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Review

Mammary Gland Infection and Its Association with Other Periparturient Diseases of Dairy Cows

Ashley Egyedy[1] and Burim N. Ametaj[1,*]

[1] University of Alberta; egyedy@ualberta.ca
* Correspondence: burim.ametaj@ualberta.ca; Tel.: +780-492-9841

Abstract: Mastitis is an inflammation of the mammary gland initiated by pathogenic bacteria. In fact, mastitis is the second most important reason for the culling of cows from dairy herds, after infertility. In this review we focus on various forms of mastitis, including subclinical and clinical mastitis. We also stress the importance of the dry-off period as an important time when pathogenic bacteria might start their insult to the mammary gland. An important part of the review is the negative effects of mastitis on milk production and composition, as well as economic consequences for dairy farms. The two most important groups of bacteria that are involved in infection of the udder, Gram-negative and Gram-positive bacteria, are also discussed. Although all cows have both innate and adaptive immunity against most pathogens, some are more susceptible to the disease than others. That's why we summarize the most important components of innate and adaptive immunity so that the reader understands the specific immune responses of the udder to pathogenic bacteria. One of the most important sections of this review is interrelationship of mastitis with other diseases, especially retained placenta, metritis and endometritis, ketosis, and laminitis. Is mastitis the cause or the consequence of this disease? Finally, the review concludes with treatment and preventive approaches to mastitis.

Keywords: dairy cow; mammary gland; mastitis; periparturient diseases

1. Mammary Gland Infections in Dairy Cows

1.1. A Brief Description of Mammary Gland Infection

Mastitis is defined as an inflammation of the mammary gland of dairy cows, which is commonly related to infection by pathogenic bacteria. Mastitis is a multifactorial disease and is classified into subclinical mastitis (SCM; with no visual signs of infection) and clinical mastitis (CM; with visual signs of infection in the udder and milk; [1,2]. Subclinical and clinical mastitis negatively affect both milk quality and yield, making mastitis a major economic concern for the dairy industry [3]. Indeed, a case of mastitis costs an average of US$253 [4]. Mastitis infection can result in production losses of up to $2 billion dollars to dairy producers in the United States alone [5]. According to the government of Canada [6] the number of cows culled in 2019 for mastitis reasons in Canada was 23,832. Given that the cost of a cull cow is C$880, then the economic loss to the dairy industry from mastitis is roughly CAD$21 million. This does not consider financial losses due to other factors such as treatment, milk loss, labor for treatments, veterinary bills, medication costs, and discarded milk.

1.2. Types of Mastitis

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As indicated, mastitis is classified as either subclinical or clinical based on the manifestation or lack of signs. Mastitis also has been classified as chronic, subacute, acute, or peracute based on its duration and severity. A summary of the signs, somatic cell counts (SCC), and severity of each mastitis type is provided in Table 1.

Table 1. A summary of signs for subclinical and clinical mastitis, including the four categories of clinical mastitis (chronic, subacute, acute, and peracute).

Subclinical Mastitis			**Clinical Mastitis**			
Signs	**SCC Level[1]**	**Severity**	**Category**	**Signs**	**SCC Level[1]**	**Severity**
Decreased production, increased SCC, changes in milk components, presence of pathogens.	>200	Can only be determined by laboratory tests. Non-visible symptoms causing potential risk of infecting entire herd.	**Chronic**	Visible alterations in the milk (clots, flakes, water), no inflammation or other visible sings.	>200	Long-term infection in which culling is recommended.
			Subacute	Minor udder inflammation, heat, changes in milk (clots, flakes, water-like appearance), some sensitivity to udder.	>200	Typically, non-life threatening.
			Acute	Sudden onset, redness/ swelling, hardness, pain, abnormal milk, decreased production, fever, decrease appetite, decrease rumen function, rapid pulse, dehydration, weakness, depression.	>200	Life-threatening and causes extreme discomfort.
			Peracute	Very rapid onset with similar symptoms to acute but more severe.	>200	Decrease in cow's overall condition, life threatening.

[1]Somatic cell counts in 10^3 cells/mL

(Adopted from: [7,8,9,10,11].

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1.3. Subclinical Mastitis

Cows affected by subclinical mastitis do not display signs of infection, such as alterations in milk appearance or udder swelling. However, SCM is associated with an increase in SCC, decreased milk production, the presence of infectious pathogens in the milk, and alterations in milk composition [8]. Subclinical mastitis is the most common udder infection found in Holstein dairy cows, affecting between 36-50% of the herd [12,13]. Subclinical mastitis can also result in milk losses of up to 70% for dairy producers due to non-visible effects unless regular measurements of SCC are part of the post-partum management of the cows [7]. Given that cows affected by SCM do not reach their maximum milk yield potential; the profitability of dairy farms is negatively affected. Somatic cell counts are the main indicator in determining a subclinical mammary gland infection. SCC less than 100,000 cells/mL in mammary gland milk is considered healthy [14,15], whereas values greater than 100,000 cells/mL are suggestive of mastitis. In Canada, a threshold concentration of 200,000 cells/mL is used for distinguishing between healthy udders and the diseased ones [16]. There are several factors that can influence the SCC, including the cow's age, breed, stage of lactation, and milk yield [17].

1.4. Clinical Mastitis

Based on duration of disease, CM is classified as: chronic, subacute, acute, and peracute (Table 1; [9,10]. During CM, signs of disease are visible on the mammary gland, including swollen or hardened udder, heat, pain, and changes in the milk consistency [11]. Chronic cases are the least severe, compared to the other types of CM, as there is a visible change in milk consistency and infection can persist for long periods of time where culling is often recommended [9,10,11]. During subacute mastitis, there is a slight inflammation and swelling to the mammary gland accompanied by a change in milk consistency [9,10]. Subacute mastitis is the most common type of CM, affecting around 10-50% of a herd. The onset of an acute clinical infection is relatively quick, and signs include the mammary gland appearing red, swollen, and hard, and changes in milk consistency. Cows with acute CM also experience systemic signs including pain, reduced appetite, reduced rumen function, increased heart rate, fever, depression, and weakness. Peracute mastitis has an extremely severe and rapid onset, which frequently results in the animal's death [10]. Both acute and peracute clinical infections are extremely dangerous for the cow as infection affects the animal systemically.

1.5. Mode of Infection of the Mammary Gland

Bacterial invasion of the mammary gland is the primary cause of intramammary infections (IMIs) in dairy cows. There are multiple microbial pathogens, including Gram-negative and Gram-positive organisms, that invade the mammary gland. Pathogens can invade the mammary gland directly from the environment or from animal-to-animal contact, and this is referred to as environmental mastitis and contagious mastitis, respectively [18].

Environmental mastitis is primarily caused by pathogens located within the cow's environment [18], including the soil, contaminated water, manure, and bedding. The most common environmental mastitis-causing pathogens include coliform organisms such as *Escherichia coli* and

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environmental streptococci such as *Streptococcus uberis*. Approximately 70-80% of CM cases are mainly caused by coliform organisms. Coliform infections have been found to have a short duration of 10 days within the mammary gland [5]. However, studies have found that 1.5% of *E. coli* infections have a duration of over 100 days [18]. The difference in duration could be based on the serotype of the pathogen that is causing infection. In comparison to streptococci organisms, the duration has been found to be 3 times longer within the mammary gland [5].

In contagious mastitis, the mode of infection for bacteria is from udder-to-udder, typically by the milker or milk machines. Therefore, it has been extremely important to wash, dry, and dip each teat during milking time in order to reduce spreading. The main pathogen of concern for contagious mastitis is *Staphylococcus aureus* [18], which has been shown to be one of the more difficult pathogens to control and treat [19]. In a study conducted by [20] it was found that most CM cases were caused by *S. uberis* and *S. aureus*.

1.6. The Importance of Somatic Cell Count for Diagnosis of Mammary Gland Infections

There are various methods used for the diagnosis of mastitis in dairy cows. The most efficient method is by determining the SCC in the milk and the presence of inflammation [21]. In healthy mammary glands, somatic cells consist of neutrophils, macrophages, lymphocytes, and shed mammary epithelial cells [22,23]. Various countries worldwide have different regulations regarding the level of somatic cells acceptable in the milk, either on a bulk-tank or per cow basis. For instance, the limit for raw milk SCC in Canada is 400,000 cells/mL [24], whereas the International Dairy Federation [21] states that SCC should be less than 200,000 cells/mL. The United States Department of Agriculture [25] allows a SCC of 750,000 cells/mL whereas other countries, like Brazil, have a limit of 1,000,000 cells/mL. According to [14], the SCC of dairy cows should be 1,000,000 cells/mL or less; anything above would indicate the presence of infection.

Detecting cows with a high SCC can be done by lab analysis or using the California Mastitis Test (CMT). The CMT was developed by [26] and is now used worldwide for identifying mastitis in dairy-producing animals. The benefit of using a CMT is that it's an easy method to quickly determine SCC in milk collected from individual quarters [27]. A sample of milk from each quarter is deposited into a well where detergent is added, causing lysis of the external membrane (lipoprotein membrane) of the somatic cells and exposing the gel-like DNA. Indication of a mastitis infection is based on the gel consistency and color that is formed, with the darker color indicating high SCC [28].

Detecting a mammary gland infection using CMT has been shown to be correlated with the quantity of SCC present in the milk, making CMT a beneficial method for determining mastitis [26]. The procedure and the scoring system for a CMT were described in a study by [29], who performed a 1:1000 dilution of milk samples with 3% sodium lauryl sulphate and bromocresol. Samples were separated into wells where the plate was then rotated. The rotation of the plate leads to a color change or gel formation, and a score is given based on the strength of the color. The scoring system for a CMT is on a scale of 0-4 where 0 indicates no reaction, 1 for a slight reaction, 2 for a weak positive, 3 for a confirmed positive, and 4 for a high positive.

Dairy cows approaching the dry off period are susceptible to new IMI (intramammary infections); therefore, the CMT can be a useful tool in determining cows with high SCC prior to dry off. Poutrel and Rainard [30] were able to accurately determine 80% of new IMI using the CMT. In

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the same study by Bhutto et al. [29], it was reported that cows had significantly higher CMT scores at dry off than a week prior to dry off. However, the CMT has been found to produce less reliable scores in determining cows with mastitis post-partum [30]. This is a major drawback as the incidence of disease in dairy cows is higher after parturition.

1.7. Additional Factors that Cause Elevation of Somatic Cells in the Mammary Gland

Other factors should be taken into consideration when looking at SCC to determine mammary gland infections, including stage of lactation, age, breed, stress level, and season [24]. According to [31], somatic cells can increase within 6 hours following bacterial invasion. Specific mammary quarters have also been found to be highly susceptible to bacterial infections [32]. For example, Harmon [32] indicates hindquarters have a higher SCC compared with the front ones, and Dhakal [33] found that the right quarters had a higher SCC than the left quarters. Therefore, the position of the mammary quarters could potentially increase the cow's susceptibility to mammary gland infections.

2. The Role of Dry Period in Dairy Cow's Health and Performance

2.1. Background and Importance of the Dry Period on Cows' Performance

The dry period can be defined as the period in which the cow is no longer lactating but is undergoing nutritional and metabolic changes and changes to the mammary gland [34]. The dry period is important for cows to prepare for the next calving and lactation. Previous research found that cows with a dry period have a higher milk yield in the following lactation than cows without a dry period [35,36]. During the dry period, the mammary gland regenerates the milk-secreting tissue that was damaged during the previous lactation [36,37].

Additionally, the length of the dry period can also significantly influence milk yield in the next lactation. The dry period typically begins at 60 d prior to parturition; however, DHI (Dairy Herd Improvement, Canada) recommends the dry period to be a minimum of 40 d prior to parturition [36]. Studies have found that cows with a dry period of 40 d significantly reduce milk yield in the next lactation compared to cows dried at 60 d [36]. Cows that are continuously milked with no dry period experience a 33% drop in production in the next lactation [35]. Funk et al. [38] found that cows with a dry period of 60-69 d had an increase in milk yield of 459 kg following parturition. A study by Watters et al. [39] showed that cows with a shorter dry period of 34 d had a significant decrease in milk yield by 2.1 kg/d compared to cows with a dry period of 55 d following parturition. Other studies have also confirmed that cows with a 40-d dry period had a decrease in milk yield [39]. We can therefore conclude that if maximum production is to be achieved, cows should have approximately a 60-day dry off period or greater.

All cows entering the dry period are administered an antibiotic that helps prevent infection of the mammary gland. The use and benefits of antibiotic treatments at dry off include administering antibiotics in higher dosages without having to discard milk, reducing infectious pathogens, regeneration of damaged mammary tissue, and reducing the incidence of CM [36]. The mammary

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gland also undergoes various physiological changes when dry cow therapy is administered. Such changes will prepare the mammary gland for parturition and the next lactation.

2.2. Physiological Changes of the Mammary Gland During the Dry Period

A cow entering the dry period experiences various physiological changes, including nutritional, metabolic, and changes to the mammary gland [40,34]. There are 3 stages through which the mammary gland undergoes during the dry period in preparation for the next lactation [41]. These stages are involution, steady-state involution, and colostrogenesis.

