Disease in one lactation is associated with a greater incidence of the same disease in the subsequent lactation in dairy cows

by

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Abstract

Objectives were to evaluate if having a disease in one lactation is associated with the risk of developing the same disease in the subsequent lactation and to identify risk factors for prevalent postpartum diseases. A total of 13,761 cow-lactations from 6,179 Holstein cows from two dairy farms located in Central California and two dairy farms located in North Florida were used in this retrospective study. Data were retrieved from farm management software from 2007 to 2013 and included prevalent postpartum disorders, such as dystocia, stillbirth, retained placenta, metritis, clinical hypocalcemia, hyperketonemia, displaced abomasum, and mastitis. Data were analyzed by logistic regression with the GLIMMIX procedure of SAS and included the fixed effects of history of the disease, parity, the interaction between the history of disease and parity, and the random effect of farm. Having dystocia increased the risk (odds ratio [OR] = 1.48; $P < 0.001$) of having dystocia in the subsequent lactation (25.5 vs. 18.8%). Having a stillbirth tended to increase the risk (OR = 1.58; $P = 0.07$) of having a stillbirth in the subsequent lactation (5.0 vs. 3.3%). Having retained placenta increased the risk (OR = 1.63; $P = 0.002$) of having retained placenta in the subsequent lactation (9.5 vs. 6.0%). Having metritis increased the risk (OR = 1.68; $P < 0.001$) of having metritis in the subsequent lactation (19.0 vs. 12.2%). Having clinical hypocalcemia increased the risk (OR = 13.32; $P < 0.001$) of having clinical hypocalcemia in the subsequent lactation (19.7 vs. 1.8%). Having hyperketonemia increased the risk (OR = 1.91; $P < 0.001$) of having hyperketonemia in the subsequent lactation (32.5 vs. 20.2%). Having displaced abomasum increased the risk (OR = 3.68; $P = 0.02$) of having displaced abomasum in the subsequent lactation (1.35 vs. 0.37%). Having mastitis increased the risk (OR = 2.10; $P < 0.001$) of having mastitis in the subsequent lactation (16.8 vs. 8.8%). For all the variables analyzed, the interaction between disease history and parity was not significant. Our data indicate that cows with a history of the

disease or disorder are more likely to develop the same condition, suggesting a genetic component in the pathogenesis of postpartum diseases and disorders. In addition, our data suggests that the adaptive immune response to infectious diseases such as metritis and mastitis may not last until a subsequent lactation or may be insufficient to reduce the risk of diseases.

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1 Literature Review: Metabolic and Infectious Diseases During the Transition Period in Dairy Cows

1.1 Abstract

The transition period, encompassing three weeks before and after parturition in dairy cows, is a critical phase characterized by heightened vulnerability to postpartum diseases, significantly impacting animal well-being, productivity, and economic outcomes for dairy farms. This literature review meticulously examines the incidence, pathophysiology, management, treatment, and prevention strategies of predominant conditions such as hypocalcemia, hyperketonemia, displaced abomasum, retained placenta, and metritis. Hypocalcemia, a prevalent metabolic disorder, threatens cow health, increasing the risk of other adverse events such as infectious diseases. Management strategies, including dietary adjustments of minerals before parturition, have successfully reduced its incidence. Hyperketonemia, triggered by a severe negative energy balance and consequent fat mobilization, significantly affects milk yield, and increase the risk of other diseases since impair the immune system of the cow, with treatment strategies focusing on energy supplementation such a propylene glycol, which is later converter into glucose. Displaced abomasum, results in considerable losses in milk production and sometimes necessitates surgical intervention, and results in a high culling rate. Retained placenta, linked to various etiological factors such as hormonal imbalance and immune dysfunction, leads to additional reproductive challenges, and increases the risk for uterine diseases. Metritis, an infectious postpartum uterine disease, manifests through systemic inflammatory responses and necessitates antimicrobial treatment, which has shown to improve clinical outcomes. The review emphasizes the critical importance of an integrated approach encompassing vigilant health monitoring, optimized nutritional strategies, and timely medical interventions for the effective management of these diseases. Despite advances in understanding and management, the persistent incidence of these

conditions highlights an ongoing need for research into improved diagnostics, genetic selection, innovative therapeutics, and particularly the development of effective preventative measures. Advancements in managing the transition period are pivotal for enhancing dairy herd health, productivity, and sustainability.

Key words: transition period, postpartum diseases, management strategies, dairy herd health

1.2 Introduction

The transition period, commonly defined as the interval between three weeks prepartum to three weeks postpartum, stands as a critical stage in the lifecycle of dairy cows. During this time, dairy cows experience pronounced changes in nutrient demand, especially when shifting the nutritional demand from a gravid uterus to the sudden increased nutrient demand by the mammary gland to support milk production. This phase is accentuated by dramatic hormonal shifts and depressed dry matter intake, posing a significant challenge in maintaining nutrient balance (Grummer, 1995).

Milk production plays a dramatic role on energy and nutrient requirements for cows during lactation. Cows during the first 105 days postpartum averaging 38.9 kg of milk yield per day have an estimated net energy requirement for lactation of 26.6 Mcal/d, while their energy requirement for maintenance is estimated to be 9.3 Mcal/d; therefore, cows need to consume 2.5-fold their maintenance requirement to support energy demand during early lactation (Nehme Marinho and Santos, 2022). Cows typically experience reduced dry matter intake during the early postpartum period. Considering that milk synthesis accounts for up to 85% of the blood glucose usage (Bauman and Bruce Currie, 1980), cows often need to mobilize their body reserves to satisfy the energy requirements of the mammary gland. This extensive mobilization of fatty acids can lead to

incomplete beta-oxidation in the liver, subsequently raising blood ketone body levels and potentially causing metabolic disorders (Bell, 1995). The depletion of glucose can also impair the immune system as it has been estimated that the activated immune system uses more than 1 kg of glucose within 12 hours (Kvidera et al., 2017). In addition, Hillreiner et al., (2016) reported that ketone bodies in blood can attenuate the innate immune response. Thus, the combination of lack of glucose availability with increased ketone bodies observed postpartum can increase the susceptibility of cows to infectious diseases.

Nearly 40% of dairy cows experience at least one disease within the first 60 days postpartum, which can be calving related disorders, disruption of metabolic pathways, and/or infectious diseases (Santos et al., 2010). Because of that, postpartum dairy cows are known to require extensive labor for disease diagnosis and treatment of those cows which are directly impactful to profitability of dairy farms. Nevertheless, the most important consequences to profitability are observed in the impairment of productive and reproductive performance. According to Carvalho et al. (2019), cows experiencing disease in the first 21 d postpartum produced 410 kg less milk, 17 kg less fat, and 12 kg less protein during 305 d of lactation when compared with cows that did not experienced diseases during the first 21d postpartum. In addition, cows that had at least one disease, either related to the uterine tract or not, have reduced pregnancy per breeding and increased pregnancy loss (Ribeiro et al., 2016). Consequently, the production efficiency of dairy cows experiencing disease are compromised and sustainability of dairy operations are highly impacted.

Culling from the herd is also a consequence of disease occurrence, usually associated with either lower production or impaired fertility. According to Carvalho et al. (2019), 22.6% of healthy cows during the postpartum period will be removed from the herd by 305 days postpartum, but

cows that experienced at least one disease have a removal rate of 38.3%. This plays an important role in the economics of dairy farms as replacement heifers are costly to raise (Vredenberg et al., 2021). Although involuntary culling is necessary to mitigate suffering of sick cows, a high proportion of involuntary culling in a herd may suggest inefficient use of animal resources and poor animal welfare, which impairs sustainability of dairy farms (Ahlman et al., 2011).

The objective of this literature review is to provide a concise overview of the most common postpartum diseases observed in dairy cows, their causes, symptoms, diagnosis, treatment, and consequences. Additionally, this chapter will offer some practical prevention strategies to reduce the incidence and mitigate the negative impacts of these disorders on cow health and productivity. The chapter will also present some novel information and current publications to help readers better understand some of the challenges and opportunities that this critical period entails.

1.3 Metabolic diseases

1.3.1 Hypocalcemia

Hypocalcemia is a prevalent metabolic disorder that occurs during the periparturient period in dairy cows and can significantly increase the risk of other adverse health events and impair production performance, and in severe cases, can be life-threatening (Martinez et al., 2012). Hypocalcemia can be categorized into clinical hypocalcemia or subclinical hypocalcemia depending on blood concentration of calcium and the presence or absence of clinical signs. Dairy cows with blood calcium levels above 2.0 mmol/L are considered normocalcemic (Martín-Tereso and Martens, 2014) and are physiologically normal. When serum concentrations of calcium are between 1.5 and 2.0 mmol/L, cows physiology can be affected although clinical signs may not be present, hence, subclinical hypocalcemia (Martin-Tereso and Martens, 2014). Clinical

hypocalcemia, or commonly called milk fever, is characterized by blood concentrations of calcium generally below 1.5 mmol/L with the presence of clinical symptoms. Oetzel (2020) categorized clinical hypocalcemia signs into three stages; stage 1: cows present signs of hypersensitivity and excitability, and mild ataxia; stage 2: cows do not stand but may maintain sternal recumbency, bloat may be observed because of smooth muscle paralysis of the gastrointestinal tract, and cows may tuck their heads into their flanks; stage 3: cows may lose their consciousness and present coma, body temperature may change due to the inability of thermoregulation, complete muscle flaccidity, and heart rate can approach 120 beats per minute with undetectable peripheral pulse.

Although clinical hypocalcemia has severe clinical symptoms, its prevalence is generally very low, and it can be <1% on well-managed dairy farms. (Martín-Tereso and Martens, 2014) reported that the incidence of clinical hypocalcemia was 1% for first lactation, 4% for second lactation, and between 6 and 13% for older cows. In the same study, prevalence of subclinical hypocalcemia in the first 48 hours was 25% for first lactation cows and greater than 41% for older cows (Reinhardt et al. 2011). While subclinical hypocalcemia impacts are not severe, it is probably more relevant than clinical hypocalcemia because of its high prevalence observed in dairy cows.

As part of colostrum synthesis when cows are getting closer to parturition, a significant amount of calcium is mobilized and sequestered by the mammary gland to increase calcium availability in the local tissue to be secreted into colostrum (Visek et al., 1953). Rodney et al., (2018) reported that first lactation cows secreted 16.9 g of calcium in colostrum compared with multiparous cows that secreted 25.5 g of calcium. These quantities are 5 to 8-fold the calcium available in the plasma pool of an adult dairy cow weighting 600 kg, which would be around 3.5 g of calcium (Rodney et al., 2018). This sudden increase in the calcium demand activates homeorhetic mechanisms to ensure that blood calcium do not fall below critical levels that would

compromise cellular function (Kimura et al., 2006). Calcium-sensing receptors located in the Chief cells of the parathyroid gland (Brown et al., 1993), will detect changes in blood calcium and trigger the release of parathyroid hormone (PTH) into the bloodstream. In addition, during hypocalcemia the intracellular breakdown of PTH is reduced, resulting in a greater availability of PTH for secretion (Morrissey and Cohn, 1979). In the kidney, PTH will stimulate the expression of the production of 1-α-hydroxylase which adds a hydroxyl group and activates the vitamin D metabolite 25-hydroxyvitamin D_3 into 1,25-dihydroxyvitamin D_3, also known as calcitriol (Fraser and Kodicek, 1970). Calcitriol increases calcium availability in blood by stimulating the uptake of calcium from the gastrointestinal tract, increasing the resorption of calcium from the urinary tract, and by increasing bone mobilization (Hoenderop et al., 2001; Kitazawa et al., 2003; Pansu et al., 1983). Despite activating the PTH-vitamin D axis to improve calcium availability, some cows are not able to maintain calcium homeostasis and succumb to clinical or subclinical hypocalcemia due to the increased and sudden demand for calcium.

Dairy cows suffering from clinical hypocalcemia are unable to regain normal calcium levels, and if the cow does not receive immediate treatment it could result in the animal's death (Oetzel G., 1988). To ensure an effective and rapid return to normocalcemia, cows are often treated with intravenous administration of 500 mL of calcium gluconate at 23% to provide 10.8 g of calcium. Additionally, as a supplementary therapy, a calcium bolus containing 43 g of calcium in the form of calcium sulfate, calcium chloride, or calcium propionate, can be given orally. The diagnosis of subclinical hypocalcemia is only possible by collecting a blood sample of cows and submitting to veterinary diagnostic laboratory and would take a few days to have the results back, therefore, it is usually used as a monitoring tool to verify calcium homeostasis on commercial dairy farms. For research purposes, there are cow-side tests available that can promptly measure

the concentration of ionized calcium, however costs are still elevated which make this commercially prohibitive. Strategies to prevent clinical and subclinical hypocalcemia have been explored extensively and are widely adopted by dairy farmers. Among them we can include the use of diets with low concentrations of calcium, feeding mineral chelators during the prepartum period, feeding acidogenic diets during the prepartum period, and the use of oral calcium during the postpartum period.

