species, several hormones (i.e., epinephrine, norepinephrine, corticosterone, and cortisol) (Bradford et al., 2015), and other immune-active compounds released during the physiological adaptation of the cow to the early lactation phase (i.e., adipokines and several fatty acids released during adipose tissue remodeling) (Zachut and Contreras, 2022) may serve as non-infectious inductors for APR.

Importantly, clinical symptoms of inflammation are rarely observed before calving, even in cows affected by abnormal trends of plasma analytes reflecting metabolic functions and redox balance (i.e., heightened concentration of oxidative metabolites and liver transaminases, or lowered concentration of antioxidants, Ca, and albumin). Conversely, inflammation (with the release of cytokines and APP) is widely documented as a physiological state of postpartum cows (Bionaz et al., 2007; Bradford et al., 2015). Although the inflammatory condition affects even healthy cows after calving (Bertoni et al., 2008), the magnitude and duration of the process, as well as the large and complex metabolic consequences on energy, protein, mineral (mainly Ca) metabolisms, redox balance, and endocrine disorders are cow specific. Research has consistently shown that cows exhibiting a lower APR postpartum tend to be more productive, fertile, healthy, and efficient (Bertoni and Trevisi, 2013; Bradford et al., 2015; Horst et al., 2021). For these reasons, the evaluation of the changes of positive and negative APP has been proposed to assess the resilience of dairy cows to the challenges of the peripartum (Trevisi and Minuti, 2018). In this context, understanding the role of PIC in the periparturient period and their involvement in the anorexic response and hepatic metabolism in early lactation is crucial.

The immune paradox of the transition period: opposite trends of cytokines and inflammatory response

Pro- and anti-inflammatory cytokines are released by different cell types during the inflammatory process and are aimed to coordinate the body's response to the threatening agent until the recovery of the homeostasis condition. The synthesis of positive APP by the liver is typically stimulated by PIC (i.e., IL-1, IL-6, and TNF-α). These cytokines not only signal the liver to produce positive APP but also affect the whole organ's metabolism by altering the synthesis of negative APP, essential proteins, and enzymes, redirecting the metabolic focus of the liver from anabolic to catabolic processes to support, concurrently with milk production, the immune response. Notably, the role of PIC in determining the APR typical of transition dairy cows remains speculative, as most of the studies exploring the pathways driving the establishment of an APR condition have been performed in animal models consequential to a local pathogenic stimulation (Vlasova and Saif, 2021). The outcome obtained in most of these studies cannot be extended to transition cows, as endocrine changes occurring in late pregnancy alter the release rates and metabolic functioning of several inflammatory mediators (including PIC). Examining trends of circulating PIC and those of the main APR biomarkers in transition cows (i.e., APP) a paradox arises. The peak in APR occurs within the first few days postpartum, but PIC follow the expected pattern (i.e., higher postpartum than prepartum levels, mimicking the trend of APP) only in cows affected by reproductive diseases (i.e., retained placenta or metritis) (Islam et al., 2013). Oppositely, in clinically healthy animals, the concentrations of circulating PIC in the first few days of lactation decrease compared with those documented in late pregnancy: IL-6 is 2.5 times lower (Ishikawa et al., 2004; Trevisi et al., 2015), while IL-1β (Koets et al., 1998; Trevisi et al., 2015) and TNFα are halved (Koets et al., 1998). The lag between the elevated PIC levels in the late dry period and the rise of APR markers postpartum appears too large to be explained by the timing of the inflammatory event, which is generally faster.

This apparent contradiction could be understood by considering the variety of physiological and regulatory mechanisms that modulate the inflammatory response in early lactation. Despite APR in early lactation is not accompanied by a clear surge in PIC (Trevisi et al., 2015; Kuhla, 2020), the immune system and the whole organism employ various mechanisms to cope with the APR established by the liver, with the final aim of minimizing damages to the host tissues and preventing the establishment of a chronic inflammatory state (Bradford et al., 2015; Minuti et al., 2020). Thus, the synthesis of anti-inflammatory cytokines, such as transforming growth factor β and IL-10 (Heiser et al., 2015), increases in early lactation to promote a quick resolution of APR. Furthermore, cortisol released at calving time dampens the inflammatory response due to its immunosuppressive properties (Oppert et al., 2005). Altogether, these mechanisms aimed at coping with the synthesis of positive APP by the liver could account for the low PIC concentration detected in early lactating cows. Besides this, some authors hypothesized that increased 17β-estradiol concentration (Rogers and Eastell, 2001) and the removal of placenta at calving time (Vitoratos et al., 2008) could have a role in the drop of PIC detected in early lactation. Nevertheless, the exact mechanisms orchestrating this apparent immune paradox are still unclear. It is certainly worth emphasizing, as correctly observed by Kuhla