Involution occurs during the first 3 weeks of the dry period, and the mammary gland ceases milk synthesis. Regression of the alveolar epithelial cells occurs when milk builds up in the mammary gland, increasing pressure and decreasing secretion [40,41]. Atrophy of epithelial cells by the increasing pressure from milk accumulation is known as pressure atrophy [42]. Pressure atrophy is defined as a force that causes the secretory cells to cease milk secretion [42]. The increase in pressure on the alveolar milk-secreting cells lowers blood flow to the capillaries, and the mammary gland appears swollen [37]. In the alveolar epithelium, milk secretion decreases, and secretory cells become non-functional for the duration of the dry period [42]. Local macrophages and neutrophils also remove apoptotic mammary epithelial cells and pathogens from the mammary gland [37].

During involution, there is a build-up of a keratin plug within the teat canal that functions as a protective barrier to prevent bacteria from entering the mammary gland during the dry period [41]. However, the involution stage poses a high risk of bacterial infection due to the accumulation of milk and bacteria not being removed, thereby increasing the potential risk of infection during the dry period. It takes approximately 21-30 days for the involution process to be completed. Previous studies have shown that at approximately 10 d into the dry period, 50% of the quarters have not formed the keratin plug in the teat canal, making the quarter susceptible to bacterial invasion. About 5% of quarters were found to be open at 60 d. Dry cow therapy treatments are administered at the beginning of the dry period; however, they are not effective throughout the entire dry period, meaning that cows are not completely protected from new IMI occurring at parturition. Full protection of the mammary gland can be achieved by administering dry cow therapy in combination with a teat sealant. The combination of dry cow therapy with a teat sealant has been shown to lower the incidence of new IMI by 7.3% [41].

Senger [42] indicates that the involution stage is crucial during the lactation cycle of dairy cows. In relation to milk yield, a shorter dry period resulting in a decreased milk yield for the next lactation could be due to a lack of restoration of the milk-secreting tissues. Thus, maximum production can be achieved in the subsequent lactation if the cow has a sufficient dry period, ensuring tissue regeneration.

The second stage in the dry off period is the steady state involution, which is when the mammary gland is protected from the entrance of bacteria from outside and has resistance to infection due to the keratin plug in the streak canal [41].

Colostrogenesis is the third and final stage of the dry period and is known as the transition period [41]. This stage occurs towards the end of gestation, when the mammary gland undergoes physiological changes opposite to those of involution. In response to prolactin, adrenal cortical hormones, and placental lactogen secreted from the pituitary gland, adrenal gland, and placenta, respectively, alveoli are stimulated to begin milk synthesis [42]. The secretory tissue undergoes

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differentiation and intense growth along with the secretion of proteins, lipids, and lactose as part of colostrum [40,43,44]. Bacterial infection can increase during colostrogenesis because of breakdown of the keratin plug within the teat canal, thus increasing exposure to foreign pathogens. Leukocytes, which are white blood cells including neutrophils, have been shown to be under a state of immunosuppression during the transition period [45]. Approximately 95% of new IMIs have been shown to occur 2-3 weeks prior to parturition [41].

2.3. Other Physiological Changes During the Dry Period and Susceptibility to New Infections

During the dry period, the fetus will enter its final stages of growth [34]. Two-thirds of fetal growth is completed during the dry period, which often causes body maintenance to prioritize the fetus over the dam. This can cause high metabolic stress towards the end of gestation, which can result in immunosuppression and increased susceptibility to new IMI and other metabolic diseases [46,47,48]. It has also been indicated that the incidence of new IMI is highest during the involution stage of the dry period and towards the end of gestation [40]. Moreover, Natzke et al. [49] indicated a positive correlation between cows dried off with a pre-existing mammary gland infection and the incidence of new IMI during the dry period. This could possibly be due to increased stress around parturition and leakage of colostrum from the udder, thus allowing bacteria to enter the mammary gland. Although bacterial infection increases around parturition, administration of dry cow therapy at dry off is beneficial in lowering the risk of new IMI.

2.4. The Impact of Mammary Gland Infection on a Dairy Herd

The incidence of a mammary gland infection in a dairy herd has a tremendous impact on milk production, cow health, and economic loss. Milk production can suffer in the subsequent lactation from a mammary gland infection if cows have a short dry-off period and tissue regeneration is not fully achieved. During lactation, milk production can decrease in cows with mastitis as bacteria utilize the milk-secreting cells. A decrease in milk production also results in an economic loss for producers. Furthermore, mammary gland infections can impact the health of the cow, including the display of abnormal behavior and systemic signs depending on the severity of infection [50].

2.5. Effects of Mammary Gland Infections on Milk Production

It has been well established that an IMI results in a decline in milk production. More specifically, the physical damage exerted on the mammary epithelial cells because of infection causes a reduction in both the synthesis and secretion of milk [51]. The lactating bovine mammary gland is composed of a network of ducts that terminate at the alveolar clusters. The alveolar clusters are lined with mammary epithelial cells that secrete milk. Connectivity of the mammary epithelial cells is achieved by the apical junction complex, which is composed of adherens and tight junctions [52]. Tight junctions link adjacent epithelial cells by forming a narrow and continuous seal surrounding each cell at the apical border. The main function of tight junctions is to coordinate the movement of materials between cells and prevent leakage of milk components into the systemic circulation [51,53].

During infection, bacteria release endotoxins that induce an influx of leukocytes into the mammary gland and the secretion of inflammatory mediators [51]. The influx of leukocytes into the

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mammary gland results in disruption of the tight junctions, causing the mammary epithelial cells to lose their integrity and decrease milk synthesis [53,54,55]. Permeability of the blood-milk barrier is also increased because of infection, leading to a decrease in volume and milk components [56]. Additionally, the role of lactose in the mammary gland is for osmotic regulation of milk volume. The reduction in lactose synthesis can therefore contribute to a further decline in milk production [57].

Physical damage to the mammary epithelium is not the only cause of the decline in milk production during mammary gland infections. Affected mammary quarters can cause a decline in milk production in healthy mammary quarters because of systemic infection [56]. The secretion of inflammatory compounds including cytokines and arachidonic acid can alter the stimulatory or inhibiting hormone concentration, causing reduced milk precursor uptake. However, the decline in milk production is evident in affected quarters as opposed to healthy ones since inflammation is localized within the sick quarters. Furthermore, in the affected quarters, local inflammatory mediator secretion, leukocytosis, and mammary edema can reduce milk production [56]. Cows may also experience a decline in milk yield during other periparturient diseases such as uterine infections [58], ruminal acidosis [59], lameness [60], ketosis [61], and retained placenta [62].

2.6. Effects of Mammary Gland Infection on Milk Composition

An infection of the mammary gland not only affects the milk production in dairy cows but alters the composition of milk. Milk is composed of various proteins, fats, carbohydrates, minerals, vitamins, hormones, enzymes, ions, cells, and water. Concentrations of milk constituents tend to fluctuate during mammary gland infections depending on the pathogen present, immune response, and severity of infection.

During a mastitis infection, the concentration of protein in milk will either increase or decrease. The increase in protein in the milk may be an influx of blood-borne proteins, including serum albumin and immunoglobulins, into the milk in response to bacterial endotoxins and tight junction disruption [54,56,57]. This is mainly due to the increase in humoral or antibody-mediated immunity that results in an increase in immunoglobulins, which are important for combating infection [54,56,57].

Lactoferrin has also been shown to increase during mammary gland infections [51]. Lactoferrin is an iron-binding protein synthesized by the mammary epithelial cells that functions by competing with bacteria for free iron to decrease bacterial growth [63]. Lactoferrin can also bind to bacterial membrane surfaces, thereby altering the integrity and permeability of the cell walls and resulting in cell destruction [64]. According to [66] the concentrations of lactoferrin have a 100-fold increase during the involution stage of the dry period and less during lactation. Additionally, transferrin, an iron-binding protein taken up from the blood, will increase during infection [66].

Caseins are phosphorylated proteins that account for 80% of the total protein found in the mammary gland [67]. The main functions of caseins are providing amino acids to the newborn and binding calcium and phosphorous within the Golgi apparatus of the mammary epithelial cells, forming casein micelles which are important for skeletal growth in neonates [67]. Casein concentrations tend to decrease during infection, unlike lactoferrin, immunoglobulins, and serum albumin, which increase. The decrease in casein is largely due to secreted proteinases by infectious pathogens and leukocytes, or in the blood because of disruption of the blood-milk barrier [57].

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Milk protein also consists of whey proteins including α-lactalbumin, β-lactoglobulin, serum albumin, and immunoglobulins. Both α-lactalbumin and β-lactoglobulin decrease in cows with a mammary gland infection largely due to the decline in synthesis and secretory activity [57]. Studies have observed a reduction in both α-lactalbumin and β-lactoglobulin in milk of high SCC cows [68]. The reduction in these proteins could be in part due to the decrease in synthesis and secretory function as well as protein leakage from the mammary gland [69]. Auldist and Hubble [57] suggest that the decline in α-lactalbumin and β-lactoglobulin could be due to protein leakage because of tight junction disruption. McFadden et al. [69] observed elevated concentrations of α-lactalbumin in the blood of high SCC dairy cows. The role of α-lactalbumin in the mammary gland is in the synthesis of lactose whereby binding the regulatory unit of lactose synthase induces synthesis [67]. Therefore, decreased synthesis of α-lactalbumin can result in a decreased synthesis of lactose in high SCC cows.

Additional enzymes found in milk have also been shown to be increased during a mastitis infection [57,70], including plasmin, a caseinolytic enzyme derived from plasminogen found in the blood [51]. The main function of plasmin in blood is to dissolve blood clots, whereas in the milk, plasmin cleaves β-casein into γ-casein [51,57,70]. It has been suggested that elevated plasmin activity in high SCC is attributed to leakage into the mammary gland from the blood [71]. Neutrophil granules contain various bactericidal peptides, including defensins, enzymes, and neutral and acidic proteases that can destroy various mastitis-causing pathogens [72]. These proteases, along with plasmin can permit chemotaxis of cells to the area of inflammation during immune response [73]. The increase in plasmin is, therefore, important in the immune host response to IMI.

Although much of the research has mainly focused on milk protein alterations during mammary gland infections, conflicting reports have found milk fat to decrease [57], whereas others have reported an increase in milk fat [55,56,67]. The synthesis of milk fat occurs in the rough endoplasmic reticulum of mammary epithelial cells [67]. Bruckmaier et al. [55] reported an increase in fat content, a reduction in lactose synthesis, and therefore a decline in milk production. Holdaway [70] also indicated that the decline in milk synthesis will eventually cause milk fat to decline. Furthermore, unlike lactose, which leaks from milk with water, milk fat is retained within the alveolar lumen due to the large size of lipid droplets, which are unable to move through the disrupted tight junctions [70]. Leukocytes produce lipase enzymes during infections that can act on the fat globules, causing oxidation of fatty acids and the breakdown of triglycerides [54,57,70]. Additionally, the phagocytotic ability of neutrophils can result in a further decline of milk fat [74] along with a decrease in synthesis and secretory function of the mammary gland [57]. Higher SCC in cows has also been associated with spontaneous lipolysis of milk fat [70].

Lactose is the main carbohydrate found in milk and is a disaccharide consisting of a glucose and a galactose molecule [67]. Lactose synthesis occurs within the Golgi apparatus of the mammary epithelial cells. Lactose synthesis consumes approximately 60-70% of plasma glucose [67]. As briefly mentioned above, lactose is the osmotic regulator for milk volume in lactating mammary glands. During a mammary gland infection, the concentration of lactose tends to decline partly due to the damaged mammary epithelium, resulting in reduced lactose synthesis [55,57]. The reduction in lactose is caused by the increased gap of the tight junctions, resulting in lactose being transported through the paracellular pathway out of the mammary gland and into the systemic circulation [54]. According to Bruckmaier et al. [55], the amount of damage to the tight junctions determines the decline in lactose concentration in milk.

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Other sources have also observed elevated levels of lactose in both blood and urine in cows with mastitis [53,55,57]. Specific bacteria can ferment lactose, causing a further decline in lactose concentration [54].

Mammary gland infections result in alterations in mineral concentrations in the milk. Potassium is the most abundant mineral found in milk [54] and is present in high concentrations compared to sodium [70]. During mastitis, potassium leaks into the blood from the mammary gland via the paracellular pathway, causing a decrease in its concentration [54,57]. Conversely, sodium leaks from the blood into the mammary gland, causing an increase in sodium concentrations in the milk [54,57]. Additionally, chloride concentrations in the milk also increase during mastitis, likely due to the influx of blood constituents into the mammary gland [57].

2.7. Effects of Mammary Gland Infections on Dairy Cow Health

Animals will often display abnormal behavior that indicates the presence of disease [50]. Invasion by pathogenic organisms is energy-demanding for the cow, especially when the immune system must be altered to combat infection and support recovery. Infection can cause a decrease in normal behavior including socialization, grooming, and feeding behaviors [75]. Acute mastitis causes visible signs of disease, such as a decrease in rumination [50]. Siivonen et al. [50] found that cows with endotoxin-induced acute mastitis displayed poor appetite and a decrease in rumen function.