Feeding prepartum dairy cows a low-calcium diet is a strategy that can stimulate calcium metabolism and has been previously reported to successfully reduce the incidence of clinical hypocalcemia (Goings et al., 1974). Dry cows require only 22 g of calcium per day (NASEM, 2021), which are sufficient for their maintenance and the development of their fetus. If cows are fed less calcium than the requirement, this would continuously stimulate the calcium homeostatic mechanisms to increase mobilization of calcium from gastrointestinal tract, urinary tract, and bones (Kichura et al., 1982), thus when a cow calves and is challenged with sudden demand for calcium to be secreted into colostrum, those overstimulated mechanisms would be more readily available to maintain calcium homeostasis and prevent hypocalcemia. Because of the common feedstuffs used to formulate a well-balanced diet for prepartum cows, more than 20 g of absorbable calcium is typically available in prepartum diets which challenges nutritionist to effectively use this prevention strategy on commercially dairy farms.

Alternatively, an approach to reduce the bioavailable calcium in prepartum diets, thereby activating the homeostatic mechanism without altering the ingredients, is to incorporate products that can bind to calcium. Aluminosilicate minerals, such as synthetic zeolite A, can be added to the diet for this purpose and has been shown to bind with calcium, phosphorus, and magnesium in rumen fluid at different pH levels using *in vitro* systems (Thilsing et al., 2006). Subsequent *in vivo*

work reported that feeding zeolite A to prepartum dairy cows resulted in reduction of blood concentration of calcium, phosphorus, and magnesium during the prepartum period, but reduced subclinical hypocalcemia postpartum (Grabherr et al., 2009). This strategy has been promising on improving calcium homeostasis, however because zeolite A is not selective to bind to calcium, cows with hypophosphatemia are usually observed and the consequences of that is unknown (Kerwin et al., 2019). Another concern is usually related to the amount of ash been offered to prepartum cows since 200 to 500 g of zeolite A is required to be added to prepartum diets, and therefore dry matter intake may be reduced.

Another strategy utilized during the prepartum period is to feed an acidogenic diet during the prepartum period to induce a state of metabolic acidosis and consequently improve calcium homeostasis and productive performance (Block E., 1984). Nutritionists can alter the dietary cation-anion difference (DCAD) of diets by including different sources of strong anions and reducing the sources of strong cations to achieve a negative DCAD. Ender et al. (1971) proposed that the DCAD can be calculated by the following equation: DCAD = ([mEq of K^+ + mEq of Na^+] – [mEq of Cl^- + mEq of S^{2-}]), which remains the most popular equation although some other equations have been proposed with the inclusion of other ions, such as Ca^{2+}, Mg^{2+}, and PO^{3-}_4 (Charbonneau et al., 2006). An acidogenic diet may be formulated by the inclusion of salts such as ammonium chloride, calcium chloride, magnesium chloride, ammonium sulfate, calcium sulfate, and magnesium sulfate, and is recommended to be fed during the final 21 or 28 days of gestation (Ender et al., 1971).Extended periods of feeding acidogenic diets , such as 42 days before parturition, can have detrimental impacts to performance during the postpartum period (Lopera et al., 2018). Due to metabolic acidosis, cow's parathyroid hormone receptors become more responsive and consequently the activation of 1-alpha hydroxylase and production of 1,25-

dihydroxyvitamin D_3 is increased, which improves the ability of cows to meet the high demand for calcium at calving driven by colostrum synthesis (Goff et al., 2014; Vieira-Neto et al., 2021).

To determine whether cows are experiencing metabolic acidosis, a straightforward method is to perform a urine pH test. According to Constable et al. (2019), a collective average urine pH value between 6.0 and 7.0 is typically indicative of moderate acidification in the herd. However, it should be acknowledged that urine pH levels may differ from cow to cow due to individual variations in feed intake, behavioral patterns like resting and rumination (Constable et al., 2019). It is important to evaluate urinary pH to prevent overfeeding of acidogenic salts, which would increase the dietary cost and reduce dry matter intake (Lopera et al., 2018). However, is important to mention that dry matter intake increases during the postpartum period in cows that where offered an acidogenic diet prepartum, likely because of reduction in diseases such as retained placenta and metritis, and therefore their milk production also increases (Santos et al., 2019). Those benefits are more evident in multiparous cows as this category is more prompt to hypocalcemia due to high colostrum yield (Santos et al., 2019).

In addition to the use of an acidogenic diet prepartum, the oral administration of calcium to cows right after parturition is also a practice that is adopted in some dairy operations. The purpose of supplementing calcium postpartum is to ensure that cows have an immediate calcium source, this bridges the gap between the time when homeostatic calcium regulation mechanisms kick in and when normocalcemia is restored (Oetzel, 2013). Oral calcium is typically given in the form of a bolus, with most commercial products containing 40 to 50 grams of elemental calcium per bolus or set of boluses designed to be administered as a single dose. Some products suggest a second dose 12 hours later, these products contain various combinations of calcium salts that are absorbed at different rates (Wilkens et al., 2020). It’s crucial to understand that the absorption of

various calcium salts by the animal differs. The absorption rates of different calcium salts vary among animals, which is a crucial aspect to consider. While studies indicate that oral calcium supplements can raise blood calcium levels, the duration and extent of this increase vary. These variations can be attributed to several factors, including the specific composition of the calcium product used, the frequency of administration, and the individual milk production capacity of the cows in question (Blanc et al., 2014; Valldecabres et al., 2018). The rumen wall absorbs calcium chloride and calcium sulfate via passive transport, while vitamin D-dependent active transport facilitates the absorption of calcium carbonate in the small intestine (Goff and Horst, 1993). In other words, the administration of calcium chloride or calcium sulfate can effectively increase the serum concentration of calcium in less time than calcium carbonate, and two boluses of calcium chloride or calcium carbonate can be applied at the same time without need of another application after 12 hours (Verhoef et al., 2021). Furthermore, the acidogenic traits of calcium chloride and calcium sulfate enhance the function of the parathyroid hormone (PTH), which in turn boosts the resorption of calcium from the bone, the reabsorption of calcium in the kidney tubules, and the absorption of calcium in the intestine (Sampson et al., 2009).

Most of the studies do not advocate for their use as a blank treatment, especially in primiparous cows that do not present high risk for hypocalcemia, the results of providing oral calcium to primiparous cows or to low producing cows might be counterproductive, since they can induce a hypercalcemia negatively affecting reproductive performance and milk production (Martinez et al., 2016; Valldecabres et al., 2018). On the other hand, these studies do provide strong evidence supporting the benefits of oral calcium supplementation for multiparous animals, especially those with higher production where a low serum calcium concentration is observed right after parturition (Martinez et al., 2016). Therefore, it is crucial to consider the formulation of oral

calcium boluses considering slow and rapid available calcium forms, it is also critical to be assertive in the time and frequency of administration to see an effective and positive response (Wilkens et al., 2020). Ensuring that the supply of calcium aligns with the cow's metabolic needs is crucial, particularly during peak lactation periods. For high-yielding dairy cows, administering a calcium bolus 48- and 72-hours post-calving—when the gap between dietary calcium intake and the body's ability to mobilize calcium is at its widest—can be more effective in supporting calcium requirements and may lead to increased milk production (Seely et al., 2023).

Hypocalcemia is often seen as an initial condition that can lead to several infectious diseases and some metabolic disorders due to the central role that calcium plays in metabolism, cellular transport, and immune function of mammals (Rodríguez et al., 2017). Some of the diseases related to low concentration of calcium in blood include disorders of the uterus, mastitis, hyperketonemia, and displacement of the abomasum (Martinez et al., 2018; Curtis et al.,1983; Rodríguez et al., 2017; Chapinal et al., 2011). Hypocalcemia not only impairs the immune system and increases susceptibility to diseases in dairy cows, but it also adversely affects reproductive performance. Specifically, cows with that were measured to have subclinical levels of hypocalcemia for three days in row were 1.8 times less likely to return to cycle when compared with normal levels of calcium in blood and 30% less likely to become pregnant (Caixeta, et al., 2017).

Notably, among all the diseases and disorders that are typical of the transition period, hypocalcemia is the one that we have been more successful in lowering the incidence in the last two decades (Bradford et al.,2015). This success can be attributed to a combination of advanced nutritional strategies, improved herd management practices, and a greater emphasis on animal health monitoring. These interventions have collectively enhanced the ability to better manage

calcium levels in dairy cows. The improvement on highlights the importance of a comprehensive and integrated approach to health management, where each strategy plays a vital role in improving overall herd health and productivity.

1.3.2 Hyperketonemia

Dairy cows' ability to adjust to the increased energy needs at the start of lactation is a key part of their transition period. During this period, the amount of nutrients required for milk production surpass the animal's feed intake capacity (Bauman and Currie, 1980) resulting in a negative difference in energetic intake and output, excessive fat mobilization beyond the liver's oxidative capacity, and production of ketone bodies (White, 2015). The elevated concentration of ketone bodies in the blood, known as hyperketonemia or hyperketonemia, may cause clinical signs such as diminished appetite, weight loss, and reduced milk production, (Duffield et al., 2009). Additionally, cows may suffer from subclinical hyperketonemia (SCK), where an excess of ketone bodies is present in the bloodstream without presenting clinical signs (Andersson, 1988). While the threshold for clinical hyperketonemia varies, it is generally accepted in the literature that blood beta-hydroxybutyric acid (BHBA) levels of 1.4 mmol/L or higher indicate hyperketonemia (McArt et al., 2012). This condition can bring consequences for the animal's health and negatively affect productivity (McArt et al., 2012). Hyperketonemia has been linked to suboptimal reproductive outcomes, with affected cows being less likely to conceive at first service (Duffield et al., 2009). Rodriguez et al. (2022) showed that cows with hyperketonemia in the first week were 30% less likely to become pregnant at the first service and 2.48 times more likely to be removed from the herd, when comparing with cows that present normal level of ketone bodies in blood during the first week. In the meta-analysis done by (Fourichon et al., 2003). On average, cows with hyperketonemia required 2.5 additional days before receiving their first service. Consequently, the

time taken for cows to conceive was extended by about 5.9 days. Additionally, affected cows needed approximately 0.13 more services for conception. The data also revealed a decreased probability of conceiving between 56 and 120 days postpartum, with a hazard ratio of 0.87. These results collectively suggest that hyperketonemia delay and reduce the efficiency of reproductive processes in dairy cattle.

Other complications are also associated with hyperketonemia such as increased risk of displaced abomasum, with odds ranging from 3 to 25 times higher than cows with lower BHBA levels (Geishauser et al., 1997). Milk production decreases in cows with high postpartum BHBA levels, Duffield et al. (2009) reported a daily reduction of 1.88 kg when BHBA levels were at 1.4 mmol/L, and a 3.3 kg decrease at 2 mmol/L. Furthermore, NEFA serum levels higher than 1 mmol/L have been associated with an increased likelihood of culling of 4.3 times when compared with cows with lower levels (Seifi et al., 2011). Subclinical hyperketonemia often goes undetected, but it affects approximately half of all dairy cows within the first month of lactation, and it has profound implications for herd health and productivity (Biswal et al., 2016). Indeed, McArt et al. (2012) found that 43.2% of cows reach levels above 1.2 mmol/L within the initial 16 DIM, were they found that with each 0.1 mmol/L of increase in BHBA increase the risk of developing DA, herd removal, or reduced milk production in the first 30 DIM increases by a factor of 1.1, 1.4 and 0.5 kg/d, respectively. Economic repercussions were estimated by Raboisson et al., (2015) where they estimate that the average cost for subclinical hyperketonemia correspond to the equivalent of $282.10 in the year of the publication, due to diminished milk yield and fertility complications, increased risk of DA along with infection diseases (Geishauser et al., 1998) Early culling can correspond to 30% of the total cost for the metabolic disease (Raboisson et al., 2015).