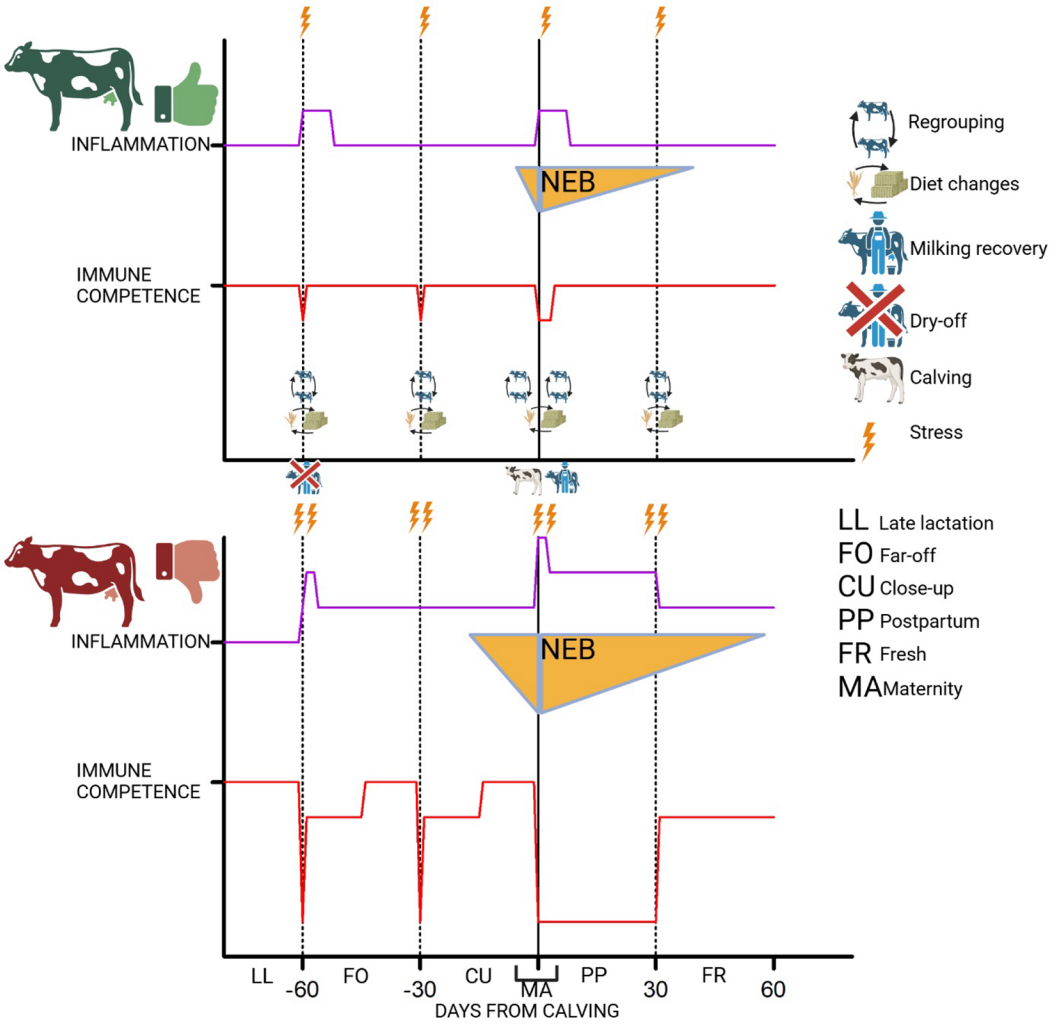

Figure 2. Schematic representation of inflammation, immune competence, and negative energy balance (NEB) trends from late to early lactation over a good (green cow) and poor (red cow) peripartum phase.

(2020), that there are concerns regarding the quality of commercial ELISA kits used for cytokine assays.

WHY DOES THE INFLAMMATORY PROCESS DERAIL IN THE PERIPARTURIENT PERIOD?.