Additionally, it has been observed that sick animals are reluctant to lie down, and those with a swollen udder and fever spend more time standing up [50]. Danzter [76] also points out that less lying down time is an indication of disease. Lying behavior is extremely important for dairy cows as it provides time for the cow to ruminate, which can maximize milk production [77]. If a cow is sick, then there is a reluctance to lie down and ruminate, negatively influencing milk production. The pain sensation in the cow's udder during infection could explain the reluctance to lie down but rather stand to avoid putting pressure on the swollen mammary gland [50]. Fogsgaard et al. [78] also found similar behavioral changes in cows with *E. coli*-induced mastitis, including a decrease in feeding, rumination, grooming, and lying behavior. Changes in normal behavior can be used as an indication of pain [79].

2.8. Economic Implications of Mammary Gland Infections

Infection of the mammary gland also contributes to economic loss for dairy producers due to the decrease in milk production. Subclinical mastitis, which is a form of mastitis infection with no visible signs, can result in a 70% decrease in milk production [7]. Aghamohammadi et al. [80] indicate that the economic loss due to mastitis can be up to CA$662 on a per cow basis. The National Mastitis Council [81] estimates 66% of production losses from a decrease in milk production due to a mastitis infection. The other 34% of losses would be for treating the sick cow, including veterinary bills, cost of treatments, extra labor, animal replacement, and discarding milk [81]. The economic loss from cows with mastitis is tremendous. Therefore, producers should routinely check their herd for mastitis and take necessary measures to prevent further production loss.

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3. Microbial Pathogens that Cause Mammary Gland Infections

3.1. Gram-positive Pathogens

Staphylococcus aureus is a major mastitis-causing pathogen, producing both subclinical and clinical infections and occurring more frequently in tie-stall housing systems [19,82]. The incidence of *S. aureus* infections has been found to be higher during early lactation and decrease further into lactation [83]. Sol et al. [84] sampled 143 *S. aureus* infected quarters 7 days prior to antibiotic treatment, the day of treatment, as well as 16 and 30 days after treatment. According to the authors, it was found at 30 d after treatment that 34% of infected quarters were still culture-negative [84]. *Staphylococcus aureus* can colonize the mammary gland and release lipoteichoic acid (LTA), a constituent of the cell wall [85]. Lipoteichoic acid can result in necrosis to the milk secreting tissue, thereby decreasing milk production [86].

The immune response has been shown to be inefficient during *S. aureus* infection due to the limited release of pro-inflammatory cytokines [87]. Cytokine expression in a *S. aureus* infection has been shown to be 5% lower compared to an *E. coli* infection [88]. Riollet et al. [89] reported a lack of pro-inflammatory cytokine expression, including interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8), in milk infected with *S. aureus*. Furthermore, Rainard et al. [90] discovered an increase in IL-1 and chemokine concentrations but no changes in TNF concentrations. During an immune response, IL-1 and TNF-α are important mediators of the host's inflammatory response. Furthermore, elevated TNF-α levels cause inflammatory signs such as heat, redness, swelling, and pain [91]. Interleukin-8's primary function is to act as a chemoattractant in neutrophil migration and degranulation while also increasing neutrophil microbicidal activity and stimulating phagocytosis [92]. Lara-Zarate et al. [93] indicated that *S. aureus* can disrupt the nuclear factor κB (NF-κB) system and decrease cytokine expression. Nuclear factor κB is known as a transcription factor that increases pro-inflammatory cytokine production and inducible nitric oxide synthase [94].

Another characteristic of *S. aureus* is the ability to produce a biofilm during pathogenesis, which protects the pathogen from phagocytosis by neutrophils and macrophages [86]. Chronic mastitis infections have also been found to be caused by Gram-positive bacteria possibly due to the formation of biofilm [95]. A recent study [96] compared the physiological and behavioral effects of pain in cows infused with LPS and LTA. Their findings displayed a higher degree of pain and discomfort in LPS-infused cows compared to LTA [96]. Naturally occurring *Staphylococcal* mastitis was found to induce chronic subclinical cases compared to acute clinical cases caused by *E. coli* [97]. These findings further support LTA's role in chronic mastitis infections, and slower immune response. Indeed, LTA has been shown to stimulate a weaker effect on vascular permeability compared to LPS [90,96].

Another Gram-positive pathogen found in pasture, free-stall, and tie-stall systems is *Streptococcus uberis* [82,98]. Countries including Canada, the United States, the Netherlands, and the United Kingdom have found *S. uberis* to produce 14 to 26% of clinical signs, where 33% of occurrence is greatest in the United Kingdom [99]. Surprisingly, 50-60% of *S. uberis* infections were discovered in cows kept in straw yards [100,101]. Research of *S. uberis* is lacking, but there have been various speculations on the exact mechanisms of infection. Different strains of *S. uberis* have also been found to be resistant to phagocytosis by neutrophils, allowing for colonization and clinical signs to be produced [102]. It is speculated that *S. uberis* strains produce a capsule for protection against

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neutrophils and macrophages; however, the exact mechanism remains unclear [103]. Thomas et al. [104] found *S. uberis* established within the secretory alveoli and ductular tissues, suggesting colonization occurs in the milk secreting tissues. *Streptococcus uberis* also produces an LTA endotoxin like that of *S. aureus* [105].

3.2 Gram-negative Pathogens

Gram-negative bacteria are major pathogens involved in environmental mastitis, where coliform pathogens are the predominate organisms, including *Escherichia, Klebsiella,* and *Enterobacter* [106]. *Escherichia coli* is harbored within the gastrointestinal tract of ruminants, often at normal, non-pathogenic coliform forming unit (cfu) levels [107]. *Escherichia coli* has also been found in the uterine tract of cows with uterine infections (metritis) [108]. Interestingly, coliform pathogens can multiply within the mammary gland without adhering to the epithelial tissue surfaces, possibly due to their ability to utilize lactose, the main carbohydrate in milk. The mammary gland itself has low oxygen levels, making this an ideal environment for coliform colonization. These factors can enable coliform bacteria numbers to increase, which is positively correlated to severity of the mammary infection. For instance, the coliform population can reach 10^8 cfu/mL of milk, whereas some Gram-negative bacteria such as *Serratia* and *Pseudomonas* are not lactose fermenters and typically don't exceed 10^4 cfu/mL of milk [107].

Susceptibility to new IMI increases during the first 2 weeks of the dry period and 2 weeks prior to parturition [40]. Certain serotypes of bacteria also require iron, which is necessary for their growth and survival but is bound, during dry-off, by lactoferrin [109]. As previously discussed in section 1.3.2 on protein alterations during mammary gland infections, lactoferrin increases during involution and remains constant throughout the dry period until colostrogenesis, when it decreases. Bacteria such as *Klebsiella pneumoniae* and *Enterobacter* can overcome the iron-binding abilities of lactoferrin and thereby cause mammary gland infection during the dry-off period [110].

The release of lipopolysaccharide (LPS) is triggered by the death of the pathogens, or the toxins are released in the form of vesicles [111]. Lipopolysaccharide can also translocate from the mammary gland into the systemic circulation, and contribute to clinical symptoms including fever, dehydration, anorexia, and diarrhea [107]. Hakogi et al. [112] found an 18-fold increase in plasma LPS concentration in cows with mastitis compared with healthy cows. Dosogne et al. [113] performed intramammary infusions of LPS, which increased plasma LPS levels in mastitis-affected cows with concentrations of 55-134 pg/mL compared to healthy cows at 10 pg/mL. Both studies confirm that infusion of LPS into the mammary gland can translocate into the blood circulation. Eckel and Ametaj [94] also suggested 3 potential sources of LPS that translocate into systemic circulation, including the rumen, mammary gland, and reproductive tract.

4. Immune Responses During Infections of the Mammary Gland Infection

During infection by bacteria, the host initiates an immune response to combat infection. The innate immune response is the non-specific initial response by the host that recognizes the pathogens and triggers the release of inflammatory mediators, including cytokines. The adaptive immune response is the secondary response by the host. This response is much more prolonged as it functions

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by memorizing a particular bacterial antigen to prevent further bacterial invasion. Both innate and adaptive immunity play major roles in the host defense during bacterial infection.

4.1 Innate Immunity

The innate immune response is the first line of defense against invading bacteria. More specifically, activation of innate immunity in the mammary gland is triggered by the recognition of invading organisms and the initiation of an inflammatory response [114]. The innate immune response of the mammary gland includes a series of cellular (e.g., leukocytes) and humoral defenses (e.g., cytokines, complement system, lactoferrin, transferrin, lysozyme, and the lactoperoxidase/myeloperoxidase systems) as well as oligosaccharides, gangliosaccharides, reactive oxygen species, acute phase proteins (APPs), ribonucleases, and various antimicrobial proteins and peptides [114].

Macrophages are the most predominant immune cells in both healthy and unhealthy mammary glands [22,115]. The proportion of macrophages varies depending on the stage of lactation. In early lactation, the number of macrophages is highest at 68% of all SCC and decreases to 21% in late lactation [116]. During early involution and in colostrum, the macrophages do not exceed 30% [115]. Blood monocytes migrate to the mammary gland where they differentiate into macrophages [92]. They migrate at a slower rate in comparison to neutrophils due to their large nuclei that provide more of a challenge to move between the endothelial cells [117]. Macrophages function in a similar manner to neutrophils where they engulf bacteria, foreign material, milk components, and cellular debris [118]. These cells also play a role in the adaptive immune response by processing and presenting antigens (Ag) to lymphocytes [119]. Bovine macrophages contain receptors for immunoglobulin G1 (IgG1) and immunoglobulin G2 (IgG2) to aid in the adaptive immune response [120]. Macrophages in the mammary gland also exhibit a bactericidal effect, which is found to be more effective during the dry-off period than during lactation [118].

Neutrophils are present in healthy mammary glands. They are important during early inflammatory stages and serve as the second line of defense during an infection [92]. The migration of additional neutrophils into the mammary gland occurs during IMI but also during milk removal [117]. The numbers of neutrophils have been found to increase during early and late lactation [121]. Furthermore, neutrophils made up 40-80% of SCC in early involutional secretions, but decreased in the second and fourth weeks of the dry period. Once the mammary gland has become fully involuted, neutrophil numbers return to lactational values [115]. Bovine neutrophils have a multilobulated nucleus which allows for easy migration across the endothelium of the mammary gland by diapedesis without causing damage to the mammary epithelium [122]. Neutrophils function in the phagocytosis of foreign invaders and remove them from infected areas. As previously discussed, neutrophils have been shown to mistakenly engulf milk components, including milk fat globules [123] and secrete proteinases, resulting in proteolysis of milk proteins such as casein [57,124]. Additional humoral components are secreted by neutrophils, including cytokines, chemokines, and hydroxyl radicals that damage the mammary epithelium and decrease milk production [124]. After completing their function, neutrophils undergo apoptosis (programmed cell death) and are removed from the mammary gland by macrophages [125,126].

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4.2 Adaptive Immunity

Adaptive immunity is a specific response to bacterial infection mediated by lymphocytes, which includes T and B lymphocytes and natural killer (NK) cells. Antigens (Ags) bind to membrane-bound receptors of lymphocytes, which in turn alter the function of those cell. The purpose of adaptive immunity is to elicit a faster response to a previously exposed threat [89] by memory lymphocytes [126]. Conditions in which adaptive immunity responds at a slower rate occur when the host has never been previously exposed to a particular threat [127]. Not much is known about the role of lymphocytes within the mammary gland, and populations tend to vary during lactation [116,128,129].

T lymphocytes are categorized into αβ T-cells and γδ T-cells [92]. The αβ T-cells include $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T-cells, where T-cells expressing the $CD8^+$ receptor are predominant within the mammary gland [129]. Activation of $CD8^+$ T cells is mediated by the major histocompatibility complex (MHC) class I molecules, which are present on majority of cells [130]. Taylor et al. [128] suggested that $CD8^+$ T cells may remove the damaged mammary epithelial cells, further increasing susceptibility to infection. $CD4^+$ T cells are activated by MHC class II molecules from Ag-presenting cells and are important for secreting various immunoregulatory compounds [89]. $CD4^+$ T cell concentrations are lower in milk compared to $CD8^+$ T cells but higher in the blood [129]. Cytotoxicity may also be mediated by γδ T-cells, which are found in secretions and the parenchyma of the mammary gland [131]. It has been suggested that γδ T-cells may also provide a barrier to the mucosal microenvironments to protect against infectious pathogens, indicating γδ T-cells' potential role in antibacterial immunity [122].

B lymphocytes play a role in humoral immunity by secreting antibodies (Ab) during infection. Levels of B lymphocytes within the mammary gland remain constant during lactation and during bacterial infection, unlike T lymphocytes, which tend to fluctuate [122,132]. Recognition of Ag by B lymphocytes is done via MHC class II molecules which bind the Ag, internalize, and process it whereby specific Ab are produced. Antibodies produced are termed immunoglobulins, including IgG1, IgG2, IgM, IgA, IgD, and IgE, where IgG is the predominant antibody in the milk of dairy cows [37].

Natural killer cells reside in the bone marrow, spleen, lymph nodes, and tonsils [133]. They are involved in the non-specific responses that recognize and cause lysis of foreign cells by various mechanisms, including Ab-dependent cell-mediated cytotoxicity, release of cytolytic factors, receptor-mediated Ag-recognition, granule exocytosis, and secretion of toxic molecules that induce apoptosis of altered cells [122]. Natural killer cells' ability to destroy both Gram-negative and Gram-positive bacteria has been demonstrated in various studies; therefore, NK cells could be crucial in preventing IMIs [134].