The central metabolic adaptions of the animal during this period mostly regard the capacity to mobilize and break down fat. The metabolic process of lipolysis assures the breaking down of large fat molecules into non-esterified fatty acids (NEFA), which then act as energy source for muscle tissue, contribute to milk fat synthesis in the mammary gland, or are taken up by the liver (Vazquez-Añon et al.,1994; Busato and Bionaz, 2020). The liver metabolizes approximately 15-20% of circulating NEFAs (Drackley and Andersen, 2006). Once in the liver, NEFA can be completely oxidized to produce energy. Alternatively, partial oxidation of NEFA produces ketone bodies such as acetone, acetoacetic acid, and BHBA. β -Hydroxybutyric acid is not only a useful energy source for peripheral tissues but also helps to mitigate the liver's metabolic limitations. Lastly, non-esterified fatty acids can be esterified into triglycerol and either be transported to adipose tissues via very low-density lipoproteins or stored within the liver itself as TAGs. For the complete oxidation of NEFAs, beta-oxidation converts them into acetyl-CoA. This molecule then combines with oxaloacetate to enter the citric acid cycle to generate ATP, supplying energy to liver cells and other tissues (Drackley, 1999). However, this process can be compromised when oxaloacetate is insufficient, which can occur due to its prioritization for gluconeogenesis to maintain blood glucose levels, or when NEFA concentrations are excessively high like postpartum dairy cows, leading to incomplete oxidation (Bergman, 1990). During incomplete oxidation, acetyl-CoA accumulates, prompting the liver to convert these excess molecules into ketone bodies. Peripheral tissues may utilize these ketone bodies as an alternative energy source. Yet, when their concentration in the blood becomes excessive, hyperketonemia arises (Herdt, 2000). Furthermore, triglycerols transportation can be challenging for some species including bovine. Dairy cows' liver has a limited capacity for synthesis of very low-density lipoproteins and therefore reduced ability to export triacylglycerol. When this threshold is surpassed, triglycerols begin to accumulate within

the liver, a condition termed hepatic lipidosis, or fatty liver disease, which can detrimentally affect liver functionality (Jorritsma et al., 2001). When it comes to treatment possibilities, since 1954 propylene glycol has been utilized as a treatment for hyperketonemia due to its effectiveness in elevating plasma glucose levels (Johnson, 1954). This elevation occurs as propylene glycol diminishes the glucose needed by peripheral tissues, consequently increasing insulin levels, which in turn inhibits lipolysis (Kristensen and Raun, 2007). Additionally, it has been shown to reduce NEFA concentrations and liver triglycerides, leading to a consequent decrease in plasma BHBA levels (Chung et al., 2009). Oral propylene glycol has been validated as an effective treatment that not only diminishes the clinical signs of hyperketonemia but also promotes the overall health and productivity of the cow, cows that present concentrations of BHBA above 1.2 mmol/L and treated with propylene glycol are 54 % less likely to develop clinical hyperketonemia and present an increase of 0.69 kg/d in milk yield (McArt et al., 2011; McArt et al., 2012). Moreover, postpartum cows receiving two drenches of propylene glycol within 24 hours after calving produced 3.1 kg/d more milk, highlighting the relevance in providing sufficient energy in the first day after calving (Stokes and Goff., 2001). A treatment regimen of 300 g of propylene glycol daily for five days has been recommended by Gordon et al. (2013), despite the labor intensity involved. Nonetheless, the authors maintain that a single daily dose is sufficient for improvement and can significantly reduce the labor required for treatment. Employing a drenching gun has been suggested to simplify the administration process, enhancing compliance among producers. For animals displaying neurological symptoms indicative of clinical hyperketonemia, the recommended treatment is to give propylene glycol orally (250–400 g, once daily for 3–5 days) in addition to one-time intravenous injection of a 50% dextrose solution (500 mL). For those who are also hypoglycemic, it is suggested to administer vitamin B12 (1.25 mg, intramuscularly, once daily for 3 days;

Duffield, 2021). The use of corticoids in the treatment of hyperketonemia has been in debate for decades now, however, there is insufficient evidence for positive outcome (Duffield, 2021). Although it has been shown to decrease BHBA concentration and reduce milk production (Andersson and Olsson, 1984) the NEFA concentration in blood increases after a corticoid treatment and the oxidative burst capacity of polymorphonuclear leukocytes is compromised by corticoids like dexamethasone (Lohuis et al., 1988) To prevent the onset of hyperketonemia, a multifaceted approach is required, beginning well before the critical transition period that spans 21 days before and after calving.

Maintaining an ideal body condition score (BCS) during the prepartum period is a key approach to control postpartum hyperketonemia. Optimal BCS should be between 3.00 and 3.50 on a 5-point scale to promote cow health, optimize milk production, and reduce the incidence of metabolic disorders (Roche et al., 2009). Cows calving with a BCS below this range are prone to decreased milk output and compromised reproductive performance. Conversely, excessive BCS, above 3.50, is associated with reduced dry matter intake, decreased milk yield in the early stages of lactation (Domecq et al., 1997), and a heightened risk of metabolic diseases (Overton and Waldron, 2004).

The prevalence of overweighted cows at calving is often not primarily due to dry period management, but rather linked to reproductive challenges (Visek et al., 1953). Cows that have more than 116 days opens are considered to have a poor reproductive performance (Inchaisri et al., 2010), this excessive time taken to achieve pregnancy leads to cows being milked an extend period, at this late stage, they are at the lower end of their lactation curve, producing less milk, which results in a positive energy balance (Ferguson, 1996; Mulligan et al., 2007). This excess

energy, rather than being used for milk production, gets stored in the body, leading to an increase in BCS.

Excessive BCS can lead to decreased insulin sensitivity, impaired glucose metabolism, and increased NEFA mobilization, escalating the risk of hyperketonemia (Samii et al., 2019). Hence, diligent monitoring and management of BCS are vital to prevent both overweight and underweight conditions during the prepartum period (Roche et al., 2009). In addition to BCS management, another crucial strategy is to maximize dry matter intake (DMI) while concurrently preventing ruminal acidosis. Ensuring sufficient DMI is essential for meeting the cow's energy requirements and averting a negative energy balance, which is a precursor to metabolic stress (Hayirli et al., 2002; Overton and Waldron, 2004). To alleviate the energy balance, dietary interventions can include the use of rumen-protected nutrients like choline. Choline has a critical role in liver function and fat metabolism, especially in the formation of very low-density proteins (VLDL) that facilitate the removal of excess TAG from the liver (Jayaprakash et al., 2016). The expression of genes related to the synthesis of VLDL especially increase ($P \leq 0.02$) when 25.8 g/d of choline ion was provided, and depending to the source and quantity of rumen protected choline provided the hepatic triglycerol was reduced over 50% (Arshad et al., 2023). However, in the metanalysis done by the same authors, rumen-protected choline improve milk yield and milk composition but had not effect on hyperketonemia(Arshad et al., 2020). This process not only improves liver function but also mitigates the risk of fatty liver syndrome, which is intimately associated with both hyperketonemia and immune function impairments (Zenobi et al., 2018; Lima et al., 2012).

1.3.3 Displaced abomasum

Displaced abomasum is a condition in dairy cows where the abomasum shifts from its normal anatomical position. This condition is usually associated with high producing dairy cows.

Usually, the displacement occurs to the left (LDA) or, less frequently, to the right (RDA), commonly within the three weeks after parturition. In extreme cases, the abomasum can rotate around itself, leading to a potentially fatal condition known as abomasal volvulus (Doll et al., 2009). A comprehensive survey across the United States revealed that the incidences rate of the of DA is 2.2 the incidence varied based on the size of the herd, with larger herds (500 or more cows) having a lower occurrence rate of 2.5%, while smaller herds (less than 500 cows) had a rate 2.3 percentage points higher (NAHMS-USDA, 2024). Economic analysis has estimated that the average expense for each diagnosis of DA exceeds $700 (McArt et al., 2015). This estimate includes both direct expenses, such as examination, treatment, medication, wasted milk, and mortality, as well as indirect costs, like future loss in milk production, weight loss, reduced reproductive efficiency, and increased likelihood of being culled from the herd (McArt et al., 2015).

Displaced abomasum in dairy cows leads to discomfort and reduced feed intake, consequently causing a significant drop in milk production, even cows that had a successful surgical outcome produced on average 1,130 kg les milk per lactation (Lima et al., 2012; Zenobi et al., 2018). This condition entails a series of adverse outcomes such as increased treatment costs and premature culling Furthermore, cows with DA are 1.8 times more likely to have another disease in comparison with cows without DA, all of which accrue substantial financial losses (Detilleux et al., 1997). Displaced abomasum has found to delay the days to the first AI. In the study done Raizman and Santos , (2002) animals diagnosed with DA took 12.7 more days to receive their first AI when compared with healthy animals. Displaced abomasum, results in elevated hyperketonemia, adding metabolic stress to the cows (Oetzel, 2020), thus can contribute indirectly to a decrease in reproduction performance.

Some of the diseases associated with DA are retained placenta, hypocalcemia, and hyperketonemia. In extreme situations, DA may even be fatal (Dirksen, 1962). Recovery from DA, whether through surgical or conservative methods, does not always restore milk production to its previous level, often leading to the cow's removal from the herd. The condition worsens as the gas-filled abomasum rises in the abdomen, worsening the patient prognosis.

The onset of DA in dairy cows is multifactorial, with several interrelated factors that can be broadly categorized into three elements: abomasal atony, a postpartum abdominal vacuum, and insufficient rumen fill (Constable et al., 1992). The displacement of the abomasum to the left for example requires the creation of space in the abdominal cavity, which naturally happens after the delivery of the calf, expulsion of the placenta, and subsequent shrinking of the uterus (Dirksen, 1962). This sudden extra room is intensified when the rumen is undersized, providing conditions for the abomasum to shift to the left (Dirksen, 1962). Simultaneously a decreased appetite following calving leads directly to a reduction in rumen fill and size, leading to increased abdominal room (Constable et al., 1992). Along with that, the abomasal hypomotility after parturition due to decreased muscle tone leads to a gas-distended abomasum, since the gas produced by digestion is not being properly expelled with the reduced and weakened contractions (Caixeta et al., 2018). Additionally, the slow gastric emptying rate associated with hypomotility exacerbates the issue, contributing to the progression of DA. Infectious diseases have been recognized to affect both rumination time dairy cows, which theoretically can increase the risk of displaced abomasum (Rial et al., 2023). This relationship implies that overall systemic health is determinant for abomasal function, although further research in this topic is warranted (Rial et al., 2023).

Moreover, specific dietary choices exacerbate the risk of DA. High ratios of non-structural carbohydrates in the diet increase fermentation rates and gas accumulation within the abomasum (Van Winden et al., 2002). Inadequate long-fiber intake can impair rumen function and reduce appetite, while finely ground rations (Dawson et al., 1992) or excess short-fiber forages, like maize, predispose cows to LDA by decreasing rumen fill and enhancing fermentation (Sexton et al., 2007). Recognizing these dietary risk factors is crucial for managing and mitigating the vulnerability to DA. In addition, certain risk factors have been identified that heighten the vulnerability to DA. Notably, hyperketonemia significantly escalates the risk, with an odds ratio (OR) of 9.27 compared to healthy cows, a figure that underscores a strong association possibly due to altered abomasal tonicity from metabolic stress (Kang et al., 2019). Additionally, cows that have undergone more than three parturitions, exhibit a higher risk of DA, with an OR of 5.23 compared with healthy cows (Kang et al., 2019), indicating a potential connection between parity and susceptibility to the condition.

The diagnosis of DA can be made by the veterinarian or trained farm staff through a thorough evaluation of the animal's history, clinical signs, and physical exam, especially by auscultation and percussion of the abdomen. When the abomasum is displaced and filled with gas, it creates an unusual percussion sound described usually described as metallic that can be detected dorsally on either side of the abdomen (Richmond, 1964). Additionally, ultrasonographic examination has been used to aid in the diagnosis of LDA, especially in equivocal cases where physical exam findings are not definitive (Li et al., 2018). Displaced abomasum in dairy cows can be approached in two ways: conservative management or surgical intervention. The former method involves procedures like casting and rolling the bovine to induce reposition of the abomasum. The initial objectives of treatment are to stabilize the animal using fluid therapy, calcium, and dextrose

solutions, reposition of the abomasum, prevent future displacement, and ensure the procedure is as minimally traumatic for the cow as possible (Tschoner et al., 2022). This strategy also includes diet modifications and treatment of any existing diseases (Niehaus, 2016). Despite these efforts, the conservative approach often sees high rates of failure and recurrence. As a result, immediate surgery is typically necessary, particularly in cases of RDA, which carries a high risk for volvulus (Tschoner et al., 2022). The two most used techniques for the surgical treatment of displaced abomasum in dairy cows are omentopexy and abomasopexy (Li et al., 2018), specifically: right flank omentopexy and laparoscopic abomasopexy. The right flank omentopexy involves creating an incision on the right side of the cow's flank. Through this incision, the surgeon accesses the greater omentum, which is a large fold of peritoneum that hangs down from the stomach. The greater omentum is then sutured to the body wall, aiming to stabilize the abomasum in its normal position and prevent future displacements (Gabel and Heath, 1969). The laparoscopic abomasopexy is a minimally invasive surgical technique used for the correction of LDA in dairy cows involves making small incisions in the cow's abdomen to insert laparoscopic instruments. This technique allows for direct visualization of the abomasum for proper positioning and fixation, which is one of its main advantages over blind techniques. The abomasum is identified, and a steel trocar and cannula are inserted into the abomasal lumen, fallow by a stainless steel toggle pin with suture material is inserted into the abomasum, then the abomasum is deflated and the suture material is tightened to position the abomasum adjacent to the body wall, and then tied to secure it in place (Newman et al., 2005).

Since this condition is multifactorial, it can be significantly influenced by periparturient management and nutrition during the transition period. Controlling clinical, and subclinical hypocalcemia, hyperketonemia and maximizing dry matter intake during the periparturient period

is essential. Therefore, it is vital that cows approach parturition in a body condition that optimizes feed intake and prevents periparturient diseases (i.e., 3.50 BCS; Barrett., 2003). Feeding of postpartum cows should include high-quality forage as opposed to large portions of concentrate (Constable et al., 1992). Adequate nutritional management is needed to minimize changes between late, dry, and early lactation ration, ensuring animals have plenty of bunk space will facilitate their access to feed, and practices should aim to maximize cow comfort and minimize stress(Shaver, 1997).