Several proinflammatory stimuli have the potential to initiate multiple inflammatory events between the dry-off and the early lactation phase (Figure 2), although the frequency and intensity of these phenomena during the peripartum are cow-specific (Bradford et al., 2015). The worst scenario consists of developing a chronic inflammatory status lasting throughout the peripartum phase. In this case, the response differs from the canonical APR, affecting multiple metabolic pathways and hindering the adaptation of the cow to the peripartum phase. Several causes concur in precluding the regulatory systems of peripartum cows to restore homeostasis following the establishment of an inflammatory event.

It is known that immune cells of adult mammals (as memory T and B cells) undergo epigenetic modifications while activated (Cronkite and Strutt, 2018), allowing a quicker activation of the cells when exposed to the same challenge (i.e., boosted proliferation, production of effector cytokines and improved capacity to carry out their effector functions). This memory-conditioned inflammatory response allows a faster resolution of infections, lowering the risk of deleterious side effects related to the inflammatory response on the host tissues. Conversely, multiple activations of the inflammatory process in peripartum cows commonly result in impaired immune functions and reduced cell-mediated immune response, especially when severe inflammation occurs before calving (Bradford et al., 2015; Trevisi and Minuti, 2018). This

peculiar immune condition of peripartum cows could depend on the different regulation of the immune system during pregnancy, which makes the repeated exposure to inflammatory processes occurring before calving a potential threat to the cow's health, as normal memory functions of lymphocytes are recovered after delivery. Among the repeated signals that activate inflammation in the peripartum, epinephrine and norepinephrine (released by the sympathetic nervous system), and corticosterone and cortisol (released via the hypothalamus-pituitary-adrenal axis) deserve attention, as they promote a sterile inflammation, with different severity and persistence (Bradford et al., 2015). Indeed, psychological stress causes "rapid central and peripheral autonomic responses that occur almost simultaneously with detection of a threatening stimulus and a slower endocrine response that develops over time" (Johnson et al., 2019). Altered gastrointestinal permeability is another factor that elicits multiple inflammatory conditions in the peripartum, especially in the most sensitive or metabolic-compromised cows of the herd. Sudden diet changes, fasting conditions, heat stress, and psychological stressors affecting the cows from dry-off to calving are predisposing factors for developing the leaky gut condition, promoting the paracellular translocation of immunostimulant molecules (i.e., bacterial LPS or lipoteichoic acid) from the gastrointestinal tract to the lymphatic system and the liver. Thus, any stressful event occurring in the peripartum phase (e.g., regrouping, overcrowding, sudden diet changes) could potentially act as a proinflammatory stimulus in transition cows, resulting in multiple activations of the immune system.

From local to systemic inflammation: the mammary gland model

The mammary gland represents a precious model allowing the monitoring of local inflammatory processes evolving toward systemic inflammations, and pathophysiological changes affecting the udder throughout the peripartum phase make it a fundamental point to consider when exploring the immune metabolism of the transition cows. Any activation of the immune response within the mammary gland against external insults originates the mastitis phenomena, starting as a local inflammatory process within the infected mammary quarter (Rainard and Riollet, 2006). As mastitis has the greatest incidence between dry-off and the peak of lactation (i.e., 40–70 DIM), systemic alterations accompanying their pathological progression can affect the severity of immune dysfunctions experienced by cows during the peripartum phase. The magnitude and duration of the inflammatory response consequential to IMI are reflected by milk SCC. As soon as the peak of lactation has been overcome, SCC is normally below 10^5/mL and is mostly represented by resident macrophages, with a low proportion of mammary epithelial cells (**MEC**) (Rainard and Riollet, 2006) and histological pieces of evidence suggested that detecting milk SCC >20,000/mL already reflects neutrophils infiltration within the udder (Schalm et al., 1971). Environmental pathogens include both Gram-positive and negative species, that are more likely to induce acute clinical mastitis. LPS associated with Gram-negative bacteria strains (i.e., *E. coli*) interact with toll-like receptor (**TLR**) 4 on MEC, activating nuclear factor κ-B and inducing a massive secretion of TNFα, IL-1, and IL-8 that is LPS dose-dependent (Wellnitz and Bruckmaier (2012). After a Gram-negative IMI, SCC raises to 10^7/mL within 12 h, triggering a massive immune response that allows a fast elimination of bacteria, followed by SCC fall to preinfectional levels within 1 d from IMI onset. This response is accompanied by altered expression of genes encoding for APR and metabolic functions at the liver level, fever, elevated heart rate, lethargy, reduced feed intake, hypersalivation, and sepsis, possibly resulting in cow death at last (Lohuis et al., 1988; Waldron et al., 2003). As the contribution of LPS translocation from the mammary gland to the bloodstream is very limited (Shangraw and McFadden (2022), systemic symptoms depend mainly on TNFα and glucocorticoids produced into the mammary gland and diffused to the other body compartments through blood (Shuster et al., 1991). Conversely, systemic alterations induced by Gram-positive IMI are largely unknown. These bacteria encompass contagious strains (i.e., *Staph. aureus*) that can invade, live and multiply into the alveolar cells of the mammary gland to evade host immune defenses (Hébert et al., 2000; Geng et al., 2020), being released into the cistern and stimulating the host immune response at some point of the lactation cycle (i.e., around calving, due to cellular stress induced by parturition process). Host response against these pathogens is driven by the recognition of lipoproteins, peptidoglycans, and lipoteichoic acid by TLR 2. This pathway elicits the release of IL-1 mainly, while neither TNFα nor IL-8 have been detected (Riollet et al., 2000; Bannerman et al., 2004). The resultant SCC response could take up to 48 h to mount and could reach 10^6 SCC/mL, commonly allowing bacteria to survive after this first wave of response (Wellnitz and Bruckmaier, 2012). For these reasons, Gram-positive bacteria could originate persistent IMI alternating clinical and subclinical stages (Oviedo-Boyso et al., 2007). These have the potential to affect dairy cows even before dry-off without sorting any clinical sign, predisposing the cow toward developing a chronic inflammatory state during the peripartum phase.