4.3 Recognition of Infectious Bacteria Within the Mammary Gland

Microbial surfaces contain various molecules that alert the immune system to the presence of an infectious organism. Such molecules are termed pathogen-associated molecular patterns (PAMPs) and are recognized by pattern recognition receptors [135] (PRRs). There are 3 known categories of PRRs, which include secreted PRRs, membrane-bound PRRs, and phagocytic PRRs

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[136]. Secreted PRRs are proteins including complements, pentraxins, peptidoglycan-recognition proteins, and lipid transferases, which are produced by hepatocytes that destroy bacteria via phagocytosis. Pattern recognition receptors are found on the surfaces of macrophages, neutrophils, and dendritic cells that bind bacteria and remove them from the host. Common PRR's would include macrophage mannose receptors, β-glucan, and scavenger receptors [134].

The most common type of PRR that recognizes PAMPs and damage-associated molecular patterns (DAMPs) are toll-like receptors (TLRs), which are the most heavily involved in the innate immune response. The central role of TLRs is binding of bacteria and secreting pro-inflammatory compounds. Macrophages, dendritic cells, and neutrophils have the majority of TLRs present on their cell surfaces, further indicating the role of these phagocytes during innate immunity [137]. Binding of bacteria to the TLRs triggers the secretion of pro-inflammatory cytokines, thus initiating an inflammatory response. Macrophages are known to have the most abundant production of cytokines during infection and inflammation. TNF- and IL-1, which are both important during the inflammatory response, are released by macrophages and other leukocytes [135].

4.4 Alterations in Serum Components During Mastitis Infections

Apart from alterations in milk composition during mammary gland infections, there are various serum metabolites, carbohydrates, and fatty acids involved in the immune responses that undergo marked changes during infection. Additionally, serum concentrations have also been shown to be altered during other periparturient diseases [62,138,139,140,141].

Tumor necrosis factor-α and IL-1 have been shown to be major indicators of diseases in dairy cows. The main functions of IL-1 are to enhance recruitment of neutrophils and their phagocytic and bactericidal abilities, stimulate secretion of additional cytokines and chemokines (e.g., IL-1, IL-6, IL-8, IL-12, TNF-α), and mediate the acute phase response (APR) [92].

Tumor necrosis factor-α has pro-inflammatory properties similar to IL-1, such as neutrophil recruitment, enhancing neutrophil activity, and mediating APR [92]. TNF-α also stimulates endothelial cells to express adhesion molecules [92]. TNF- and IL-1 can both cause systemic effects in the host, such as fever, increased heart rate, and appetite loss [137]. Dervishi et al., [138] found significant increases in TNF- in the serum of SCM cows four weeks before calving and during disease diagnosis. Concentrations of TNF-α in the milk and serum of cows are higher in both naturally occurring and experimentally induced E. coli mastitis [142,143]. Increased concentrations of TNF- α and IL-1 in milk suggest a link between neutrophil recruitment in the mammary gland and the development of SCM [144]. Other studies on innate immunity and disease also found increased concentrations of pro-inflammatory cytokines in cows with SCM, retained placenta, metritis, ketosis, and lameness [62,138,139,140,141].

Tumor necrosis factor-α and IL-1 are important pro-inflammatory cytokines in activating the APR which involves the secretion of APPs including haptoglobin (Hp) and serum amyloid A (SAA) from liver hepatocytes [89,144]. The study by Dervishi et al. [138] additionally found serum SAA concentrations to be greater in cows with SCM at multiple time points prior to and after parturition. The authors speculated that elevated TNF-α and SAA throughout the study means that IMI had begun at dry-off and that cows were in a state of chronic endotoxemia during the dry period [138]. The function of SAA is to expedite the removal of endotoxin, bound to lipoproteins, from systemic

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circulation through liver hepatocytes [48]. Serum amyloid A is also present in the mammary epithelial cells of infected mammary glands and may be important in protecting the tissues against pathogenic bacteria in the early stages of infection [145].

Haptoglobin also plays a major role in innate immunity as it binds free hemoglobin to decrease the availability of iron, which bacteria require for growth [146]. Haptoglobin was observed to be lower at 8 and 4 weeks prior to parturition, possibly making cows more susceptible to disease during the dry period [136]. Previous studies on lameness, metritis, and ketosis witnessed significant increases in Hp during the week of diagnosis [138,140,141]. Dervishi et al. [138] suggest that lower concentrations of Hp in the blood of SCM cows prior to calving are a result of Hp moving into the mammary gland to assist in immune response. Aside from binding of free hemoglobin, Hp can also facilitate neutrophil recruitment, free radical quenching, and help tissue repair and regeneration during inflammation [147].

Apart from innate immunity alterations during disease, there have also been alterations in carbohydrate and lipid metabolism in dairy cows with SCM. Cows with SCM post-partum were shown to have significantly higher levels of serum lactate at -8 weeks relative to calving [138]. Research has found that concentrations of lactate in the blood can serve as a useful indicator of the severity of illness [148]. Moreover, assessment of lactate concentrations in the milk can also be a useful indicator for udder health [149]. Davis et al. [149] previously reported that there is a close relationship between SCC and high lactate in the milk, further supporting lactate as an indicator for udder health. Concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) also have been shown to be elevated at -4 weeks in dairy cows diagnosed with SCM [138]. Increased blood NEFA and BHBA is a result of negative energy balance and reduced feed intake which causes mobilization of fatty acids in the adipose tissue and conversion of NEFA into ketone bodies in the liver [150]. Increased BHBA and NEFA concentrations have been linked to an increased risk of periparturient disease such as ketosis, displaced abomasum, milk fever, mastitis, and retained placenta [151,152,153].

5. Relation of Mastitis to Other Periparturient Diseases

It is being proposed that mastitis possibly contributes to the development of other periparturient diseases in dairy cows. These diseases include retained placenta, metritis/endometritis, lameness, and ketosis; however, there has been no research conducted on whether mastitis does in fact contribute to other diseases. Previous studies have found an elevation in the SCC of dairy cows diagnosed with periparturient disease [62,138,140,141]. Eckel and Ametaj [94] have suggested that endotoxins, such as LPS and LTA, can translocate from 3 different organs of cattle, including the rumen, reproductive tract, and mammary gland. Reports from [112] and [113] showed that plasma LPS increased during experimentally induced or naturally occurring mastitis. It is suggested that translocation of LPS occurs by two possible mechanisms [154]. The first mechanism is paracellular transport, where endotoxin disrupts the epithelium's tight junctions, increasing permeability and allowing for passage of endotoxin across the epithelium into the bloodstream [114,154]. The second possible mechanism of endotoxin transport is transcellular transport, where endotoxin binds to TLR's located on the epithelial surfaces where it is internalized, transported to the Golgi apparatus, and chylomicrons are produced, which are transported into systemic circulation

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[155]. Endotoxin transport via paracellular and/or transcellular transport could provide some insight into how bacteria causing an IMI could enter systemic circulation and cause or worsen other diseases.

5.1. Retained Placenta

The incidence of retained placenta has increased substantially over the years, occurring in 2-5% of dairy cows postpartum [156]. Normal expulsion of the fetal membranes should be within 6 hours following parturition. The placenta is considered retained if the cow fails to expel the fetal membranes after 24 hours. Multiple factors increase the likelihood of a cow developing a retained placenta, including abortion, short gestation, twins, and dystocia. Retained placenta also contributes to an increased risk of postpartum metritis and/or endometritis, an increased time to first heat, an increased number of days open, decreased fertility, delayed uterine involution, and an increased number of times bred. The prevalence of retained placenta has also been linked to the development of other periparturient diseases in dairy cows, such as ketosis, metritis, endometritis, and mastitis [156]. Retained placenta is associated with a decrease in milk production [157].

Dervishi et al. [62] found elevations of blood metabolites including TNF-α, IL-1, IL-6, SAA, and lactate beginning at 8 and 4 weeks prior to parturition, indicating an inflammatory state and activation of innate immunity. Concentrations of Hp also increased 10-fold in retained placenta cows during diagnosis week [62]. Ametaj et al. [158] suggest that *E. coli* LPS has some involvement in the low milk yield of dairy cows with retained placenta. Increased concentrations of TNF-α during gram-negative bacterial infections inhibit prolactin production in the pituitary gland, thereby decreasing production [159]. Lactate levels have also been noted to increase in cows with retained placenta [62]. Lactate has previously been elevated during diseases like mastitis, and the sources of this metabolite may be the mammary gland or muscle tissue [149].

6.2 Metritis and Endometritis

Metritis and endometritis refer to inflammation of the uterine tract where metritis is the inflammation of the uterus and endometritis is inflammation of the endometrium [156]. Metritis and endometritis are mainly caused by bacterial infection; however, inflammation of the uterine tract post-partum is considered normal. A visual indication of metritis is a purulent or reddish-brown discharge, often with a foul odor, that occurs within 21 days post-partum [160,161]. Metritis incidence in dairy cows has been reported to be around 18.5% [162], but on some farms it can reach up to 40% of the cows [163]. Studies performed using American and European dairy herds have also found that cows with retained placentas were 6 times more likely to develop metritis, and those with metritis had a 2-fold higher risk of developing ketosis [156].

Metritic cows also exhibited activation of innate immunity and subsequent inflammation prior to calving [139]. Multiple studies have reported upregulation of pro-inflammatory cytokines IL-6 and TNF-α in cows with metritis [139,164], whereas concentrations of IL-1 are decreased. The lowered levels of IL-1 could be caused by negative feedback exerted by IL-6-type cytokines [165]. Pro-inflammatory cytokines have also been shown to activate APP and SAA as early as 8 weeks prior to parturition [139]. The involvement of SAA in uterine tissue damage has been observed after the

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fetus and placenta are expelled from the uterus [166]. There is also evidence that Hp is involved in the pathogenesis of multiple reproductive diseases [167]. It has additionally been observed that metritis can decrease milk production and increase SCC within the mammary gland [139].

To our best knowledge, there have been no studies conducted on the association of SCC and metritis; however, some studies have observed a negative correlation between mastitis and pregnancy success [168,169]. Moore et al. [168] reported that cows with CM were more likely to develop irregular estrous cycles. Fetal abortion was also 3 times higher in cows that developed CM within the first 45 DIM [170]. Endotoxins have also been found to be present in tissues of the ovary [171], endometrium [172], and hypothalamus [173]. Herath et al. [171] found that follicular growth was disrupted by bacterial infection, whereas [172] found LPS prolonged the luteal phase with an increase in estrogen production. Intramammary infections have also been linked to hypothermia (fever) induction and embryo loss as a result of pro-inflammatory cytokines released from the mammary gland [169].

6.3 Ketosis

Ketosis is a metabolic disorder that commonly occurs during early and peak lactation (3-6 weeks postpartum) [174,175]. Ketosis first appears in subclinical form, characterized by an elevation of ketone bodies (β-hydroxybutyric acid (BHBA), acetoacetate, and acetone) in the urine, milk, and blood. Subclinical ketosis (SCK) affects approximately 40% of dairy cows in North America, often reaching 80% in some dairy herds [174]. Around 2-15% of SCK cases can progress to clinical ketosis (CK) characterized by excess ketone bodies in the blood, urine, and milk, decreased appetite, decreased milk production, significant decrease in body condition, and dry manure [176]. Cows tend to experience a NEB during early lactation, where a decrease in feed intake cannot compensate for the high demand for milk production, resulting in ketosis [175]. Additionally, cows at peak lactation require large amounts of energy for milk production, resulting in NEB and reduced feed intake [175].

Previous research has shown an association between ketosis and the incidence of other periparturient diseases, including mastitis and metritis [177]. Moreover, alterations in blood metabolites suggest that innate immunity as well as carbohydrate and lipid metabolism may have a role in the development of ketosis [141]. The latter authors observed a significant increase in BHBA, lactate, IL-6, TNF-α, Hp, and SAA in dairy cows beginning at 8 and 4 weeks prior to calving, indicating activation of innate immunity. Elevation in IL-6 was observed in human subjects during hyperketonemia, possibly due to IL-6 effects on multiple metabolic pathways including oxidative stress, oxidation of fatty acids, lipoprotein metabolism, and protein degradation [178]. Furthermore, [179] proposed that elevated IL-6 and TNF- and decreased appetite could stimulate adipose tissue breakdown, leading to insulin resistance and lipolysis.

In vitro studies have also determined a correlation between elevated plasma BHBA and *E. coli* mastitis [180]. Intramammary infusion of LPS has been previously demonstrated to affect metabolism, immune response, and overall performance of dairy cows [181]. Several studies have provided evidence that LPS challenge induces both a metabolic response and an mRNA abundance of inflammatory mediators [181,182]. Moreover, infusion of BHBA has resulted in an increase in APP mRNA abundance within the mammary gland [183]. Zarrin et al. [184] induced hyperketonemia by BHBA infusion for 56 h and then infused LPS into the mammary gland in mid-lactating dairy cows.

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The authors observed a decline in plasma glucose concentrations and confirmed that the mammary tissues used BHBA as an alternative energy source. Reduction in glucose concentrations could negatively impact the immune system and reduce cow performance if glucose concentrations are depleted for long periods of time [184]. While this study established the metabolic effects of induced hyperketonemia and LPS-infusion in the mammary gland, it remains unclear whether mastitis is associated with ketosis.