1.4 Uterine diseases

1.4.1 Retained Placenta

Retained placenta (RP) is characterized by the failure to expel the fetal membranes within 24 hours after parturition. Typically, these membranes are expelled within 3 to 8 hours post-delivery (Roberts, 2022). The USDA National Animal Health Monitoring System's 2014 survey indicates that the condition affects approximately 4.5% of dairy cows within U.S. herds. The financial impact of RP averages $480 per affected animal, as reported by Mahnani et al., (2021). This cost accounts for losses predominantly from decreased milk production and subsequent reproductive challenges. It's important to note that these costs can vary internationally, reflecting differences in management practices, milk prices, and healthcare costs across countries. Mahnani et al. (2021) elaborate on this variation, analyzing the economic losses associated with RP across different herds in Iran. To elucidate the pathogenesis and etiology of this condition, several factors need to be considered. These include gestation length, hormonal imbalance, inflammatory response, immune dysfunction, and metabolic disturbances. The gestation length can also be associated with the incidence of retained placenta, as demonstrated by (Vieira-Neto et al., 2017). They found that primiparous cows with a shorter gestation length were at a higher risk of RP

compared with those with normal and longer gestation length (18.0% and 4.5% and 4.5% respectively), similarly to multiparous cows (28.6%, 3.7%. and 4.6% for short, medium, and long gestations, respectively). The reasons for these differences are not entirely clear, but it has been suggested that cows with a shorter gestation length secrete fewer calving-related hormones, making them more susceptible to difficult births and stillbirths (Kindahl et al., 2002). On the other hand long gestation length are associated with difficulty at calving probably associated with increased calf size and weight (Jenkins et al., 2016) ;Atashi and Asaadi) and difficulty at calving increases the risk for retained placenta to odds ratio of 4.36 in multiparous cow (Mahani et al., 2021).

Another factor contributing to the incidence of RP is its association with a pro-inflammatory response. It has been reported that there is an increase in the gene expression of IL-β in cows with RP when compared with cows without RP (Ramos et al., 2022). An effective immune response necessitates a carefully coordinated regulation of immune cells and the inflammatory signals they produce reactions. Furthermore, in a study conducted by (Nelli et al., 2019), the effect of various macrophage types on the expulsion of the placenta was examined. They discovered that cows with the condition showed a diminished expression of CD14+/CD16+ in the caruncle monocyte/macrophage population and an increased expression of genes associated with the anti-inflammatory M2 macrophages. This implies that an overabundance of M2 macrophages could result in decreased movement of immune cells into the caruncle, hindering the inflammatory, phagocytic, and proteolytic processes that are crucial for the expulsion of the placenta (Nelli et al., 2019). The successful expulsion of the placenta involves the coordinated breakdown of collagen within the placental tissues by matrix metalloproteinases (MMPs). These enzymes play a key role in remodeling the extracellular matrix, allowing for the placental release

postpartum (Walter and Boos, 2001). In cases of RP, a decrease in MMP activity is observed (Maj and Kankofer, 1997). Additionally, this process encompasses the programmed cell death of binucleate and trophoblastic cells, and the entry of phagocytic white blood cells (Attupuram et al., 2016). These processes are crucial for the detachment of the placenta from the caruncle and its eventual expulsion. As an example of these imbalances, the study done by (Maj and Kankofer, 1997) found differences in MMP-2 activity between cows with and without placental retention, been more active in cows without RP, which may have affected collagen hydrolysis and the release of fetal membranes.

The normal process of placental expulsion is a complex interplay between hormonal changes and immune cell activities. Research by Nelli et al. (2019) underscores the critical role of a balanced immune response in this process. Disturbances in the equilibrium between pro-inflammatory and anti-inflammatory immune cells can lead to RP, indicating that proper immune cell activation is crucial for placental release. Hormonal fluctuations contribute to this balance; metabolic stress, for instance, induces the release of corticotropin-releasing hormone, increasing plasma corticosteroids, which modulate the immune system (Mormede et al., 2007). During parturition, there is also a surge in adrenaline that activates myometrial adrenoceptors, potentially leading to uterine relaxation. This hormonal shift can impact the uterus's ability to contract and expel the placenta effectively, as illustrated by Mordak and Stewart (2015). The suppression of the immune system and reduced movement of the uterus in cows are of great importance, as they can pose risks during parturition, specifically during the expulsion of the fetus and placenta. As a result of these alterations, the typically effective maternal immune response and the expulsion of fetal membranes are delayed, leading to the retention of these membranes in cows (Kimura et al., 2003).

Other difficulties in calving are also considered to be risk factors for RP, Hossein-Zadeh and Ardalan (2011) demonstrated that dystocia, stillbirth, and twinning increases the incidence of RP. Furthermore, immune suppression, infectious diseases, elevated lipid mobilization, and a depleted status of antioxidants such as vitamin E are strongly correlated as well (Qu et al., 2014). Moreover, vitamin E and β-carotene are recognized as antioxidants at the cellular level, while selenium contributes to the antioxidant system through its function in the enzyme glutathione peroxidase (Spears and Weiss, 2008), they are crucial antioxidants that play a significant role in enhancing the immune response in dairy cows, particularly around the time of parturition. The positive influence to the reduction of RP was proven by Harrison et al., (1984) in which they saw a reduction of RP from 17% to 0% when both vitamin E and selenium were supplemented to cows.

Concerning treatments for RP, authors discuss the manual removal of the placenta and whether the use of antibiotics can be beneficial or not when applied locally or systemically. The review done by Beagley et al., (2010) provide helpful insights discussing these controversies. When the RP is removed manually, it usually increases the chances of uterine infections, leading to a reduction in fertility rates (Bolinder et al., 1988). Showing not beneficial outcomes (Moller et al., 1967). These results are not in agreement with Maletić et al. (2021) where they found that animals present a better reproductive performance when the RP was manually removed and an intramuscular dose of ceftiofur was applied to the cow in comparison with the animals that were only treated with antibiotics coupled with natural detachment of the fetal membranes. There is also discussion about whether the use of antibiotics is beneficial or not, and if they should be utilized locally or systemically. The use of local antibiotics does not present any benefits in terms of reducing the time of placenta detachment nor preventing future related metritis condition according to Peter (1966). However, other studies have found beneficial results such as increase

feed intake and milk production when systemic antibiotics were used, especially when the animal presented a fever (Beagley et al., 2010; Risco and Hernandez et al., 2003). Continuing with treatment, the use of collagenases is another interesting therapeutic finding that needs to be further investigated. It shows promising responses shortening the time in which the placenta is released, however, most of the time they require veterinary assistance (Eiler and Hopkins, 1993). This is not the case for α- chymotrypsin which acts as a proteolytic enzyme, when associated with PGF2α can help with accelerating the placental shedding, decreasing the postpartum metritis, and improving the reproductive performance (Amin et al., 2023).

When it comes to the economic impact of this condition and its consequences, some are directly related. For example, the use of antibiotics and veterinary assistance impart direct financial expenses. However, there are also indirect economic consequences of RP, such as a reduce of 294 kg in milk production over 305 days, a reduction in fat production, and an increase of 20 days in open days (Mahnani et al., 2021). Moreover, an increased risk of metritis, where 54.8% of cases had a history of RP has been already describe by Sandals et al. (1979).

The occurrence of RP as stablished before is intricately linked to the cow's immune status, which is significantly influenced by metabolic health. Nutritional deficiencies, particularly in the periparturient period, can compromise the cow's immune response, thereby escalating the risk of RP. Specifically, inadequate feed intake, which is common around the time of calving, can lead to a negative energy balance that suppresses immune function. Additionally, selenium and vitamin E are critical antioxidants that play a vital role in immune defense mechanisms. Deficiencies in these micronutrients have been documented to weaken the immune system, increasing susceptibility to RP and associated complications (LeBlanc, 2008; Hogan et al., 1993; Baldi et al., 2000). Therefore, managing these dietary factors is crucial for optimizing immune competence and

reducing the incidence of RP, which in turn can improve overall reproductive efficiency and herd health.

1.4.2 Metritis

Metritis is an infection of the uterus associated with an inflammation of the same organ, typically affecting cows within the first three weeks after parturition. It is characterized by the presence of an abnormal, often fetid vaginal discharge, which is usually reddish-brown and watery. This discharge is expelled from the uterus and can be more readily observed after rectal palpation. Clinical signs of metritis may include fever, lethargy, and a noticeable reduction in dry matter intake, which can lead to decreased milk production and overall poor health status in the affected cow (Chenault et al., 2004; Sheldon et al., 2006). The reported incidence of metritis in dairy operations across the United States averages around 15 and 20% (LeBlanc, 2008). Still, actual rates may vary widely due to factors such as diagnostic practices and cow parity. While some farms report rates as low as 8%, others may experience rates exceeding 20%, variations are frequently attributed to underdiagnosis (Kaneene and Miller, 1994), as not all cows with clinical signs of uterine discharge are identified or reported. When thorough examinations are conducted, approximately 20% of cows may exhibit discharge indicative of metritis. It is important to note that incidence rates differ between primiparous and multiparous cows, and those with retained placenta (RP) also show distinct patterns of metritis occurrence, reflecting variations in vulnerability and disease manifestation across these groups (LeBlanc, 2008; Galvão, 2012; Vieira-Neto et al., 2016). This condition comes along with negative economic implications. The economic impact was studied by Pérez-Báez et al. (2021), who estimated an average cost of $511 due to losses in milk production, treatment, and replacement costs. Therefore, with high incidence and elevated costs, metritis is a relevant subject to the dairy industry.

The development of metritis in dairy cows is a multifaceted process that begins postpartum. It involves a complex interplay between infectious agents and the cow's immune response. The cow's immune system is crucial in responding to these pathogens but can be compromised due to metabolic stress and hormonal changes associated with calving. During the calving process, the uterus is subjected to trauma and is inevitably exposed to a variety of pathogens. These pathogens can then infect the damaged areas of the uterus, leading to the development of metritis (Sheldon et al., 2009). A series of events such as the damage of the mucosa, the presence of the adequate amount of pathogens and poor immune response must occur for the bacteria to successfully establish in the uterus, leading to the beginning of the condition. Some pathogens have already been correlated with the disease, such as *Escherichia coli, Trueperella pyogenes*, and *Fusobacterium necrophorum* (Bonnett et al., 1991). Moreover, other agents that could cause metritis may include *Bacteroides pyogenes, Porphyromonas levii, and Helcococcus ovis* (Cunha et al., 2018) and an imbalance of imbalance in the uterine microbiota, marked by a high prevalence of Bacteroides, Porphyromonas (Jeon and Galvão, 2018).

The crutial factor to in preventing metritis is efficiently handling the infection immediately after parturition, and this should be done by the immune system (Casaro et al., 2023). Indeed, the battle between the immune system and disease-causing organisms occurs when cows are transitioning into their lactation phase. This is a time of significant physiological transformations that impact the effectiveness of the immune system (Hammon et al., 2006). Cows with metritis present alteration in blood markers related to inflammation and immune response and even carbohydrate metabolism weeks before parturition, greater concentrations of lactate, Interleukin 6 (IL-6), Tumor Necrosis Factor (TNF), and serum amyloid A (Dervishi et al., 2016), more specifically, four weeks before parturition cows with metritis have 3.5 time the serum

concentration of IL-6, 3.8 time of TNF, 1.7 serum amyloid A and 1.5 lactate (Dervishi et al., 2016). This previous alteration in serum markers might altered the cow's capacity to response to infection.

The nutritional condition of the cow at parturition is also critical. Animals with a high BCS and greater fat loss were more prone to develop metritis when studying metabolic and immune changes postpartum (Casaro et al, 2023). This scenario of high fat mobilization and metabolic stress affected the proper functioning of the immune response (Sordillo and Aitken, 2009). Cows with metritis usually present a widespread inflammatory condition, evidenced by increased activation of B cells, higher levels of pro-inflammatory cytokines, and more extensive cell damage before and after giving birth (Casaro et al., 2023). Hormonal changes and fluctuations can also affect the immune response of the mucosal immune system (Bondurant, 1999). For example, cortisol serum concentrations can be increased at least 2 times above base line during this period (Mormède et al., 2007) suppressing leukocyte function. Moreover, progesterone, while primarily recognized for its vital hormonal functions during pregnancy, also possesses intriguing abilities to modulate the immune system (Raghupathy and Szekeres-Bartho, 2022). It can suppress the initiation of inflammatory responses, stimulate immune cells, and produce cytokines, which are essential for orchestrating immune reactions (Shah et al., 2019; Fedotcheva et al., 2022).

Intending to establish a common criterion for the definition and diagnosis of metritis, Garzon et al., (2022)compiled various characterizations and descriptions of the clinical signs from the literature. According to their review, most authors described the disease by observing clinical signs such as fever > 39.5°C, decreased milk yield, apathy, reduced appetite, and foul-smelling, watery, red-brown vaginal discharge. Along with these clinical signs, behavioral changes are also noticed. Cows suffering from metritis show a significant reduction in their rumination time,

physical activity, and resting time (Rial et al., 2023). To finalize the diagnosis, a veterinarian can palpate the uterus through the rectum, followed by an examination of the vaginal discharge, and an assessment of the cow's overall health status can also be utilized to evaluate the uterus status (Garzon et al., 2022).