Besides mastitis, another process taking place in the mammary gland and impacting the systemic inflammatory state of peripartum cows is active involution. It

consists of the remodeling of the secretory tissues taking place between 2 and 23 d after dry-off and aimed at turning the udder to a nonlactating state. It is accompanied by a lowered synthesis of milk components, increased apoptosis of the alveolar secretory cells, augmented permeability of the tight junctions (becomes leaky after 20–21 h of milk accumulation), and activation of proteases enzymatic complexes (Zhao et al., 2019). In this phase, leukocytes infiltrate the mammary gland across the disrupted tight junction's barrier, attracted by mediators released by senescent MEC, β-casein 1–28 fragments, and serotonin (Zhao et al., 2019). Leukocytes are involved in the clearance of casein micelles, lipid droplets, and cellular debris and in the remodeling of the udder's secretory tissues. Thus, in the 7–14 d following dry-off, SCC in mammary secretions reaches about 2–5 × 10^6/mL, and neutrophils represent up to 40% of total SCC during the early stages of involution (2–4 d after dry-off) (Rainard and Riollet, 2006). In the current decade, a growing number of studies documented that dairy cows experience systemic inflammations, APR, and oxidative stress conditions following dry-off, mimicking those experienced by transition dairy cows following delivery (Putman et al., 2018; Mezzetti et al., 2020). The severity of systemic alterations experienced by the cows was positively related to the milk yielded at dry-off, suggesting that the risk of affecting body homeostasis is proportional to the effort sustained by leukocytes in reabsorbing milk residuals and secretory tissues during the involution phase. A study mimicking mammary gland involution in early lactating cows through reducing the milking frequency revealed that activation of leukocytes within the mammary gland upregulated the expression of genes encoding for immune response, inflammation, oxidative stress, and PIC (Piantoni et al., 2010). The latter suggests that leukocytes release proinflammatory mediators during the involution phase, suggesting that these mediators directly contribute to determining the systemic inflammatory conditions documented in dairy cows after dry-off. Another hypothesis could be the direct implication of milk residuals diffusing into the bloodstream through the disrupted mammary epithelium in determining proinflammatory effect, as most of the milk components exert a documented antigenic role. Those assumptions remain largely speculative, as the pattern of compounds and mediators released during the involution process and diffusing into the bloodstream remains an unexplored topic to date, and further investigations aimed at elucidating their proinflammatory action should be performed to minimize the risk of developing chronic inflammatory conditions at dry-off by peripartum cows.