6.4. Laminitis

Laminitis is defined as the aseptic inflammation of the corium layer, commonly occurring in both horses and bovines. Laminitis is one of the top 3 major diseases of dairy cows, following infertility and mastitis [185]. Physical characteristics of cows affected by laminitis include abnormal gait, arched back, abnormal claw morphology, favoritism on one leg, decreased body condition, decreased feed intake, and decreased milk production [186]. Inflammation in the hoof region is also a major indicator of laminitis. The relationship between grain-overload and the development of subclinical ruminal acidosis to the incidence of laminitis has been well established; however, the pathogenesis of laminitis remains poorly understood [158]. Furthermore, research has primarily focused on lameness contributing to a mastitis infection but not mastitis contributing to lameness.

A study by [192,193] investigated the relationship between locomotion scores and SCC in 7 UK dairy herds. Their results indicated a negative association between SCC and locomotion score, where lame cows had lower SCC compared to non-lame cows [192]. Archer et al. [193] then concluded that lame cows that spent more time standing decreased exposure of the mammary gland to infectious pathogens, thus maintaining a low SCC. To our best knowledge, this is the only study that aimed to establish a relationship between SCC and lameness. While these findings observed the overall behavior and locomotion scoring of lame cows, they did not, however, look at the changes in metabolites that are associated with lameness and mastitis.

It was previously demonstrated that the upregulation of blood metabolites in dairy cows with lameness could be used as potential biomarkers for predicting lameness incidence [140]. Zhang et al. [140] reported increased serum concentrations of TNF-α, IL-1, and IL-6 in dairy cows at 8 and 4 weeks prior to calving. Previous reports in horses suggested that the development of clinical lameness would result in an increase in IL-6 in blood [194]. In previous reports, it was shown that IL-6 was a potent biomarker for cows with mastitis, retained placenta, and metritis [195,196]. This indicates that IL-6 may play an important role in the clinical stages of lameness and other metabolic diseases. It was also suggested that endotoxins might play a role in stimulating cytokine production and increasing Hp and SAA concentrations [197]. Zhang et al. [140] also reported increased SCC levels in lame cows and a positive correlation between lactate, IL-6, TNF-α, and SAA, further suggesting that mammary gland infection prior to dry off could contribute to the development of diseases post-partum.

6. Current Approaches to Treatment of Mammary Gland Infections

6.1 Application of Antibiotics and Bacterial Resistance

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Treatment and prevention of disease in food-producing animals has been maintained using antibiotics, which is one of the most effective ways of reducing IMI. The highest risk for a cow to develop a new IMI has been shown to be at the beginning of dry-off and around parturition [40]. Dry cow therapy (DCT) is an antibiotic treatment administered to cows at dry off. The benefits of DCT are related to decreasing the number of infectious organisms within the mammary gland, thus reducing the incidence of new IMI post-partum [36]. According to [36], farmers who do not treat their cows with DCT increase the incidence rate of new IMI by 10-15%. The dry cow therapy has been shown to have an efficiency of 90-93% against *S. agalactiae* infections, 70-80% against *S. aureus* infections, and 70-90% against environmental streptococci bacteria. In comparison to treating mastitis infections during lactation, the dosage of antibiotic is less compared to DCT due to the risk of antibiotic residues within the milk. Therefore, cows receiving DCT at dry off is more beneficial since higher dosages can be administered and incidence of new IMI is decreased.

Recent suggestions have been put forward that antibiotic use for treatment of disease within the agriculture industry is resulting in increased antibiotic resistance by infectious organisms [198]. Indeed, the mechanisms by which bacteria become resistant to antibiotics include preventing entry or export of the drug, secretion of enzymes that alter or destroy the antibiotic, or making changes to the antimicrobial target [199]. Countries such as the United Kingdom, Denmark, and Norway have implemented a ban on certain antimicrobials in swine and poultry systems with the goal of decreasing populations of resistant organisms. Conflicting reports showed that some populations of resistant organisms had decreased after the ban, while other populations had remained unchanged [200,201]. An example is the resistance of *S. aureus* against penicillin [10], which has made it increasingly difficult to control and treat sick animals. Cows treated for a staphylococcal infection during lactation have been shown to be <50% effective at reducing infectious pathogens [36]. Oliver and Sordillo [202] concluded that DCT is not entirely effective at decreasing the incidence of new IMI. There is now an increasing interest in new alternative methods of reducing IMI and reducing other periparturient diseases of dairy cows.

8. Summary

The incidence of a mammary gland infection is highly problematic within the dairy industry as it results in production loses for producers and poor cow performance. Both Gram-negative and Gram-positive organisms can produce endotoxins that contribute to mammary gland infections and activation of the immune system. There have been multiple studies on immunosuppression during the transition period, and disease incidence post-partum. To our best knowledge, there is no research yet on the association of mammary gland infections at the time of dry off being related to other periparturient diseases in dairy cows. The incidence of mammary gland infection at dry off and its possible association with periparturient diseases including metritis, laminitis, ketosis, retained placenta, and milk fever will be the subject of interest.

References

1. Shamra, N. Alternative approach to control intramammary infection in dairy cows- A review. *Asian J. Anim. Vet. Adv*. **2007**, *2*, 50-62.

2. Koeck, A., F. Miglior, D. F. Kelton, F. S. Schenkel. 2012. Alternative somatic cell count traits to improve mastitis resistance in Canadian Holsteins. *J. Dairy Sci*. **2012**, *95*, 432-439.
3. Seegers, H.; Fourichon, C.; Beaudeau, F. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet. Res*. **2003**, *34*, 475-491.
4. Pinzón-Sánchez, C.; Cabrera, V.E.; Ruegg, P.L. Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation. *J. Dairy Sci*. **2011**, *94*, 1873-1892.
5. Harmon, R.J. Symposium: Mastitis and Genetic Evaluations for Somatic Cell Count. *J. Dairy Sci*. **1994**, *77*, 2103-2112.
6. Government of Canada. Breed Improvement and Genetic Evaluation: Culling and Replacement Rates in Dairy Herds in Canada. Available from: https://www.dairyinfo.gc.ca/eng/dairy-statistics-and-market-information/dairy-animal-genetics/culling-and-replacement-rates-in-dairy-herds-in-canada/?id=1502475693224 [Accessed Jan 2nd,2021], 2019.
7. Kirk, J.H.; Bartlett, P.C. Economic impact of mastitis in Michigan Holstein dairy herds using computerized records system. Agricultural Practises. 1: 3. Philpot, W.N. 1984. Mastitis Management. Oak Brook III.: Babson Brothers, 1988.
8. Tylor J.W.; Cullor, J.S. Mammary gland health and disorders. In: Smith BP, editor. Large animal internal medicine. 3rd ed. London: Mosby. 1990, 1019-1022.
9. Klocke, P.; Walkenhorst, M.; Butler, G. 11-Redecuing antibiotic use for mastitis treatment in organic dairy production systems. Handbook for Organic Food Safety and Quality. Woodland Publishing Series in Food Science, Technology and Nutrition. 2007, 199-220.
10. Nickerson, S.C. Managing Clinical Mastitis in Dairy Cows to Reduce Somatic Cell Counts and Improve Milk Quality. Available from: http://www.ads.uga.edu/2011AnimalDairyScienceReports.html [Accessed 2016 July 4], 2011.
11. Roberson, J.R. Treatment of Clinical Mastitis. *Vet. Clin. North Am.: Food Anim. Pract*. **2012**, *28*, 271-288.
12. Wilson, D.J.; Gonzales, R.N.; Das, H.H. Bovine mastitis pathogen in New York and Pennsylvania: prevalence and effects on somatic cell count and milk production. *J. Dairy Sci*. **1997**, *80*, 2592-2598.
13. Pitkala A.; Haveri, M.; Pyorala, S.; Myllys, V.; Honkanen-Buzalski, T. Bovine mastitis in Finland 2001- prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci*. **2004**, *87*, 2433-2441.
14. Kromker V.; Graowski, N.T.; Redetzky, R.; Hamann, J. Detection of mastitis using selected quarter milk parameters. Canada: 2nd Intl Symp Bovine Mastitis and Milk Quality Vancouver. 2001, 486-487.
15. Djabri, B.; Bareille, N.; Beaudeau, F.; Seegers, H. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. *Vet. Res*. **2002**, *33*, 335-357.
16. Madouasse, A.; Huxley, J.N.; Browne, W.J.; Bradley, A.J.; Green, M.J. Somatic cell count dynamics in a large sample of dairy herds in England and Wales. *Prev. Vet. Med*. **2010**, *96*, 56-64.

17. Nyman, A.K.; Persson Waller, K.; Bennedsgaard, T.W.; Larsen, T.; Emanuelson, U. Associations of udder-health indicators with cow factors and with intramammary infection in dairy cows. *J. Dairy Sci*. **2014**, *97*, 5459-5473.
18. Hogan, J.; Smith, K.L. Managing environmental mastitis. *Vet. Clin.: Food Anim. Pract*. **2012**, *28*, 217-224.
19. Barkema, H.W.; Schukken, Y.H.; Zadoks, R.N. *Invited Review:* The Role of Cow, Pathogen, and Treatment Regimen in the Therapeutic Success of Bovine *Staphylococcus aureus* mastitis. *J. Dairy Sci*. **2006**, *89*, 1877-1895.
20. McDougall, S., D. G. Arthur, M. A. Bryan, J. J. Vermunt, A. M. Weir. 2007. Clinical and bacteriological response to treatment of clinical mastitis with one of three intramammary antibiotics. New Zealand Veterinary Journal. 55:161-170.
21. Smith, K.L.; Hogan, J.S. A world standard for milk somatic cell count: is it justified? Bulletin of the International Dairy Federation. **1999**, *345*, 7-10.
22. Napel, J.T.; de Haas, Y.; de Jong, G.; Lam, T.J.G.M.; Ouweltjes, W.; Windig, J.J. Characterization of distributions of somatic cell counts. *J. Dairy Sci*. **2009**, *92*, 1253-1264.
23. Rainard, P.; Foucras, G.; Boichard, D.; Rupp, R. Invited review: Low milk somatic cell count and susceptibility to mastitis. *J Dairy Sci*. **2018**, *101*, 6703-6714. doi: 10.3168/jds.2018-14593. PMID: 29803421.
24. Shamra, N., N. K. Singh, M. S. Bhadwal. 2011. Relationship of Somatic Cell Count and Mastitis: An Overview. *Asian-Austral. J. Anim. Sci*. **2011**, *24*, 429-438.
25. United States Department of Agriculture. Determining U.S. Milk Quality Using Bulk-tank Somatic Cell Counts. Available from: http://www.aphis.usda.gov [Accessed 2016 June 10], 2012.
26. Schalm, O.W.; Noorlander, D.O. Experiments and observations leading to development of the California mastitis test. *J. Am. Vet. Med. Assoc*. **1957**, 130, 199-204.
27. Sanford, C.J.; Keefe, G.P.; Sanchez, J.; Dingwells, R.T.; Barkema, H.W. Leslie, K.E.; Dohoo, I.R. Test characteristics from latent-class models of the California Mastitis Test. *Prev. Vet. Med.* **2006**, *77*, 96-108.
28. Pradieé, J.; Moraes, C.R.; Gonçalves, M.; Vilanova, M.S.; Corrêa, G.F.; Lauz, O.G.; Osório, M.T.M.; Schmidt, V. Somatic Cell Count and California Mastitis Test as a Diagnostic Tool for Subclinical Mastitis in Ewes. *Acta Sci. Vet*. **2012**, *40*, 1038.
29. Bhutto, A.L.; Murray, R.D; Woldehiwet, Z. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. *Res. Vet. Sci*. **2012**, *92*, 13-17.
30. Poutrel, B.; Rainard, P. California Mastitis test guide of selective dry cow therapy. *J. Dairy Sci*. **1981**, *64*, 241-248.
31. Kehrli Jr, M.E.; Shuster, D.E. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. *J. Dairy Sci*. **1994**, *77*, 619-627.
32. Harmon, R.J. Mastitis and genetic evaluation for somatic cell count. *J. Dairy Sci*. **1994**, *77*, 2103-2112.
33. Dhakal, I.P. Normal somatic cell count and subclinical mastitis in Murrah buffaloes. J. *Vet. Med, B. Infect. Dis. Vet. Public Health*. **2006**, *53*, 81-86.