The treatment of the disease typically involves a combination of systemic antimicrobials, fluid therapy, and anti-inflammatory drugs. Ceftiofur is the most used antimicrobial and has proven effective in several trials (Reppert et al., 2015) by improving the clinical signs of animals with metritis. Cows exhibiting signs of postpartum fever and uterine discharge showed notable improvements in healing rates, milk production, and body temperature when treated with ceftiofur (Zhou et al., 2001). According to Zhou et al. (2001), cows treated with ceftiofur had a significantly higher probability of recovery compared to the control group, with cure rates of 56.0% versus 28.9%, respectively, at 9 to 10 days after enrollment. Milk yield improved 2.27 kg in the first milking after treatment was initiated compared to the control group. Concerning the dosage, a single subcutaneous injection of ceftiofur hydrochloride at a dosage of 1 mg/kg body weight is used, and its levels found in the plasma, uterine tissues, and lochia fluid surpassed the minimum inhibitory concentration of the primary pathogens identified in metritis (Okker et al., 2002). Other antimicrobials, such as ampicillin trihydrate, are also effective for treating metritis (Lima et al., 2014).

Supportive care is critical for cows with metritis exhibiting severe symptoms such as lethargy, lack of appetite, and fever. Intravenous (IV) administration of dextrose solutions is essential for managing metritis since most cows with metritis that present these signs are ketonic (Lima, 2022). Additionally, IV hypertonic saline is recommended to correct dehydration. These IV treatments are vital in stabilizing the cow's condition and promoting recover (Lima, 2022). The

use of anti-inflammatory drugs is an option for treatment along with antimicrobial drugs. However, some studies found no positive benefits in clinical cure, milk yield, or reproductive performance when flunixin meglumine or meloxicam were utilized in single doses (Drillich et al., 2001; Lomb et al., 2018).

Research has consistently shown the multifaceted impact of metritis on dairy cows. A study by Pérez-Báez et al., (2019) found that cows with metritis exhibit a reduced dry matter intake, with an observed decrease by 0.4% of body weight compared to healthy cows. Milk production is also affected, as showed by Mikulková et al., (2020) who reported that within the first 30 days in milk (DIM), cows suffering from metritis produced significantly less milk, averaging a decrease of 17.1 kg/d when the control group was producing on average 50.5 kg/d. This reduction persists over time, as further evidenced by Pérez-Báez et al. (2021), who noted that by 305 days in milk, affected cows produced 814 kg less milk than their healthy counterparts.

The repercussions of metritis extend to reproductive performance as well. Lima et al. (2019) observed a 7% reduction in the pregnancy rate at 21d after voluntary waiting period post-treatment for cows treated for metritis compared to unaffected cows. This decline in fertility was also evaluated over 305 days in milk, where a 10 % reduction in pregnancy was seen for cows with metritis when comparing with cows without metritis (Pérez-Báez et al., 2021). Furthermore, the presence of metritis significantly raises the risk of culling. Wittrock et al., (2011) highlighted that cows with metritis were approximately 30% more likely to be removed from the herd, suggesting that metritis can lead to serious decisions regarding herd composition and suggesting that the effects over milk production and fertility can be underestimated.

Several publications in the literature discuss prevention and risk factors for metritis. A study by Machado et al., (2014) examined a vaccine, finding a decrease in metritis and a fever

reduction compared to the control group. In this study, heifers who received subcutaneous vaccines twice in late pregnancy showed less metritis than the control group, and 0.18 ◦C lower body temperature at six days postpartum. The vaccine used in the study was designed to develop immunity against the most common infectious agents encountered during metritis in dairy cows, such as *Escherichia coli*, *Fusobacterium necrophorum*, and *Trueperella pyogenes*. The intravaginal infusion of probiotics containing *Lactobacillus* strains and *Pediococcus* strains has also proven effective in reducing the incidence of metritis and uterine diseases, lowering the concentration of lipopolysaccharide-binding protein, and modulating the immune response (Deng et al., 2015). Heifers and cows with a history of calving difficulties should be bred with bulls that are likely to produce smaller offspring. This can reduce the incidence of calving difficulties, since difficulty at calving can decrease reproductive performance and increase the incidence of uterine diseases, including metritis as discussed before (Wiltbank, 1983; Morek-Kopeć et al 2021).

Nutritional interventions are also important for preventing metritis, and they can start before calving. Providing an adequate amount of vitamin E and minerals such as selenium can improve reproductive performance and lower the incidence of retained placenta (RP), which is a risk factor for metritis (Pontes et al 2015; Dubuc et al 2010; Aréchiga et al., 1994).

From an economic perspective, the combination of these health and productivity issues culminates in a substantial financial burden. Pérez-Báez et al. (2021) quantified this impact, revealing that cows with metritis are $520.7 less profitable in the United States, factoring in the direct costs of treatment and the indirect costs of lost production and reproductive inefficiency. Effective management and prevention strategies are essential and include, the selection of more resilient animals, optimizing nutrition, and closely monitoring cows during the peri-partum period. Early diagnosis and intervention are crucial to managing metritis effectively, reducing the need for

antibiotics, and mitigating the risk of developing antibiotic resistance. Furthermore, implementing preventive measures, such as vaccination against common pathogens causing uterine infections, can significantly reduce the incidence and severity of metritis, leading to improved animal welfare and economic outcomes for dairy farms.

1.5 Conclusions

The postpartum period is delicate in the lifecycle of dairy cows, marked by an increased vulnerability to a range of diseases that can significantly damage their health, milk production, and reproductive performance. Effective management of these diseases' hinges on a multifaceted approach that integrates proactive actions such as vigilant monitoring, nutritional optimization, and strategic antibiotic and treatment interventions. Despite our efforts to prevent and manage these diseases, the persistent incidence rate observed on many dairy farms highlight the need for ongoing research. Future studies should focus on refining diagnostic criteria, genetic selection, and developing novel therapeutics to support the immune system of dairy cows during this critical period. Ultimately, the advancement of dairy herd health requires a commitment to continuously improving animal husbandry practices, particularly in managing transition period challenges.

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2. Disease in one lactation is associated with a greater incidence of the same disease in the subsequent lactation in dairy cows

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2.1 Abstract

Objectives were to evaluate if having a disease in one lactation is associated with the risk of developing the same disease in the subsequent lactation and to identify risk factors for prevalent postpartum diseases. A total of 13,761 cow-lactations from 6,179 Holstein cows from two dairy farms located in Central California and two dairy farms located in North Florida were used in this retrospective study. Data were retrieved from farm management software from 2007 to 2013 and included prevalent postpartum disorders, such as dystocia, stillbirth, retained placenta, metritis, clinical hypocalcemia, hyperketonemia, displaced abomasum, and mastitis. Data were analyzed by logistic regression with the GLIMMIX procedure of SAS and included the fixed effects of history of the disease, parity, the interaction between the history of disease and parity, and the random effect of farm. Having dystocia increased the risk (odds ratio [OR] = 1.48; $P < 0.001$) of having dystocia in the subsequent lactation (25.5 vs. 18.8%). Having a stillbirth tended to increase the risk (OR = 1.58; $P = 0.07$) of having a stillbirth in the subsequent lactation (5.0 vs. 3.3%). Having retained placenta increased the risk (OR = 1.63; $P = 0.002$) of having retained placenta in the subsequent lactation (9.5 vs. 6.0%). Having metritis increased the risk (OR = 1.68; $P < 0.001$) of having metritis in the subsequent lactation (19.0 vs. 12.2%). Having clinical hypocalcemia increased the risk (OR = 13.32; $P < 0.001$) of having clinical hypocalcemia in the subsequent lactation (19.7 vs. 1.8%). Having hyperketonemia increased the risk (OR = 1.91; $P < 0.001$) of having hyperketonemia in the subsequent lactation (32.5 vs. 20.2%). Having displaced abomasum increased the risk (OR = 3.68; $P = 0.02$) of having displaced abomasum in the subsequent lactation (1.35 vs. 0.37%). Having mastitis increased the risk (OR = 2.10; $P < 0.001$) of having mastitis in the subsequent lactation (16.8 vs. 8.8%). For all the variables analyzed, the interaction between disease history and parity was not significant. Our data indicate that cows with a history of the

disease or disorder are more likely to develop the same condition, suggesting a genetic component in the pathogenesis of postpartum diseases and disorders. In addition, our data suggests that the adaptive immune response to infectious diseases such as metritis and mastitis may not last until a subsequent lactation or may be insufficient to reduce the risk of diseases.

2.2 Introduction

The transition period is defined as the period from three weeks prepartum until three weeks postpartum. This period is characterized by marked changes in nutrient demand from a gravid uterus to the mammary gland, with dramatic changes in the hormonal patterns of cows accompanied by compromised dry matter intake, challenging cows to maintain nutrient balance (Grummer, 1995). Because of that, and the complications of parturition, cows are more prone to metabolic and infectious diseases, resulting in about 40% of cows being affected by at least one disease within the first 60 d postpartum (Santos et al., 2010; Ribeiro et al., 2016: Pinedo et al., 2020). In addition to increasing treatment and labor costs and impairing animal welfare, those diseases are known to impair productive and reproductive performance and, consequentially, herd profitability (Ingvartsen et al., 2003; Lima et al., 2019: Pérez-Báez et al., 2021).

Genetic evaluation and selection for traits related to health performance have become more accurate and used by the dairy industry in the United States; nevertheless, genetic progression with health traits is generally categorized as having low heritability (Lopes et al., 2020; Heringstad et al., 2005). Another challenge is that health traits have low sire reliabilities due to a lack of phenotypic data in combination with low heritability. A potential alternative to improve sire reliability is used of single-step genomic analysis, which can increase sire reliability by 9 to 15 percentage points for health traits, and perhaps may facilitate the use of younger bulls with more reliable data (Gaddis et al., 2014). While there is space for improvement in how to use genomics

to improve health, several genomic studies have demonstrated strong genetic correlations with postpartum metabolic disorders (Klein et al., 2020; Cavani et al., 2021; Pacheco et al., 2018) and infectious diseases (Fang et al., 2017; Guarini et al., 2019), and consequently, genetic correlation with health traits is more evident as new studies are developed.

Previous observational studies have evaluated disease recurrence in Holstein cows. Data from 34 dairy farms in Canada, including 750 records, reported that cows with retained placenta and mastitis were more likely to be affected by the same disorders in the subsequent lactation (Bigras-Poulin et al., 1990); however, recurrence of hyperketonemia, clinical hypocalcemia, and metritis was not significant. Rowland et al. (1986) evaluated disease recurrence in 2,109 lactations over six years in 894 cows in Great Britain. They reported that cows with a history of hypocalcemia and mastitis were more likely to develop the same disease in the subsequent lactation. While some studies have noted an increased risk of certain diseases reoccurring in subsequent lactations for cows previously affected, these findings were based on limited datasets. As a result, a more extensive investigation into the connection between disease occurrence and recurrence is necessary to establish a clearer understanding.

We hypothesized that cows with a prior history of a specific disease or disorder might be at an increased risk of developing the same disease or disorder in the subsequent lactation. This hypothesis implies a potential genetic link between disease/disorder occurrences in postpartum cows, and that the adaptive immune response to previous infections may be insufficient in duration to prevent recurring infectious diseases in subsequent lactations. Therefore, the objectives of this retrospective study are to evaluate if having a disease in one lactation would affect the risk of developing the same disease in the subsequent lactation and to identify risk factors for prevalent postpartum disease in dairy cows.

2.3 Materials and Methods

2.3.1 Cows, Experimental Design, and Farms

Data from 6,179 Holstein cows were collected, resulting in a total of 13,761 cow-lactations from commercial dairy farms located in North Florida (n = 2) and Central California (n = 2). The study followed a retrospective observational design and data collected was from cows that calved between January 2007 and December 2013. Those dairy farms were selected to be included in the study because of the history of keeping track of postpartum diagnosis information over the years evaluated. Farm workers in farms located in Central California were trained and assisted by the Veterinary Medicine Teaching & Research Center (VMTRC) of the University of California – Davis, while farms located in North Florida were trained and assisted by the Food Animal Reproduction and Medicine Service (FARMS) of the University of Florida. Descriptive statistics of incidence of disease in cows enrolled in the study are presented in Table 1 according to parity group and farm. All the cows in the dataset (n = 6,179) were used for the analysis of recurrence of disease, on the other hand only (n = 3,545) cows from the farms located in FL were used for the analysis of risk factors for diseases because the dataset from CA had limited information on the induction of parturition, clinical hypocalcemia, and ketosis, and those were key risk factors that we wanted to explore.