Other causes of immune dysfunctions in peripartum cows

Other potential causes for immune dysregulation in peripartum cows include immune mediators released by adipose tissue. Protein (i.e., TNFα, IL-6, adiponectin, leptin, resistin, visfatin) and lipid adipokines (i.e., endocannabinoids and steroids) are continuously secreted by the adipose tissue and with their autocrine and paracrine effects play a role in the crosstalk between tissues as brain, liver, skeletal muscle, and immune system (Lee et al., 2019; Häussler et al., 2022). During the transition from late gestation to early lactation, the magnitude and composition of fat depots and the intensity of the lipid mobilization process can affect the adaptive immune response by altering the profile of circulating adipokines (De Koster et al., 2018; Häussler et al., 2022). Circulating leptin concentration is directly related to the size of fat reserves and circulating insulin, whereas adiponectin is inversely related to fat depots, and its secretion is reduced during the transition period. While leptin is mainly pro-inflammatory (Kiernan and MacIver, 2021), adiponectin appears to have an anti-inflammatory and immune-modulatory role in dairy cows (Kabara et al., 2014). The reduced circulating adiponectin observed around parturition promotes lipolysis and reduces peripheral tissue insulin sensitivity and can account for the impaired oxidation of NEFA by skeletal muscle and the liver. Thus, the meaningful contribution of adipokines in modulating immune system functions and FA metabolism could explain the greater risk for developing ketosis, fatty liver, and many other metabolic disorders in early lactation attributed to cows entering the dry period with excessive body fat reserves. Data from Drackley's lab suggest that a prolonged dietary excess of energy during the dry period has the potential to establish a low-grade inflammation (Janovick et al., 2022), suggesting that even the energy plan adopted during the dry phase impacts the magnitude of the immune dysregulations experienced by the cow in early lactation.

Another metabolic interference leading to immune dysfunctions could be the ability of immune cells to produce, stock, and interact with hormones having autocrine, paracrine, and endocrine regulations (i.e., ACTH, estrogens, endorphin) (Blalock and Smith, 2007). As mobile cells, leukocytes can release them locally, adapting specific immune functions. The most relevant interactions documented between immune cells and hormones include the accumulation of endorphin with an analgesic effect in CD11b+ leukocytes (Shi et al., 2023), and the increased release of ACTH in lymphocytes from pregnant cows in comparison to those of non-pregnant ones (Dixit and Parvizi, 2001). Although hormones produced by immune cells under a normal inflammatory process

exert their effect locally, intense and prolonged immune activation (as those affecting peripartum cows) could extend the endocrine effect of the immune system. In this context, the simultaneous release of inflammatory mediators (i.e., PIC) and hormones (i.e., ACTH, endorphin) from circulating immune cells could affect the metabolism of the periparturient cow. The cross-talking among immune and endocrine systems could explain the apparent paradox that occurs in the post-calving, with the activation of the APR in the liver despite the decrease in circulating PIC (Ishikawa et al., 2004; Trevisi et al., 2015), assuming a possible interference on PIC activities before calving. Although this mechanism is fascinating, the patterns of inflammatory mediators and hormones released by leukocytes of periparturient cows remain largely unknown, and further research efforts are required to quantify the role of these mediators in affecting the interaction between the immune system and metabolism occurring during this physiological phase.

Ultimately, the alteration of the severity of the immune response experienced by dairy cows during the peripartum phase could be under genetic control. Passamonti et al. (2024) identified alternative genotypes encoding for some biomarkers involved in the adaptative response of dairy cows to the peripartum period (i.e., paraoxonase and γ-glutamyl transferase). The adaptive response to the transition period of the identified genotypes has then been tested by monitoring plasma concentrations of the biomarkers as well as their performances during the peripartum phase. Even though different genotypes did not show any difference in performances, concentrations of plasma biomarkers effectively differed among alternative genotypes, suggesting that a genetic contribution to the systemic response of the cow to the peripartum challenges could be hypothesized.

CONCLUSIONS

In periparturient cows, immune functions show alterations before parturition, while other aspects are impaired only after calving (i.e., energy balance or oxidative stress). Nevertheless, the immune functions before calving are not usually suppressed but instead can be dysregulated both at local and systemic levels. Several factors can affect the immune response in this period, and a growing body of evidence suggests that events occurring around dry-off may have a relevant role. Moreover, an overextended APR at the onset of lactation predisposes the cow to metabolic and infectious disorders, and some alterations affecting the immune response before parturition can affect the severity of the systemic inflammation occurring after calving, even though most of the mechanisms proposed to account for the "immune derailment" of peripartum cows remain largely speculative. These "shadows" highlight the complexity of this topic and the uncertainty that is still present in this field, whereas the "lights" provided by the recent research efforts in elucidating the mechanisms underlying the immune-metabolic responses during the transition period should guide future investigations to improve animal management in this critical phase.

NOTES

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Nonstandard abbreviations used: APP = acute phase protein; APR = acute phase response; NEFA = nonesterified fatty acids; MEC = mammary epithelial cells; PIC = proinflammatory cytokines; TLR = toll-like receptor; TNFα = tumor necrosis factor α.

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