34. Dingwell, R.T.; Kelton, D.F.; Leslie, K.E.; Edge, V.L. Deciding to Dry-Off: Does Level of Production Matter? National Mastitis Council Annual Meeting Proceedings. **2001**, 69-79.
35. Swanson, E.W.; Poffenbarger, J.I. Mammary gland development of dairy heifers during their first gestation. *J. Dairy Sci*. **1979**, *62*, 702-714.
36. Jones, G.M. Proper Dry Cow Management Critical for Mastitis Control. Virginia Polytechnic Institute and State University, College of Agriculture and Life Sciences. 2009, 404-212.
37. Sjaastad Ø.V.; O. Sand, O.; Hove, K. Physiology of Domestic Animals. 3rd edition. Oslo: Scandinavian Veterinary Press, 2016.
38. Funk, D.A.; Freeman, A.E.; Berger, P.J. Effects of previous days open, previous days dry, and present days open on lactation yield. *J. Dairy Sci*. **1987**, *70*, 2366–2373.
39. Watters, R.D.; Guenther, J.N.; Brickner, A.E.; Rastani, R.R.; Crump, P.M.; Clark, P.W.; Grummer, R.R. Effects of Dry Period Length on Milk Production and Health of Dairy Cattle. *J. Dairy Sci*. **2008**, *91*, 2595-2603.
40. Oliver, S.P.; Bushe, T. Inhibition of coliform mastitis pathogen growth during involution of the bovine mammary gland. In Proc. Int. Symp. Mastitis Control Hygienic Prod. Milk, Espoo, Finland. 25, 1986.
41. Arnold, M. Management of the Dry Cow to Prevent Mastitis. (https://afs.ca.uky.edu/dairy/management-dry-cow-prevent-mastitis) University of Kentucky College of Agriculture, Lexington, KY, 40546, 2012.
42. Senger, P.L. Pathways to Pregnancy and Parturition. 3rd edition. Innovative Technologies: , 2012, 381.
43. Sordillo, L.M.; Nickerson, S.C.; Oliver, S.P. Morphological evaluation of bovine mammary tissue throughout involution and colostrogenesis. *J. Dairy Sci*. **1987**, 70, 247. (Abstr.)
44. Sordillo, L.M.; Nickerson, S.C. Morphologic changes in the bovine mammary gland during involution and lactogenesis. *Am. J. Vet. Res*. **1988**, *49*, 1112-1120.
45. Kehrli Jr., M.E.; Nonnecke, B.J.; Roth, J.A. Alterations in bovine neutrophil function during the periparturient period. *Am. J. Vet. Res*. **1989**, *50*, 207-214.
46. Goff, J.P.; Horst, R.L. Physiological changes at parturition and their relationship to metabolic disorders. *J. Dairy Sci*. **1997**, *80*, 1260-1268.
47. Mallard, B.A.; Dekkers, J.C.; Ireland, M.J.; Leslie, K.E.; Sharif, S.; Vankampen, C.L.; Wagter, L.; Wilkie, B.N. Alteration in immune responsiveness during the peripartum period and its ramification on dairy cow and calf health. *J. Dairy Sci*. **1998**, *81*, 585-595.
48. Ametaj, B.N.; Bradford, B.J.; Bobe, G.; Nafikov, R.A.; Lu, Y.; Young, J.W.; Beitz, D.C. Strong relationships between mediators of the acute phase response and fatty liver in dairy cows. *Can. J. Anim. Sci*. **2005**, *85*, 165-175.
49. Natzke, R.P.; Everett, R.W.; Bray, D.R. Effect of drying off practices on mastitis infection. *J. Dairy Sci*. **1975**, *58*, 1828-1835.
50. Siivonen, J.; Taponen, S.; Hovinen, M.; Pastell, M.; Lensink, B.J.; Pyörälä, S.; Hänninen, L. Impact of acute clinical mastitis on cow behaviour. *Appl. Anim. Behav. Sci*. **2011**, 132, 101-106.
51. Petrovski, K.R.; Stefanov, E. Milk composition changes during mastitis. DeLaval. Available from: www.milkproduction.com/Library/Scientific-articles/Animal-Health/Milk-composition-changes [Accessed 2017 Nov 23], 2006.

52. McManaman, J.L.; Neville, M.C. Mammary physiology and milk secretion. *Adv. Drug Del. Rev.* **2003**, *55*, 629-641.
53. Nguyen, D.; Neville, A.D.; Margaret, C. Tight Junction Regulation in the Mammary Gland. *J. Mamm. Gland Biol. Neopl.* **1998**, 3, 233-246.
54. Auldist, M.J., S. Coats, G. L. Rogers, G. H. McDowell. Changes in the composition of milk from healthy and mastitic dairy cows during the lactation cycle. *Aust. J. Experim. Agri*. **1995**, *35*, 427-436.
55. Bruckmaier, R.M.; Ontsouka, C.E.; Blum, J.W. Fractionized milk composition in dairy cows with subclinical mastitis. *Vet. Med-Czech*. **2004**, *8*, 283-290.
56. Shuster, D.E.R.J.; Harmon, J.A.; Jackson, R.E.; Hemken, R.E. Suppression of Milk Production During Endotoxin-Induced Mastitis. *J. Dairy Sci*. **1991**, *74*, 3763-3774.
57. Auldist, M.J.; Hubble, I.B. Effects of mastitis on raw milk and dairy products. The Aust. J. Dairy Technol. **1998**, *53*, 28-36.
58. LeBlanc, S.J.; Duffield, T.F.; Leslie, K.E.; Bateman, K.G.; Keefe, G.P.; Walton, J.S.; Johnson, W.H. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *J. Dairy Sci*. **2002**, *85*, 2223-2236.
59. Nocek, J.K. Bovine acidosis: implications for laminitis. *J. Dairy Sci*. **1997**, *80*, 1005-1028.
60. Warnick, L.D.; Janssen, D.; Guard, C.L.; Grohn, Y.T. The effect of lameness on milk production in dairy cows. *J. Dairy Sci*. **2001**, *84*, 1988-1997.
61. Gordon, J.L. Risk Factors for and Treatment of Ketosis in Lactating Dairy Cows (Doctoral Thesis) University of Guelph, Guelph, ON, Canada. 2013a, 1-162.
62. Dervishi, E.; Zhang, G.; Hailemariam, D.; Dunn, S.M.; Ametaj, B.N. Occurrence of retained placenta is preceded by an inflammatory state and alterations of energy metabolism in transition dairy cows. J. Anim. Sci. Biotechnol. **2016**, *7*, 26.
63. Ward, P.P.; Uribe-Luna, S.; Conneely, O.M. Lactoferrinand host defense. *Bioch. Cell. Biol*. **2002**, *80*, 95-102.
64. Diarra, M.S.; Petitclerc, D.; Lacasse, P. Effect of lactoferrin in combination with penicillin on the morphology and the physiology of *Staphylococcus aureus* isolated from bovine mastitis. *J. Dairy Sci*. **2002**, *85*, 1141–1149.
65. Smith, K.L.; Oliver, S.P. Lactoferrin: a component of nonspecific defense of the involuting bovine mammary gland. *Adv Exp Med Biol*. **1981**; *137*, 535-54. PMID: 7036686.
66. Ollivier-Bousquet, M. Transferrin and prolactin transcytosis in the lactating mammary epithelial cell. *J. Mamm. Gland Biol. Neopl*. **1998**, *3*, 303-313.
67. Sjaastad Ø.V.; O. Sand, O.; Hove, K. Physiology of Domestic Animals. 3rd edition. Oslo: Scandinavian Veterinary Press, 2016.
68. Rogers, S.A.; Slattery, S.L.; Mitchell, G.E.; Hirst, P.A.; Grieve, P.A. The relationship between somatic cell count, composition and manufacturing properties of bulk milk. 3. Individual proteins. *Aust. J. Dairy Tech*. **1989**, *44*, 49-52.
69. McFadden, T.B.; Akers, R.M.; Capuco, A.V. Relationship of milk proteins in blood with somatic cell counts in milk of dairy cows. *J. Dairy Sci*. **1988**, *71*, 826-834.
70. Holdaway, R.J. A comparison of methods for the diagnosis of bovine subclinical mastitis within New Zealand dairy herds. Thesis (Ph. D.)-Massey University, Palmerston North, New Zealand, 1990.

71. Zachos, T.; Politis, I.; Gorewit, R.C.; Barbano, D.M. Effect of mastitis on plasminogen activator of milk somatic cells. *J. Dairy Res*. **1992**, *59*, 461-67.
72. Linde, A.; Ross, C.R.; Davis, E.G.; Dib, L.; Blecha, F.; Melgarejo, T. Innate immunity and host defense peptides in veterinary medicine. *J. Vet Inter*. Med. **2008**, *22*, 247-265.
73. Moussaoui, F.; Vangroenweghe, F.; Haddadietal, K. Proteolysis in milk during experimental *Escherichia coli* mastitis. *J. Dairy Sci*. **2004**, *87*, 2923-2931.
74. Opdebeeck, J.P. Mammary gland immunity. *J. Am. Vet. Med. Ass*. **1982**, *181*, 1061-1065.
75. Johnson, R.W. The concept of sickness behavior: A brief chronological account of four key discoveries. *Vet. Immunol. Immunopathol*. **2002**, *87*, 443-450.
76. Danzter, R. Cytokine-induced sickness behavior: where do we stand? *Brain Behav. Immun*. **2001**, *15*, 7-24.
77. Jensen, M.B.; Munksgaard, L.; Pederson, L.J.; Ladewig, J.; Matthews, L. Prior deprivation and reward duration affect the demand function for rest in dairy heifers. *App. Anim. Behav. Sci*. **2004**, *88*, 1-11.
78. Fogsgaard, K.K.; Røntved, C.M.; Sørensen, P.; Herskin, M.S. Sickness behaviour in dairy cows during *Escherichia coli* mastitis. *J. Dairy Sci*. **2011**, *95*, 630-638.
79. Weary, D.M.; Niel, L.; Flower, F.C.; Fraser, D. Identifying and preventing pain in animals. *Appl. Anim. Behav. Sci*. **2006**, *100*, 64-76.
80. Aghamohammadi, M.; Haine, D.; Kelton, D.F.; Barkema, H.W.; Hogeveen, H.; Keefe, G.P.; Dufour, S. Herd-Level Mastitis-Associated Costs on Canadian Dairy Farms. *Front Vet. Sci*. **2018**, *5*, 100. [DOI: 10.3389/fvets.2018.00100] [PMCID: PMC5961536]
81. National Mastitis Council. Current Concepts of Bovine Mastitis. 4th ed. National Mastitis Council, Madison, WI, 1996.
82. Olde Riekerink, R.G.M.; Barkema, H.W.; Kelton, D.F.; Scholl, D.T. Incidence rate of clinical mastitis on Canadian dairy farms. *J. Dairy Sci*. **2008**, *91*, 1366-1377.
83. Persson Waller, K.; Bengtsson, B.; Lindberg, A.; Nyman, A.; Ericsson Unnerstad, H. Incidence of mastitis and bacterial findings at clinical mastitis in Swedish primiparous cow-influence of breed and stage of lactation. *Vet. Microbiol*. **2009**, *134*, 89-94.
84. Sol, J.; Sampimon, O.C.; Snoep, J.J.; Schukken, Y.H. Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by *Staphylococcus aureus. J. Dairy Sci*. **1997**, *80*, 2803-2808.
85. Ryan, K.J.; Ray, C.G. Sherris Medical Microbiology: An Introduction to Infectious Diseases. 4th ed. McGraw Hill Publishers. 937, 2004.
86. Kerro Dego, O.J.; van Dijk, E.; Nederbragt, H. Factors involved in the early pathogenesis of bovine *Staphylococcus aureus* mastitis with emphasis on bacterial adhesion and invasion. A review. *Vet. Quart*. **2002**, *24*, 181-198.
87. Bannerman, D.D.; Paape, M.J.; Lee, J.W.; Zhao, X.; Hope, J.C.; Rainard, P. *Escherichia coli* and *Staphylococcus aureus* elicit differential innate immune responses following intramammary infection. *Clin. Diagn. Lab. Immunol*. **2004**, *11*, 463-472.
88. Guntler, J.; Liu, S.; Esch, K.; Schuberth, H.J.; Seyfert, H.M. Stimulated expression of TNF-[alpha] and IL-8, but not of lingual antimicrobial peptide reflects the concentration of pathogens contacting bovine mammary epithelial cells. *Vet. Immunol. Immunopathol*. **2010**, *135*, 152-157.