Farm A milked about 500 Holstein cows during the study period, where cows were milked twice daily during the entire lactation. The rolling herd average during the study period was 10,000 kg of 3.5% FCM. Cows were housed in freestall beds and walking alleys were cleaned twice daily. Clean and dry sand was added to freestall beds twice weekly. Barns were equipped with fans placed above the beds and water soakers line with nozzles above the feed lane. Primiparous and multiparous cows were housed separately. Cows were fed twice a day, and amounts were

calculated for an estimated 2 to 3% leftover. Farm B milked about 4,800 Holstein cows during the study period, where cows were milked three times daily during the entire lactation. The rolling herd average during the study period was 10,500 kg of 3.5% FCM. Cows were housed in tunnel-ventilated freestall barns, which were cleaned thrice daily, and sand bedded twice weekly. Primiparous and multiparous cows were housed separately. Cows were fed twice a day, and amounts were calculated for an estimated 2 to 3% leftover. Farm C milked about 1,600 Holstein cows during the study period, where cows were milked four times daily during the first 30 d postpartum and then twice daily for the rest of the lactation. The rolling herd average during the study period was 12,500 kg of 3.5% FCM. Cows were housed in dry-lot pens with shading in the central area and above the feed bunk. Bedding under the shaded area was raked daily, while dried composted manure was added under the shades as bedding material twice weekly. Primiparous and multiparous cows were housed separately. Cows were fed twice a day, and amounts were calculated for an estimated 2 to 3% leftover. Farm D milked about 5,200 Holstein cows during the study period, where cows were milked three times daily during the entire lactation. The rolling herd average during the study period was 13,635 kg of 3.5% FCM. Cows were housed in freestall barns with sand-bedded stalls. Barns were equipped with fans placed above the beds and water soakers line with nozzles above the feed lane. Primiparous and multiparous cows were housed separately. Cows were fed twice a day, and amounts were calculated for an estimated 2 to 3% leftover.

2.3.2 Data Collection

Data for health events were collected from the dairy management software of each farm, which included AfiFarm (Afimilk Ltd., Kibbutz Afikim, Israel) for farm A, PCDART (Dairy Records Management Services, Raleigh, NC) for farm B, and Dairy Comp 305 (Valley

Agricultural Software, Turale, CA) for farm C and D. Data regarding calving information including dystocia, stillbirth, twin, sex of the calf, induction of parturition, abortion, and season of calving were recorded. Induction of parturition was recorded only at farm B and the protocol included the administration of 25 mg of dexamethasone and 25 mg of prostaglandin F2$_{\alpha}$ (Dinoprost). Local information from weather stations located in Gainesville, FL and Hanford, CA, were used to classify season of calving based on temperature observations. Therefore, the hot season was between June 1 and October 15, whereas the remainder of the year was considered the cool season. Cows were classified as having calving problems when they had at least one of the following conditions: dystocia, stillbirth, presence of twins, induction of parturition, and/or abortion. Cows were evaluated daily for diagnosis of diseases by the farm staff following standard operating protocols for diagnosis and therapy that were discussed with veterinarians from the University of Florida (Gainesville, FL) and the University of California – Davis (Davis, CA). Cows were diagnosed with clinical hypocalcemia by identification of recumbent cows usually within 72 h after parturition that were reluctant to move due to flaccid muscular paralysis and good response to intravenously treatment with calcium. Cows were diagnosed with hyperketonemia using a urine ketone strip and any color change was considered positive for hyperketonemia. Diagnosis of displaced abomasum was based on auscultation and percussion of the left and right cranial areas adjacent to the paralumbar fossa followed by confirmation during corrective surgery. Cows were diagnosed as having retained placenta if the placenta was not expelled within 24 h of calving. Metritis was defined watery, reddish, or brownish vaginal discharge with foul smell with presence or not of fever within the first 21 d postpartum (McLaughlin et al., 2012). Cows were diagnosed as having mastitis by presence of abnormal milk and inflammation of the gland.

2.3.3 Statistical Analysis

Sample size was calculated using the POWER procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC). The sample size was based on the ability to detect a difference of 5 percentage points in the incidence of metritis between groups (20% in cows with history of metritis vs. 15% in cows without history of metritis; $\alpha = 0.05$; $\beta = 0.20$), resulting on a need of at least 906 cows per group. We end up having a much larger sample size as we would like to have at least four different farms included in the analysis of at least two different locations.

Categorical data were analyzed by logistic regression using the GLIMMIX procedure of SAS version 9.4 (SAS Institute Inc.) fitting a binary distribution. The model included the fixed effects of history of the disease (yes vs. no), parity (Prim-Mult vs. Mult-Mult), the interaction between the history of disease and parity, and the random effect of the farm. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the *F* tests in the statistical models. The LINK statement was used with the LOGIT function and the OR statement was used to generate odds ratio and 95% confidence intervals. Requests estimates of odds ratios and their confidence limits, provided the link function is the logit. Nonsignificant interactions ($P > 0.10$) were removed sequentially by backward elimination based on the largest *P*-value.

2.4 Results and Discussion

2.4.1 Dystocia

The incidence of dystocia in the current and subsequent lactation was 30.2% (1,988/6,575) and 23.5% (795/3,379; Table 2), respectively. Having history of dystocia increased ($P < 0.001$) the incidence of having dystocia in the subsequent lactation (Disease history = 25.5% vs. No Disease History = 18.9% ± 6.6%; Table 3; Adj. OR = 1.48 [1.21-1.80] Table 4), regardless of parity (Table 4). In addition, incidence of dystocia in the subsequent lactation was not affected by

parity (Prim-Mult = 22.9% vs. Mult-Mult = 21.1% ± 6.6%; *P* = 0.30; Table 3). The lack of difference in parity between Prim-Mult and Mult-Mult could be due to the grouping characteristic, since primiparous alone had not been evaluated for recurrence but the data from primiparous alone with the second parity (being to consecutive lactations), the incidence of dystocia and the other diseases and condition discussed in this data set does not compare strictly primiparous vs multiparous.

The incidence rate in the current data average 38.5% for primiparous and 21.1% for multiparous. The current rates of dystocia are higher than those reported from 2003 to 2005 for over 3,000 herds in the United States, where it was 22.6% for primiparous cows and 13.7% for multiparous cows (Gevrekçie et al., 2006). The discrepancy in the current data with the data of Gevrekci et al., (2006) could be related to several factors such as the lack of uniformity in scoring this condition, usually dystocia rate are affected by calving assistance, that vary from farm to farm, unnecessary human intervention, and breeding plans (Mee, 2004). Garry (2004) hypothesized that the greater dystocia rates in the U.S. could be related to the selection for productivity and not enough emphasis in selecting for herd health traits, such as calving ease, since breeding strategies favoring yield traits could negatively impact calving ease performance (Maturana et al., 2007). In this context, certain European countries that have prioritized calving ease as a national selection goal and have reported lower dystocia rates than the United States. Thus far there is not enough data to understand the exact reasons of the discrepancy in different countries. However, the scenario is rapidly changing due to the genomic selection in the national territory towards health traits (Wiggans and Carrillo, 2022)

The recurring nature of dystocia in successive lactations could be attributed to several factors, such as discrepancies between calf size and dam pelvic area (Statham, 2023), and genetics

of the dam and selected sire (Garry, 2004; Maturana et al., 2007). One square decimeter increase in pelvic area is linked to an 11% reduction in the odds of dystocia (Johanson and Berger, 2003); therefore, the pelvic area of the dam can contribute to recurring dystocia in animals that have smaller overall pelvic area. Additionally, there is sire influence for calf birth weight, with risk for dystocia increasing by 13% for each kg increase in calf birth weight (Johanson and Berger, 2003). The continued use of sires that increase calf birth weight can necessarily increase dystocia rates, but this factor likely is less responsible for recurrent dystocia from the dam perspective since other calving ease sires can be selected. Taken together, we are not aware of any data that clearly demonstrates the overall influence of dam and sire factors to recurrent dystocia as highlighted in this paper despite the logical associations presented herein.

A more modern approach to lowering the incidence of dystocia can be leveraged with genomic selection. Cows and bulls with a greater PTA for birthweight likely contribute to greater calf size and resulting dystocia because of the high heritability of the trait (0.51; Johanson and Berger, 2003; Johanson et al., 2011). Furthermore, based on the findings of Sugimoto et al. (2012), it was determined through a genome-wide association analysis that a single nucleotide polymorphism (SNP), specifically the A-326G variation in the SLC44A5 gene's 5' untranslated region, is associated with birth weight. They discovered that the A polymorphism correlates with higher birth weights and increased rates of dystocia, whereas the G polymorphism is linked to lower expression levels of SLC44A5, resulting in smaller calf sizes due to decreased cell proliferation. Hence, selecting for the G polymorphism in dairy cows is likely to result in offspring with reduced birth weights, subsequently lowering the risk of dystocia.

2.4.2 Twins

Examining the incidence of twins during current and subsequent lactations, rates were found to be 4.5% (344/7,617) and 6.3% (479/7,622), respectively (Table 2). These observations are consistent with Schambow et al. (2021), who reported a 4.7% twinning incidence in Holsteins. Notably, there was a higher probability of twinning in subsequent lactations for cows that had previously birthed twins. In our study, such cows (Disease History) had a 9.2 % incidence of twinning, significantly higher ($P < 0.001$; Table 3) than the 4.8 ± 1.9% incidence in cows without a twinning history (No Disease History). Our analysis revealed an adjusted odds ratio of 2.02 (1.33-3.05; Table 4) indicating that the odds of animals that delivered twins in one lactation doing so again were twice as high compared to those that did not.

These data are supported by other researchers where the likelihood of twin births doubled (Markusfeld, 1990) or quadrupled in subsequent lactations after having one incidence of twins (Nielen et al., 1989). Two factors may be causative of recurrent twinning that we and others have observed, 1) genetic traits for twinning, and 2) elevated milk production creating susceptibility to twinning. The genetics of twinning is heritable and can be selected for over time (Komisarek and Dorynek, 2002), although it has a relatively low heritability (0.1420; Lett and Kirkpatrick, 2018). In a study done by Gregory et al., (1997); it was demonstrated a substantial increase in the twinning rate, rising from 3.4% in 1982 to 28.5% in 1993, where they purposefully selected this trait in beef cattle over the years . Breeders can reduce the incidence, and potentially the recurrence, of twinning by utilizing Predicted Transmitting Abilities (PTA). The range of PTA values for twinning are -7.5 to 20.8, which is indicative of individuals more or less likely to pass on the twinning trait to progeny. By using PTAs in our breeding programs, it is possible to selectively breed against twinning without negatively affecting other beneficial genetic attributes (McGovern

et al., 2021). Hence, PTAs not only aid in achieving breeding objectives to reduce twinning, but likely also reduce the recurrence of twinning since it is a heritable trait.

High producing cows may be predisposed to greater incidence and recurrence of twinning. The relationship between twinning and milk production may be influenced by a physiological process that involves the levels of progesterone and estradiol in the bloodstream. Compared with lower producing cows, high-producing cows experience a decrease in circulating progesterone because of increased metabolism of steroids by the liver due to a higher feed intake and greater blood flow to the liver, (Rabiee et al., 2001; Sangsritavong et al., 2002; Wiltbank et al., 2011). The decrease in circulating progesterone disrupts the hypothalamic-pituitary axis's negative feedback mechanism and increasing the secretion of gonadotropin-releasing hormone (Schambow et al., 2021) . The consequent lower levels of progesterone and estradiol in the bloodstream allow for an increase in gonadotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone, resulting in follicular codominance and a higher likelihood of dual ovulation (Lopez et al., 2005; Schambow et al., 2021). The Double Ovsynch protocol utilized to synchronize dairy cows for AI, usually results in two corpus luteus increasing the progesterone concentrations (Ayres et al., 2013), as a result the likelihood of double ovulation can decrease the consequent chances of twinning. Furthermore, given that multiparous cows typically produce more milk than primiparous cows, they are more likely to encounter this disruption in the feedback loop. This is consistent with the observed increase in twinning rates, from 1.0% in primiparous cows to 8.0% in multiparous cows (Ryan and Boland, 1991). Our study did not find evidence of an effect of parity on these outcomes, as both primiparous and their subsequent lactations were analyzed together, combining Prim-Mult and Mult-Mult groups (P = 0.20, Table 3).

2.4.3 Stillbirth

In the analysis of stillbirth incidence across lactations, the current data indicates that the incidence of stillbirth was 6.0% in the current lactation and 3.4% in the subsequent lactation (Table 2). Lithuanian and Russian herds had a stillbirth incidence of 5.37% and 3.33% respectively (Juozaitiene et al., 2017; Pozovnikova et al., 2020).There is a tendency for greater incidence of stillbirth in cows with a history of stillbirth (P = 0.07; Disease History = 5.0% vs. No Disease History = 3.2% ± 0.7%; Table 3; Adj. OR = 1.58 [0.96-2.59] Table 4), regardless of parity. Several factors may be causative of recurrent stillbirth. Calving difficulties such as trauma associated with assisted delivery are responsible for 46.1% of stillbirth incidents, while 31.6% of stillbirths remain unexplained (Berglund et al., 2003). Furthermore, there was a notable genetic correlation between direct effects for stillbirth and calving difficulty at 0.79, and a substantial correlation for their maternal effects at 0.62 (Heringstad et al., 2007). Commonly high incidence of unexplained stillbirths obscures the ability to establish a direct correlation with recurring stillbirths. Genomic research leveraging data from multiple calving of the same cow is vital for extracting genetic insights for stillbirths (Fonseca et al., 2022). For example, at least five functional genes related to fertility and regulation of fetal hemoglobin, which are involved in predicting stillbirth occurrences, may help to identify causes of recurrent stillbirth in the future. Furthermore, In Ayrshire cattle the presence of the AH1-C gene is correlated with an increased occurrence of stillbirth, particularly when bulls carrying this gene are mated with cows also carrying the AH1-C variant (Pozovnikova et al., 2020). Overall, factors related to calving difficulty and certain genetic components appear to be primary causative agents of stillbirth and may be critical in recurrent incidence of the disorder.

2.4.4 Induced Calving

The incidence of induced calving in dairy cows during the current and subsequent lactations was observed at 7.6% (173/2,263) and 10.3% (232/2,263; Table 2), respectively. A history of induced calving significantly increased ($P < 0.001$; Table 3) the likelihood of induced calving in subsequent lactations, with the rates being 16.9% for cows with a history of disease versus 8.5% ± 2.9% for those without, indicating an Adj. OR of 2.19 (1.45-3.33; Table 4), irrespective of the cow's parity. Furthermore, parity alone did not significantly impact the incidence of induced calving in subsequent lactations (Prim-Mult = 11.7% vs. Mult-Mult = 12.5% ± 2.7%; $P = 0.71$; Table 3).

While there is literature available outlining the reasons and outcomes for induction of parturition in dairy cattle (Villarroel and Lane, 2010), to our knowledge there is not data available on incidence across herds. The induction of parturition in this discussion is referred to the use of exogenous corticosteroids in cows with prolonged gestation, with the intention of preventing the negative repercussion of prolonged gestation, such as increase in calf size that can lead to dystocia.

Making assumptions about possible reasons for recurrence of induced calving in dairy cows is complex due to its multifactorial nature and the unpredictable human intervention and the difference in the decision of when the appropriate time is to artificially induce calving. This could be one of the reasons for a high frequency of cows being induced to calve in our study. However, we cannot discard the different biological differences that could be rising the incidence of cows having prolonged gestations. Genetic factors are frequently documented as the primary cause of prolonged gestation (Norman et al., 2009). Other biological contributing factors could include infections, exposure to phytogenous toxins, iatrogenic causes (Mee, 2022). Given that our data likely resulted from management decisions vs. biological responses, drawing conclusions from these data is challenging.

2.4.5 Retained Placenta

The incidence of retained placenta (RP) in the current study was 6.6% (507/7,631) and 6.7% (515/7,631; Table 2) in current and subsequent lactations, respectively. This incidence rate is greater than the national average (4.5%) reported by the USDA National Animal Health Monitoring System's 2014 survey. Having history of RP increased the incidence of RP in the subsequent lactation (P = 0.002; Disease History = 9.5% vs. No Disease History = 6.0% ± 1.1%; Table 3). In addition, incidence of RP in the subsequent lactation was not affected by parity (P = 0.26; Prim-Mult = 7.0% vs. Mult-Mult = 8.2% ± 1.1%; Table 3. The adjusted OR was 1.63 for cows with disease history vs. no disease history (1.20-2.22; Table 4), regardless of parity. This odds ratio indicates that cows with history of RP are 63% more likely to experience the condition in their subsequent lactation when compared to their counterparts without such a history. Previous studies have also underscored the recurring nature of RP which found a similar risk of recurrences (1.7; Markusfeld, 1990).

Several factors such as the duration of pregnancy, hormonal disparities, inflammatory reactions, malfunctioning of the immune system, and metabolic irregularities have been implicated in RP occurrences (Kimura et al., 2003; Moretti et al., 2015; Vieira-Neto et al., 2017; Mahnani et al., 2021).The link between the length of gestation and RP has been established, where they discovered that primiparous cows with shorter gestation lengths had a higher risk of RP compared to those with normal and longer gestation lengths, with incidence rates of 18.0%, 4.5%, and 4.5% respectively. This pattern was also observed in multiparous cows, with RP rates of 28.6%, 3.7%, and 4.6% for short, medium, and long gestations, respectively (Vieira-Neto et al., 2017). Shorter gestation may impede the secretion of hormones vital for birthing, increasing vulnerability to birthing challenges and stillbirths (Kindahl et al., 2002). Conversely, longer gestations could lead

to dystocia due to larger calf sizes (Jenkins et al., 2016; Atashi and Asaadi, 2019), and cows experiencing dystocia are 4.36 times more likely to have RP (Mahani et al., 2021).

The incidence of RP also links to an enhanced pro-inflammatory response, with raised IL-β gene expression observed in affected cows (Ramos et al., 2022). The pro-inflammatory status of the animal itself could increase the risk of recurrence, in other words animals that poses a less regulated inflammatory status could be at more risk of presenting RP. On the other hand, a well-regulated immune response, including the appropriate balance of immune cells and their inflammatory signals, is essential to prevent health related disorders. A study in cows with RP highlighted that an altered expression of certain monocyte/macrophage populations, suggest that an overrepresentation of anti-inflammatory M2 macrophages which may inhibit necessary inflammatory actions for placental expulsion (Nelli et al., 2019). The expulsion of the placenta requires a coordinated activity of matrix metalloproteinases (MMPs), essential for extracellular matrix remodeling. In RP cases, there is a noted decrease in MMP activity, inhibiting placental release (Maj and Kankofer, 1997) , and this could also be related to the genetics of the animal.

The interplay between immune response and hormonal fluctuations is key to placental expulsion. Metabolic stress can trigger hormonal responses that modulate the immune system and affect myometrial contractions, illustrating the impact of hormonal balance on the process of parturition (Mormède et al., 2007; Mordak and Stewart, 2015). The effective expulsion of fetal membranes can be compromised, leading to RP (Kimura et al., 2003). Additional birthing challenges, such as dystocia and twinning, are also risk factors for RP (Hossein-Zadeh and Ardalan, 2011). Kimura et al., (2003) showed an association between low levels of neutrophils in blood before and after parturition with an increase incidence of RP. In the same study, a decrease in interleukin-8 in blood was also identify in animals with RP, suggesting a potential deficiency in

the signaling necessary for neutrophil recruitment and function (Kimura et al., 2003) that could lead to RP. Moreover, immune suppression, infectious diseases, high lipid mobilization, and antioxidant depletion are also associated with the condition (Qu et al., 2014). Tailored diets are not always a possibility on dairy farms, and some outliers can suffer from the formulation considered to be appropriate for most of the herd a consequently suffer with more recurrence of diseases and metabolic conditions. As an example of this, is well understood that antioxidants like vitamin E and β-carotene, along with selenium's role in glutathione peroxidase, are crucial for bolstering the immune system, particularly during parturition. This is evidenced by research that has shown the supplementation of vitamin E and selenium significantly reduces RP rates (Harrison et al., 1984). However, not all the cows have the same requirement for vitamins nor do they have same metabolism and antioxidant demands (Horst et al., 1994) this suggest that some cows are more susceptible to recurrence due do a possible different demand of nutrients and vitamins.

Lastly, genetics may underlie the predisposition to RP, potentially by influencing the biosynthesis and regulation of hormones critical for parturition. For example, it has been noted that cows with RP exhibit lower plasma concentrations of estrogen on the day of parturition, with levels falling below 1,000 pg/mL compared to cows without RP, where levels were at least 1,500 pg/ml (Farzaneh et al., 2006). This suggests a possible genetic linkage between the regulation of estrogen and the occurrence of RP. Although further research is necessary to identify specific genetic markers or pathways that may predispose cows to these hormonal imbalances. Genetic components have also been study by several authors across different herds in the world. Heritability estimations range from to be 0.09 to 0.22 (Hossein-Zadeh and Ardalan, 2011; Benedictus et al., 2013) suggesting that this values varies from herd to herd. Genetic correlations between RP and other reproductive disorders like stillbirth and dystocia were observed, with

values of 0.32 and 0.34, respectively (Pozovnikova et al., 2020). Although is feasible to select against this trait, it might be more effective to select in favor of a stronger immune system (Thompson-Crispi and Atalla, 2014).

2.4.6 Metritis

The incidence of metritis in the current and subsequent lactations was 24.6% (1,874/7,631) and 13.4% (1,021/7,631; Table 2), respectively. The prevalence of metritis in U.S. dairy herds is typically reported to be between 15% and 20% and can vary due to underdiagnosis (LeBlanc, 2008; Vieira-Neto et al., 2016).

In our dataset, history of metritis significantly increased ($P < 0.001$) the incidence of metritis in the subsequent lactation regardless of parity (Disease History = 19.0% vs. No Disease History = 12.2% ± 2.7%; Table 3; Adj. OR = 1.68 [1.44-1.97]; Table 4). This observation aligns with Markusfeld's (1990) findings, which indicated a recurrence risk of 1.7. Moreover, the incidence of metritis in the subsequent lactation was increased ($P < 0.001$) in Prim-Mult compared to Mult-Mult cows (17.5% vs. 13.2% ± 2.7%). An OR of 0.30 for parity in the context of metritis in dairy cows suggests that as parity increases, meaning that cows with higher parity are 70% less likely to develop metritis compared to cows with lower parity, assuming all other factors are constant. This indicates that primiparous cows might be inherently more prone to metritis, primarily due to higher complication rates during parturition than multiparous (Wittrock et al., 2011).

One potential reason for metritis recurrence is the establishment of imbalanced microbiota in the uterus. Jeon and Galvão, (2018) studied the bacteria characterizing the uterus of metritic cows, finding an abundance of Bacteroides, *Porphyromonas*, and *Fusobacterium* species. Healthy cows have a more diverse uterine microbiota than cows with metritis (Santos and Bicalho, 2012),

although, the ideal composition of microbes in the uterus of a postpartum cow has not been identified. Taken together, it is plausible that the cow's natural microbiome may contribute to dysbiosis given challenging circumstances, such as parturition, and may be responsible for recurring metritis in subsequent lactations if a similar profile of microbiota exists after the subsequent parturition.

The metabolic challenges cows face during this period can also increase susceptibility to infectious diseases. A significant positive correlation exists between elevated blood levels of β-hydroxybutyric acid (BHBA) and the proliferation of *Fusobacterium necrophorum* (Jeon et al., 2016), a common causative agent in metritis cases (Jeon et al., 2016). This implies a link between hyperketonemia and metritis, where hyperketonemia may contribute to recurring metritis incidents by compromising immune systems and enhancing the survival and multiplication of *Fusobacterium necrophorum* in those with raised BHBA levels Although this could be a bidirectional scenario where the cow decreases the dry matter intake and worsening the fat mobilization or even leading the cow to hyperketonemia. In our data, cows with hyperketonemia were 5.30 times more likely to have metritis (Table 7). Although the correlation for greater concentration of BHBA in blood and metritis may stem from a reduced DMI in the weeks before parturition and the reduction of DMI due to the infection diseases itself witch demands more energy mobilization (Huzzey et al., 2007). Lower glucose levels and higher concentrations of NEFA and BHBA have been linked to compromised immune defense mechanisms (Hammon et al., 2006). Higher blood glucose levels due to insulin resistance and higher cortisol levels after parturition is also proven to be detrimental for the immune response making the cow more susceptible to uterine infections (Bicalho et al., 2017)

An elevated inflammatory status prior to parturition may contribute to the recurrence of metritis in subsequent lactations, and some cows might be more prone to have an high inflammatory status than others, as a clear example of this IL-6 blood concertation is more than 4 time greater in cows with metritis, period to calving than in healthy cows (Dervishi et al., 2016). Pomeroy et al. (2017) and Sordillo and Raphael (2013) highlighted the overproduction of pro-inflammatory cytokines, such as TNF-α and IL-1β, which exacerbate disease severity. Pomeroy et al. (2017) found that animals with increased levels of CD14+ monocytes pre-parturition, correlated with proinflammatory pathways (Hussen et al., 2013), were more susceptible to post-parturient infections, including metritis, with an odds ratio of 9.03 at 14 days before the due date. Conversely, they noted that an increase of 5 cells/μL in CD14− monocyte count, typically low in circulation, was associated with a proanti-inflammatory cytokine response (Corripio-Miyar et al., 2015), indicating a complex interaction of immune responses in metritis susceptibility and recurrence.

2.4.7 Displaced Abomasum

The observed incidence rates of displaced abomasum (DA) were reported as 0.5% (39/7,631) during the current lactation and 0.7% (54/7,631; Table 2) in the subsequent lactation. This data is underestimated since some cows were culled after their first diagnosis of DA, which can hold to be true for some of the other conditions as well. The literature point to higher incidence of DA, especially in the late 90's where Cameron et al. (1998) found a 6 % incidences across different dairy farms in Michigan and a 3.3 % incidence found in Norway (Stengärde et al., 2011) . In our study, having a DA significantly increased the risk of its recurrence in the next lactation (P = 0.02; Table 4), with incidence rates of 1.4% for cows with a history versus 0.4% for those without, and an Adj. OR of 3.68 (1.21-11.20; Table 4). The interaction between DA history and parity was not analyzed due to the sample's low incidence rate. Additionally, DA incidence was

greater in Mult-Mult than I n Prim-Mult (P= 0.008; Prim-Mult = 0.5% vs. Mult-Mult = 10.8% ± 0.9% ; Table 3).