89. Riollet, C.; Rainard, P.; Poutrel, B. Cells and cytokines in inflammatory secretions of bovine mammary gland. *Adv. Exp. Exp. Med. Biol*. **2000**, *480*, 247-258.
90. Rainard, P.; Fromageau, A.; Cunha, P.; Gilbert, F.B. *Staphylococcus aureus* lipoteichoic acid triggers inflammation in the lactating bovine mammary gland. *Vet. Res*. **2008**, *39*, 52. PMID:18593548.
91. Khan, M.M. ole of Cytokines. Immunopharmacology. 2nd ed; Springer Science + Business Media, New York. **2008**, 33-59.
92. Alnakip, M.E.; Quintela-Baluja, M.; Böhme, K.; Fernández-No, I.; Caamaño-Antelo, S.; Calo-Mata, P.; Barros-Velázquez, J. Review Article: The Immunology of Mammary Gland of Dairy Ruminants between Healthy and Inflammatory Conditions. *J. Vet. Med*. **2014**, 659801. 31.
93. Lara-Zarate, L.; Lopez-Meza, J.E.; Ochoa-Zarzosa, A. *Staphylococcus aureus* inhibits nuclear factor kappa B activation mediated by prolactin in bovine mammary epithelial cells. *Microb. Pathog*. **2011**, *51*, 313-318.
94. Eckel, E.F., Ametaj, B.N. *Invited Review:* Roles of bacterial endotoxins in the etiopathogenesis of periparturient diseases of transition dairy cows. *J. Dairy Sci*. **2016**, *99*, 1-24.
95. Gilbert, F.B.; Cunha, P.; Jensen, K.; Glass, E.J.; Foucras, G.; Robert-Granie, C.; Rupp, R.; Rainard, P. Differential response of bovine mammary epithelial cells to *Staphylococcus aureus* or *Escherichia coli* agonists of the innate immune system. *Vet. Res*. **2013**, *44*, 40.
96. Giovannini, A.E.J.; van den Borne, B.H.P.; Wall, S.K.; Wellnitz, O.; Bruckmaier, R.M.; Spadavecchia, C. Experimentally induced subclinical mastitis: are lipopolysaccharide and lipoteichoic acid eliciting similar pain response? *Acta Vet. Scandinav*. **2017**, *59*, 40.
97. Wall, S.K.; Wellnitz, O.; Hernandez-Castellano, L.E.; Ahmadpour, A.; Bruckmaier, R.M. Supraphysiological oxytocin increases the transfer of immunoglobulins and other blood components to milk during lipopolysaccharide- and lipoteichoic acid-induced mastitis in dairy cows. *J. Dairy Sci*. **2016**, *99*, 9165-9173.
98. Petersson-Wolfe, C. *Streptococcus uberis:* A Practical Summary for Controlling Mastitis. College of Agriculture and Life Science, Virginia Polytechnic Institute and State University, USA, 2012.
99. Hogan, J.S.; Smith, K.L. Occurrence of clinical and sub-clinical environmental streptococci mastitis. In: Proceedings of the Symposium on Udder Health Management for Environmental Streptococci. **1997**, 59-75.
100. NIRD. National Institute for Research in Dairying Annual Report. 1970, 65.
101. NIRD. National Institute for Research in Dairying Annual Report. 1974, 56.
102. Leigh, J.A. *Streptococcus uberis:* A Permanent Barrier to the Control of Bovine Mastitis? *Vet. J*. **1999**, *157*, 225-238.
103. Leigh, J.A.; Field, T.R. Killing of *Streptococcus uberis* by bovine neutrophils following growth in chemically defined media. *Vet. Res. Comm*. **1991**, *15*, 1-6.
104. Thomas, L.H.; Hader, W.; Hill, A.W.; Cook, R.S. Pathologic findings of experimentally induced *Streptococcus uberis* infection in the mammary gland of cows. *Am. J. Vet. Res*. **1994**, *55*, 1723-1728.
105. Leigh, J.A.; Egan, S.S.; Ward, P.N.; Field, T.R.; Coffey, T.J. Sortase anchored proteins of *Streptococcus uberis* play major roles in the pathogenesis of bovine mastitis in dairy cattle. *Vet. Res*. **2010**, *41*, 63.

106. Koneman, E.W.; Allen, S.D.; Dowell, V.R.; Sommer, H.M. The Enterbacteriacea, in: Color Atlas and Textbook of Diagnostic Microbiology, J.B. Lippincott Company, New York, New York, USA. 1983, 57-124.
107. Hogan, J.; Smith, K.L. Review article: Coliform Mastitis. *Vet. Res*. **2003**, 34, 507-519.
108. Sheldon, I.M.; Noakes, D.E.; Rycroft, A.N.; Pfeiffer, D.U.; Dobson, H. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. *Reproduction* **2002**, *123*, 837-845.
109. Smith, K. L., and F. L. Schanbacher. Lactoferrin as a factor of resistance to infection in the mammary gland. *J. Vet. Am. Vet. Med. Assoc*. **1976**, *170*, 1224-1227.
110. Todhunter, D.A.; Smith, K.L.; Hogan, J.S.; Schoenberger. P.S.; Gram-negative bacterial infection of the mammary gland in cows. *Am. J. Vet. Res*. **1991**, 52, 184-188.
111. Mashburn-Warren, L.R.; McLean, R.J.; Whiteley, M. Gram-negative outer membrane vesicles: Beyond the cell surface. *Geobiology* **2008**, 6, 214-219.
112. Hakogi, E.; Tamura, H.; Tanaka, S.; Kohata, A.; Shimada, Y.; Tabuchi., K Endotoxin levels in milk and plasma of mastitis-affected cow measured with a chromogenic limulus text. *Vet. Microbiol*. **1989**, *20*, 267-274.
113. Dosogne, H.; Meyer, E.; Struk, A.; van Loon, J.; Massart-Leen, A.M.; Burvenich, C. Effect of enrofloxacin treatment of plasma endotoxin during bovine *Escherichia coli* mastitis. *Inflamm. Res*. **2002**, *51*, 201-205.
114. Rainard, P.; Riollet, C. Innate immunity of the bovine mammary gland. *Vet. Res*. **2006**, 37, 369-400.
115. Lee, C.S.; Wooding, F.B.; Kemp, P. Identification, properties, and differential counts of cell populations using electron microscopy of dry cows secretions, colostrum and milk from normal cows. *J. Dairy Res*. **1980**, *47*, 39-50.
116. Park, Y. H., L. K. Fox, M. J. Hamilton, W. C. Davis. 1992. Bovine mononuclear leukocyte subpopulations in peripheral blood and mammary gland secretions during lactation. *J. Dairy Sci*. **1992**, *75*, 998-1006.
117. Paape, M.J.; Guidry, A.J. Effect of milking on leucocytes in the subcutaneous abdominal vein of the cow. *J. Dairy Sci*. **1969**, *52*, 998-1002.
118. Denis, M., Parlane, N.A.; Lacy-Hulbert, S.J.; Summers, E.L.; Buddle, B.M.; Wedlock, D.N. Bactericidal activity of macrophages against *Streptococcus uberis* is different in mammary gland secretions of lactating and drying off cows. *Vet. Immunol. Immunopathol*. **2006**, *114*, 111-120.
119. Fitzpatrick, J. L.; Cripps, P.J.; Hill, A.W.; Bland, P.W.; Stokes, C.R. MHC class II expression in the bovine mammary gland. *Vet. Immunol. Immunopathol*. **1992**, *32*, 13-23.
120. Rossi, C.R.; Kiesel, G.K. Bovine immunoglobulin G subclass receptor sites on bovine macrophages. *Am. J. Vet. Res*. **1977**, *38*, 1023-1025.
121. Miller, R.; Paape, M.; Fulton, L.; Schutz, M. The relationship of milk somatic cell count to milk yields for Holstein heifers after first calving. *J. Dairy Sci*. **1993**, 76, 728-733.
122. Paape, M.J.; Shafer-Weaver, K.; Capuco, A.V.; Van Oostveldt, K.; Burvenich, C. Immune surveillance of mammary tissue by phagocytic cells. *Adv. Exp. Med. Biol*. **2000**, *480*, 259-277.
123. Opdebeeck, J.P. Mammary gland immunity. *J. Am. Vet. Med. Ass*. **1982**, *181*, 1061-1065.
124. Prin-Mathieu, C.; Le Roux, Y.; Faure, G.C.; Laurent, F.; Béné, M.C.; Moussaoui, F. Enzymatic activities of bovine peripheral blood leukocytes and milk polymorphnuclear neutrophils

 doi:10.20944/preprints202208.0041.v1

during intramammary inflammation caused by lipopolysaccharide. *Clin. Diag. Lab. Immunol*. **2002**, *9*, 812-817.

125. Paape, M.J.; Mehrzad, J.; Zhao, X.; Detilleux, J.; Burvenich, C. Defense of the bovine mammary gland by polymorphnuclear neutrophil leukocytes. *J. Mamm. Gland Biol. Neopl*. **2002**, 7, 109-121.
126. Paape, M.J.; Bannerman, D.D.; Zhao, X.; Lee, J.W. The bovine neutrophil: structure and function in blood and milk. *Vet Res*. **2003**, 34, 597-627.
127. Stelwagen, K.; Carpenter, E.; Haigh, B.; Hodgkinson, A.; Wheeler, T.T. Immune components of bovine colostrum and milk. *J. Anim. Sci*. **2009**, 87, 3-9.
128. Taylor, B.C.; Dellinger, J.D.; Culler, J.S.; Stott, J.L. Bovine milk lymphocytes display the phenotype of memory T cells and are predominantly $CD8^+$. *Cell. Immunol*. **1994**, *156*, 245-253.
129. Shafer-Weaver, K.A.; Sordillo, L.M. Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the post-partum period. *Vet. Immunol. Immunopathol*. **1997**, *56*, 53-64.
130. Broere, F.; Apasov, S.G.; Sitkovsky, M.V.; van Eden, W. T cell subsets and T cell-mediated immunity. F. P. Nijkamp and M. J. Parnham (eds.), *Principles of Immunopharmacology*: 3rd revised and extended edition. **2011**, 15-26.
131. Mackay, C.R.; Hein, W.R. Marked variations in γδ T cell numbers and distribution throughout the life of sheep. *Curr. Top. Microbiol. Immunol*. **1991**, *173*, 107-111.
132. Shafer-Weaver, K.A.; Pighetti, G.M.; Sordillo, L.M. Diminished mammary gland lymphocyte functions parallel shifts in trafficking patterns during the postpartum period. Exp. Biol. Med. **1996**, *212*, 271-279.
133. Roitt, I.; Brostoff, J.; Male, D. Immunology. Mosby, Philadelphia, Pa, USA. 480, 2001.
134. Shafer-Weaver, K.A.; Sordillo, L.M. Bovine CD8+ suppressor lymphocytes alter immune responsiveness during the post-partum period. *Vet. Immunol. Immunopathol*. **1997**, *56*, 53-64.
135. Medzhitov, R.; Preston-Hurlburt, P.; Janeway, C.A. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* **1997**, *388*, 394-397.
136. Gao, B.; Jeong, W.I.; Tian, Z. Liver: An organ with predominant innate immunity. Hepatology **2008**, *47*, 729-736.
137. Cruvinel, W.M.; Junior, D.M.; Araujo, J.A.P.; Catelan, T.T.T.; Souza, A.W.S.; Silva, N.P.; Andrade, L.E.C. Immune System – Part 1: Fundamentals of innate immunity with emphasis on molecular and cellular mechanisms of inflammatory response. *Rev. Bras. Reumatol*. **2010**, *50*, 434-461.
138. Dervishi, E., G. Zhang, D. Hailemariam, S. M. Dunn, B. N. Ametaj. Innate immunity and carbohydrate metabolism alterations precede occurrence of subclinical mastitis in transition dairy cows. *J. Anim. Sci. Technol*. **2015**, *57*, 46.
139. Dervishi, E.; Zhang, G.; Hailemariam, D.; Goldansaz, S.A.; Deng, Q.; Dunn, S.M.; Ametaj, B.N. Alterations in innate immunity reactants and carbohydrate and lipid metabolism precede occurrence of metritis in transition dairy cows. *Res. Vet. Sci*. **2016b**, *104*, 30-39.
140. Zhang, G.; Hailemariam, D.; Dervishi, E.; Deng, Q.; Goldansaz, S.A.; Dunn, S.M.; Ametaj, B.N. Alterations of Innate Immunity Reactants in Transition Dairy Cows before Clinical Signs of Lameness. *Animals* **2015**, *5*, 717-747.
141. Zhang, G.; Hailemariam, D.; Dervishi, E.; Goldansaz, S.A.; Deng, Q.; Dunn, S.M.; Ametaj, B.N. Dairy cows affected by ketosis shown alterations in innate immunity and lipid and

carbohydrate metabolism during the dry off period and postpartum. *Res. Vet Sci*. **2016**, *107*, 246-256.

142. Hoeben, D.; Burvenich, C.; Trevisi, E.; Bertoni, G.; Hamann, J.; Bruckmaier, R.M.; Blum, J.W. Role of endotoxin and TNF-α in the pathogenesis of experimentally induced coliform mastitis in periparturient cows. *J. Dairy Res*. **2000**, *67*, 503-514.
143. Hisaeda, K., K. Hagiwara, J. Eguchi, H. Yamanaka, R. Kirisawa, H. Iwai. Interferon-gamma and tumor necrosis factor-alpha levels in sera and whey of cattle with naturally occurring coliform mastitis. *J. Vet. Med. Sci*. **2001**, *63*, 1009-1011.
144. Blum, J.W.; Dosogne, H.; Hoeben, D.; Vangroenweghe, F.; Hammon, H.M.; R. M. Bruckmaier, R.M.; Burvenich, C. Tumor necrosis alpha and nitrite/nitrate responses during acute mastitis induced by *Eschericia coli* infection and endotoxin in dairy cows. *Dom. Anim. Endocrinol*. **2000**, *19*, 223-235.
145. Ametaj, B.N.; Hosseini, A.; Odhiambo, J.F.; Iqbal, S.; Sharma, S.; Deng, Q.; Lam, T.H.; Farooq, U.; Zebeli, Q.; Dunn, S.M. Application of acute phase proteins for monitoring inflammatory states in cattle. In: Veas, F. (Ed.), Acute Phase Proteins as Early Non-Specific Biomarkers of Human and Veterinary Diseases. Intech Europe, Croatia. **2011**, 299-354.
146. Wassell, J. Haptoglobin: function and polymorphism. *Clin. Lab*. **2000**, *46*, 547-552.
147. Quaye, I.K. Haptoglobin, inflammation and disease. *Trans. Royal Soc. Trop. Med. Hyg*. **2008**, 102, 735-742.
148. Allen, S.E.; Holm, J.L. Lactate: physiology and clinical utility. *J.Vet. Emerg. Crit. Care* **2008,** *18*, 123-132.
149. Davis, S.R.; Farr, V.C.; Prosser, C.G.; Nicholas, G.D.; Turner, S.A.; Lee, J.; Hart, A.L. Milk L-lactate concentration is increased during mastitis. *J. Dairy Sci*. **2004**, *71*, 175-181.
150. Gordon, J.L. Risk Factors for and Treatment of Ketosis in Lactating Dairy Cows (Doctoral Thesis) University of Guelph, Guelph, ON, Canada. 2013a, 1-162.
151. Schröder, U.J.; Staufenbiel, R. Invited review: methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. *J. Dairy Sci*. **2006**, 89, 1-14.
152. Duffield, T. F., LeBlanc, S.J. Interpretation of serum metabolic parameters around the transition period. Available from: http://animal.cals.arizona.edu/swnmc/Proceedings/2009/11Duffield_2_09.pdf. [Accessed 2018 Jan 26], 2009.
153. LeBlanc, S. Monitoring metabolic health of dairy cattle in the transition period. *J. Repro. Develop*. **2010**, *56*, S29-35.
154. Mani, V.; Weber, T.E.; Baumgard, L.H.; Gabler, N.K. Growth and development symposium: Endotoxin, inflammation, and intestinal function in livestock. *J. Anim. Sci*. **2012**, *90*, 1452-1465.
155. Ghoshal, S.; Witta, J.; Zhong, J.; de Villiers, W.; Eckhardt, E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J. Lip. Res*. **2009**, *50*, 90-97.
156. Opsomer, G. Metritis and endometritis in high yielding dairy cows. *Rev. Bras. Reprod. Anim. Belo Horiz*. **2015**, *39*, 164-172.
157. Lucey, S.; Rowlands, G.J.; Russell, A.M. Short-term associations between disease and milk yield of dairy cows. *J. Dairy Res*. **1986**, *53*, 7-15.

158. Ametaj, B.N.; Zebeli, Q.; Iqbal, S. Nutrition, microbiota, and endotoxin-related diseases in dairy cows. *Rev. Brasil. Zootec*. **2010**, *39*, 433-444.
159. Theas, M.S.; De Laurentiis, A.; Lasaga, M.; Pisera, D.; Duvilanski, B.H.; Seilicovich, A. Effect of lipopolysaccharide on tumour necrosis factor and prolactin release from rat anterior pituitary cells. *Endocrine* **1998**, *8*, 241-245.
160. Sheldon, I.M.; Lewis, G.S.; LeBlanc, S.; Gilbert, R.O. Defining postpartum uterine disease in cattle. *Theriogenology* **2006**, *65*, 1516-1530.
161. Sheldon, I.M.; Cronin, J.; Goetze, L.: Donofrio, G.; Schuberth, H.J. Defining postpartum uterine disease and the mechanisms of infection and immunity in the Female reproductive tract in cattle. *Biol. Repro*d. **2009**, *81*, 1025-1032.
162. Drillich, M.; Beetz, O.; Pfutzner, A.; Sabin, M.; Sabin, H.J.; Kutzer, P.; Nattermann, H.; Heuwieser, W. Evaluation of a systemic antibiotic treatment of toxic puerperal metritis in dairy cows. *J. Dairy Sci*. **2001**, *84*, 2010-2017.
163. Markusfeld, O. Periparturient traits in seven high dairy herds. Incidence rates, association with parity, and interrelationships among traits. *J. Dairy Sci*. **1987**, 70, 158-166.
164. Kasimanickam, R.: Kasimanickam, V.; Kastelic, J.P. Mucin 1 and cytokines mRNA in endometrium of dairy cows with postpartum uterine disease of repeat breeding. *Theriogenology* **2014**, *81*, 952-958.
165. Jordan, M.; Otterness, I.G.; Ng, R.; Gessner, A.; Rollinghoff, M.; Beuscher, H.U. Neutralization of endogenous IL-6 supress induction of IL-1 receptor antagonist. *J. Immunol*. **1995**, *154*, 4081-4090.
166. Chan, J.P.W.; Chang, C.; Hsu, W.L.; Liu, W.B.; Chen, T.H. Association of increase serum acute-phase protein concentration with reproductive performances in dairy cows with postpartum metritis. *Vet. Clin. Pathol*. **2010**, *39*, 72-78.
167. Dubuc, J.; Duffield, T.; Leslie, K.; Walton, J.; LeBlanc, S. Definitions and diagnosis of postpartum endometritis in dairy cows. *J. Dairy Sci*. **2010**, *93*, 5225-5233.
168. Moore, D.A.; Cullor, J.S.; BonDurant, R.H.; Sischo, W.M. Preliminary field evidence for the association of clinical mastitis with altered interestrus intervals in dairy cattle. *Theriogenology* **1991**, *36*, 257-265.
169. Hansen, P.J.; Soto, P.; Natzke, R.P. Mastitis and fertility in cattle—Possible involvement of inflammation or immune activation in embryonic mortality. *Am. J. Repro. Immunol*. **2004**, *51*, 294-301.
170. Risco, C.A.; Donovan, G.A.; Hernandez, J. Clinical mastitis associated with abortion in dairy cows. *J. Dairy Sci*. **1999**, *82*, 1684-1689.
171. Herath, S.; Williams, E.J.; Lilly, S.T.; Gilbert, R.O.; Dobson, H.; Bryant, C.E.; Sheldon, I.M. Ovarian follicular cells have innate immune capabilities that modulate their endocrine function. *Reproduction* **2007**, *134*, 683-693.
172. Herath, S.; Lilly, S.T.; Fischer, D.P.; Williams, E.J.; Dobson, H.; Bryant, C.E.; Sheldon, I.M. Bacterial lipopolysaccharide induces an endocrine switch from prostaglandin $F_{2\alpha}$ to prostaglandin E_2 in bovine endometrium. *Endocrinology* **2009**, 150, 1912-1920.
173. Nugent, A.M.; Hatler, T.B.; Silvia, W.J. The effect of the intramammary infusion of *Escherichia coli* endotoxin on ovulation in lactating dairy cows. *Rep. Biol*. **2002**, *2*, 295-309.

174. Duffield, T.F. Subclinical ketosis in lactating dairy cattle. *Vet. Clin. North Amer: Food Anim. Pract*. **2000**, *16*, 231-253.
175. Çağdas, C. Physiological and metabolic changes during the transition period and the use of calcium propionate for prevention or treatment of hypocalcemia and ketosis in periparturient cows. *J. Biol. Environ. Sci*. **2013**, *7*, 9-17.
176. Gordon, J.L.; LeBlanc, S.J.; Duffield, T.F. Ketosis treatment in lactating dairy cattle. *Vet. Clin. North Amer.: Food Anim. Practice*. **2013**, *29*, 433-445.
177. Erb, H.N.; GroÈhn, Y.T. Epidemiology of metabolic disorders in the periparturient dairy cows. *J. Dairy Sci*. **1988**, *71*, 2557–2571.
178. Loor, J.J.; Everts, R.E.; Bionas, M.; Dann, H.M.; Morin, D.E.; Oliviera, R.; Rodriguez-Zas, S.L.; Drackley, J.A.; Lewin, H.A. Nutrition-induced ketosis alters metabolic and signaling gene networks in liver of periparturient dairy cows. *Physiol. Gen*. **2007**, *32*, 105-116.
179. Kushibiki, S.; Hodate, K.; Shingu, H.; Obara, Y.; Touno, E.; Shinoda, M.; Yokomizo, Y. Metabolic and lactational responses during recombinant bovine tumour necrosis factor-α treatment in lactating dairy cows. *J. Dairy Sci*. **2003**, *86*, 819-827.
180. Kremer, W.D.J.; Burvenich, C.; Noordhuizen-Stassen, E.N.; Grommers, F.J.; Schukken, Y.H.; Heeringa, R.; Brand, A. Severity of experimental *Escherichia coli* mastitis in ketonemic and non-ketonemic dairy cows. *J. Dairy Sci*. **1993**, *76*, 3428-3436.
181. Bruckmaier, R.M.; Schällibaum, M.; Blum, J.W. *Escherichia coli* endotoxin-induced mastitis in dairy cows: Changes and importance of insulin-like growth factor1 and oxytocin. *Milchwissenschaft*, **1993**, *48*, 374-378.
182. Vernay, M.C.M.B.; Wellnitz, O.; Kreipe, L.; van Dorland, H.A.; Bruckmaier, R.M. Local and systemic response to intra-mammary lipopolysaccharide challenge during long-term manipulated plasma glucose and insulin concentrations in dairy cows. *J. Dairy Sci*. **2012**, *95*, 2540-2549.
183. Zarrin, M.; Wellnitz, O.; van Dorland, H.A.; Bruckmaier, R.M. Induced hyperketonemia affects the mammary immune response during lipopolysaccharide challenge in dairy cows. *J. Dairy Sci*. **2014**, *97*, 330-339.
184. Zarrin, M.; Wellnitz, O.; van Dorland, H.A.; Gross, J.J.; Bruckmaier, R.M. Hyperketonemia during lipopolysaccharide-induced mastitis affects systemic and local intramammary metabolism in dairy cows. *J. Dairy Sci*. **2014**, *97*, 3531-3541.
185. Weaver, A.D.; St. Jean, G.; Steiner, A. Bovine Surgery and Lameness, 2nd ed; Blackwell: Oxford, UK.198-258, 2005.
186. Boosman, R., C. W. Mutsaers, A. Klarenbeek. The role of endotoxin in the pathogenesis of acute bovine laminitis. *Vet. Quart*. **1991**, *13*, 155-162.
187. Cook, N.B.; Bennett, T.B.; Nordlund, K.V. Effect of Free Stall Surface on Daily Activity Patterns in Dairy Cows with Relevance to Lameness Prevalence. *J. Dairy Sci*. **2004**, *87*, 2912-2922.
188. Chaplin, S.J.; Tierney, G.; Stockwell, C.; Logue, D.N.; Kelly, M. An evaluation of mattress and mats in two dairy units. *Appl. Anim. Behav. Sci*. **2000**, *66*, 263-272.
189. Tucker, C.B.; Weary, D.M.; Fraser, D. Effects of three types of free stall surfaces on preferences and stall usage by dairy cows. *J. Dairy Sci*. **2003**, 86, 521-529.

190. Whay, H.R.; Waterman, AA.E.; Webster, A.J.F.; O'Brien, J.K. 1998. The influence of lesion type on the duration of hyperalgesia associated with hindlimb lameness in dairy cattle. *Vet. J.* **1998**, *156*, 23-29.
191. Nordlund, K. V., and N. B. Cook. 2003. A Flowchart for Evaluating dairy cow free stalls. *Bov. Pract*. **2003**, *37*, 89-96.
192. Archer, S.C.; Green, M.J.; Madouasse, A.; Huxley, J.N. Association between somatic cell count and serial locomotion score assessments in UK dairy herds. *J. Dairy Sci*. **2011**, *94*, 4383-4388.
193. Archer, S. C., F. McCoy, W. Wapenaar, M. J. Green. Association between somatic cell count during the first lactation and the cumulative milk yield of cows in Irish dairy herds. *J. Dairy Sci*. **2014**, *97*, 2135-2144.
194. Boontham, P.; Chandra, P.; Rowlands, B.; Eremin, O. Surgical sepsis: dysregulation of immune function and therapeutic implications. *Surgeon*. **2003**, 1, 187-206.
195. Hagiwara, K.; Yamanaka, H.; Hisaeda, K.; Tahara guchi, S.; Kirisawa, R.; Iwai, H. Concentrations of IL-6 in serum and whey from healthy and mastitis cows. *Vet. Res. Commun*. **2001**, *25*, 99-108.
196. Ishikawa, Y.; Nakada, K.; Hagiwara, K.; Kirisawa, R.; Iwai, H.; Moriyoshi, M.; Sawamukai, Y. Changes in interleukin-6 concentration in peripheral blood of pre- and post-partum dairy cattle and its relationship to postpartum reproductive diseases. *J. Vet. Med. Sci*. **2004**, *66*, 1403-1408.
197. Emmanuel, D.G., Dunn, S.M.; Ametaj, B.N. Feeding high proportions of barley grain stimulates an inflammatory response in dairy cows. *J. Dairy Sci*. **2008**, 91, 606-614.
198. Oliver, S.P.; Murinda, S.E. Antimicrobial resistance of mastitis pathogens. *Vet. Clin. North Am.: Food Anim. Pract*. **2012**, *28*, 165-185.
199. Holmes, A.H.; Moore, L.S.P.; Sundsfjords, A.; Steinbakk, M.; Regmi, S.; Karkey, A.; Guerin, P.J.; Piddock, L.V.J. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* **2016**, *387*, 176-187.
200. Smith H.W. Persistence of tetracycline resistance in pig *E. coli*. *Nature* **1975**, *258*, 628-30.
201. Aarestrup, F.M.; Seyfarth, A.M.; Emborg, H.D.; Pedersen, K.; Hendriksen, R.S.; Bager, F. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. *Antimicrob. Ag. Chemother*. **2001**, *45*, 2054-2059. [DOI: 10.1128/AAC.45.7.2054-2059.2001] [PMCID: PMC90599]
202. Oliver, S.P.; Sordillo, L.M. 1988. Udder health in the perparturient period. *J. Dairy Sci*. **1988**, *71*, 2584-2606.