Identifying reasons for DA recurrence is challenging due to its multifactorial nature. Animals less metabolically adaptable might experience recurrence in the following lactation despite the same dietary and management practices. The relationship between DA and hyperketonemia is well-established, with hyperketonemia acting as both a risk factor and a consequence of DA (Stengärde et al., 2012). The metabolic stress faced by cows around parturition, especially due to low dry matter intake (DMI), forces the animal to mobilize energy from fat storage. This increases NEFA and BHB blood concentrations, which have been associated with an increased risk of DA (LeBlanc, 2010). However, it is important to consider the direction of this relationship, which can be bidirectional and multifactorial. The data in this study reveal that cows with hyperketonemia, which is characterized by high NEFA and BHB levels, have a significantly increased risk of developing DA. Yet, this correlation does not necessarily indicate causation. It is plausible that cows may exhibit higher NEFA and BHB due to lower feed intake, which in turn could predispose them to DA. Alternatively, the development of DA might lead to decreased feeding behavior, subsequently causing an energy deficit that results in the mobilization of NEFA.

In the data analyzed in this study, cows with hyperketonemia were 60.2 times more likely to have DA (95% CI: 29.2 to 145.6; P < 0.001; Table 7). Similarly, in the period from 0 to 6 days before calving, cows with a NEFA concentration of 0.5 mEq/L or higher were found to be 3.6 times more likely to develop left (DA post-calving. For cows with BHBA levels ≥1,200 μmol/L, the odds of LDA were up to 8 times greater (LeBlanc et al., 2005) . Improving the cow's metabolic status through dietary intervention and supplementation can reduce fat mobilization, lowering

blood concentrations of NEFA and BHB, and could reduce the incidence of DA since its relationship is consider bidirectional (Stengärde et al., 2012). Animals at the correct body weight and those better adapted to the metabolic stress of transition may be less prone to DA recurrence (Østergaard and Gröhn, 1999). Thus, proper management of prepartum body condition in dairy cows not only contributes to the immediate reduction in DA incidence but also has a profound impact on their metabolic health and reproductive success (Roche et al., 2007) . Cows that maintain a healthy weight before parturition are less likely to experience severe negative energy balance postpartum, which is a critical factor in metabolic issues (Gillund et al., 2001). These cows tend to have better reproductive performance, which in turn reduces their risk of becoming overweight before the next dry period. This creates a healthy cycle where weight management contributes to better overall health and lowers the risk of DA recurrence. A healthy body score, especially before parturition, and selecting for animals with stronger metabolic adaptation in the herd could reduce the incidence of displaced abomasum. Other identified risk factors for DA recurrence include high levels of milk production (Cameron et al., 1998), insulin resistance affecting smooth muscle contraction in the abomasum, particularly in overweight animals (Pravettoni et al., 2004), and genetic predispositions related to calcium metabolism, insulin resistance, and fat metabolism pathways (Mömke et al., 2013; Huang et al., 2019).

Displaced abomasum has greater heritability (0.22) compared to other health traits, suggesting pathways for genetic selection for this trait (Gaddis et al., 2014; Neuenschwander et al., 2012).

2.4.8 Hypocalcemia

The incidence of clinical hypocalcemia in the current lactation and the following lactation was 0.8% (17/2,263) and 2.9% (65/2,263; Table 2), respectively. Martín-Tereso and Martens

(2014) reported that the prevalence of hypocalcemia tends to be low, typically below 1%. However, Martín-Tereso and Martens (2014) noted a progressive increase with age and lactation number, with a 1% incidence in first-lactation cows, 4% in the second lactation, and a range of 6% to 13% in older cows.

A history of clinical hypocalcemia significantly increased ($P < 0.001$) the likelihood of experiencing clinical hypocalcemia in the subsequent lactation (Disease History = 19.7% vs. No Disease History = 1.8% ± 5.3%; Table 3; Adj.OR = 13.32 [4.72-37.59]; Table 4). The incidence of clinical hypocalcemia in the subsequent lactation was significantly higher ($P < 0.001$) in cows with multiple lactations compared to those transitioning from their first to second lactation (Prim-Mult = 2.4% vs. Mult-Mult = 15.3% ± 3.3%). The interaction between the history of the disease and the number of lactations could not be evaluated due to the low incidence of clinical hypocalcemia in cows during their first lactation. Moreover, with a smaller date set of lactations, Rowlands et al. (1986) found that the chances of recurrences of hypocalcemia double for cows that presented hypocalcemia the previous lactation. The data suggests that previous episodes of hypocalcemia predispose animals to future episodes, especially in the next lactation. The cow's genetic merit may influence the recurrence of hypocalcemia. Cavani et al. (2022) explored the possible genetic traits with a higher risk of hypocalcemia by mapping genes related to calcium metabolism, finding heritability values of 0.083 and repeatability estimates of 0.444 for calcium concentration. Blood calcium concentrations exhibit heritability ranging from 0.23 to 0.32 in the first 8 days postpartum, indicating a stronger genetic component compared to hypocalcemia and suggesting a potential for selective breeding to reduce the risk of this metabolic condition (Tsiamadis et al., 2016). Pacheco et al. (2018) analyzed a wide-ranging genomic to uncover genetic factors contributing to hypocalcemia. Their study incorporated gene mapping and gene-set

analysis, revealing that the risk of hypocalcemia includes a significant genetic component. Key genomic regions were identified on chromosomes BTA6 and BTA16, which explained an important amount of genetic variance in calcium concentration and included genes crucial in vitamin D metabolism and calcium transportation (Pacheco et al. 2018). The identification of these genes could help in the selection of more resilient animal and could also explain the recurrence of hypocalcemia in animals under the same treatment.

The analysis of risk factor identifies dystocia as a potential risk factor for hypocalcemia, although just a tendency has been observed ($P < 0.07$; OR: 1.60 [0.95 to 2.64]; Table 5). Cows with dystocia have a 60% higher risk of developing hypocalcemia compared to cows without dystocia (Table 5). This association suggests a complex interaction where dystocia could either contribute to hypocalcemia due to the exhaustive demand on calcium reserves for muscle contractions during a difficult birth, or conversely, existing hypocalcemia could impair uterine contractions, leading to dystocia. Determining the causal direction of this relationship requires more detailed investigation, possibly involving larger cohorts or longitudinal studies that could provide insight into the temporal sequence of these events.

2.4.9 Hyperketonemia

The incidence of hyperketonemia in the current and subsequent lactations was 8.3% (188/2,263) and 18.6% (421/2,263; Table 2), respectively. The prevalence of clinical hyperketonemia in the collected data is 15.5 % although not all the evaluated herds had kept records of clinical hyperketonemia, the prevalence of clinical hyperketonemia in postpartum has been described to be 23% across western Europe (Berge and Vertenten, 2014).

History of hyperketonemia significantly increased ($P < 0.001$) the incidence of hyperketonemia in the subsequent lactation (Disease History = 32.5% vs. No Disease History =

20.2% ± 13.9%; Table 3 Adj. OR = 1.90 [1.30-2.79]; Table 4), regardless of parity. Markusfeld et al. (1990) and Rowlands et al. (1986) also evaluated the recurrence of hyperketonemia, finding that cows were twice as likely to experience hyperketonemia in the following lactation, which aligns with our data. Moreover, in our data the incidence of hyperketonemia in the subsequent lactation significantly increased ($P < 0.001$) in Mult-Mult cows compared to Prim-Mult cows (Prim-Mult = 18.6% vs. Mult-Mult = 34.7% ± 13.8% Table 3). Others have shown an increase in hyperketonemia rates from 4.6% in second lactation to 9.9% in the sixth lactation (Detilleux et al., 1994).

Hyperketonemia is primarily an issue in high-producing cows and is directly correlated with milk production (Lei and Simões, 2021). Elevated ketone bodies in the blood during the first week of lactation are not only related to high milk production and low DMI but also to an inferior adaptive response to metabolic changes experienced during the transition period (McArt et al., 2012). This highlights the importance of selecting metabolically efficient animals, as elevated ketone bodies are associated with several risk factors. Several factors could influence the recurrence of hyperketonemia in a dairy cow, although the limitations of data collection prevent accessing production data to associate with recurrences. Risk factors for hyperketonemia include the quantity of first colostrum production, BCS, season of calving, length of previous lactation, and dry period length (Vanholder et al., 2015). They estimated that for each 1-liter increase in colostrum production, the likelihood of hyperketonemia grows by factors of 1.2. Cows with a BCS equal to or higher than 4 had an 8.7 times higher risk than thinner cows (BCS ≤ 3). Moreover, cows that gave birth during the last quarter of the year in the northern hemisphere were found to have a lower risk of developing hyperketonemia compared to other times of the year. The data indicates that with each additional day of lactation, the risk of clinical hyperketonemia increases

by 0.5%, and for each extra day in the dry period, the risk of hyperketonemia increases by 2.3% (Vanholder et al., 2015). Awareness of these production factors can help explain some management reasons that may increase the incidence of hyperketonemia and influence diseases associated with this condition. While management can reduce the incidence of hyperketonemia and possibly its recurrence, genetic factors can also increase disease incidence. Soares et al. (2021) discussed genes (ACAT2 and IGF) on chromosomes 6 and 20 related to fatty acid metabolism, lipid metabolism, and inflammatory response, raising the possibility of selecting against these traits to improve herd health. Moreover, Klein et al. (2020) exposed the genetics of hyperketonemia in dairy cows by identifying SNPs associated with milk BHB levels. Their study revealed that genetic variations on bovine chromosomes 4, 10, 11, and 29 significantly influence acetone levels, while chromosomes 1 and 16 impact BHB levels. These SNPs are linked to genes involved in lipid and glucose metabolism pathways. The genetic correlations established between these SNPs and hyperketonemia emphasize the possibility of using genetic selection to breed cows with a lower propensity for this metabolic disorder. By integrating routine measurements of ketone bodies in milk through Fourier-transform infrared spectroscopy with this genetic information, it becomes feasible to enhance herd management and selection strategies. This approach can help identify individuals with a genetic predisposition to hyperketonemia.

The risk of occurrence for ketosis varies significantly depending on several factors, including parity, induced parturition, hypocalcemia, and metritis. Prim-Mult cows exhibited a 18.6% incidence of hyperketonemia, while Mult-Mult groups cows had a significantly higher incidence at 34.7 ± 13.8% ($P < 0.001$; Table 3). The adjusted odds ratio for multiparous cows was 3.67 (95% CI: 2.74–5.01; $P < 0.001$; Table 6). Similarly, in an observational study done in the Netherlands with over 1,700 cows as parity increases the incidence of both subclinical and clinical

ketosis increased by 16 and 4% respectably (Vanholder et al., 2015). Induced parturition increased the risk of ketosis, with an incidence of 21.0% and an adjusted OR of 1.71 (95% CI: 1.25–2.31; P < 0.001; Table 6). Cows experiencing hypocalcemia had a higher incidence of ketosis at 34.9%, with an adjusted OR of 2.81 (95% CI: 1.58–4.87; *P*<0.001; Table 6). Finally, metritis was associated with a significantly higher incidence of ketosis (32.5%) and an adjusted OR of 5.10 (95% CI: 3.97–6.53; P < 0.001; Table 6), indicating a strong association between metritis and subsequent ketosis.

2.4.10 Mastitis

The incidence of mastitis in the current and subsequent lactations was 5.8% (443/7,631) and 9.4% (714/7,631; Table 2), respectively. After evaluating 17,513,429 quarters in different cows from eight commercial dairy herds in Central New York , Alanis et al. (2022) found the monthly incidence rate at the cow level was 4.8 cases per 100 cows. In our data, a history of mastitis was associated with an increase in the incidence of mastitis in the subsequent lactation (*P* < 0.001; Disease History = 16.8% vs. No Diseases History = 8.8% ± 4.8%; Table 3; Adj. OR = 2.10 [1.57-2.80]; Table 4), regardless of parity.

Our recent analysis demonstrated that cows with a history of mastitis in previous lactations were more than twice as likely to have mastitis in the following lactation (Adjusted OR: 2.10 [1.57 to 2.80]; P < 0.001; Table 4). Although there is not data available comparing mastitis in subsequent lactation there is data supporting the recurrence of mastitis within the same lactation. An example of this is the 23% recurrence identified by Abebe et al. (2023) within the same lactation, the results detail that of the 79 cows that developed mastitis during the study period, 18 cows experienced multiple cases of mastitis, either in the same quarter or different quarters. Furthermore, recurrence

within the same lactation was also analyzed by other authors and found that animals without complete bacteriological cure and with somatic cell counts >200,000 were more prone to recurrence 60 days after treating clinical mastitis in a single quarter of cows on Wisconsin farms (Pinzón-Sánchez and Ruegg, 2011). It was also noted that cows with more than three lactations had a lower bacteriological cure rate (60.7%) compared to cows in their first lactation (83.3%), which indicates a potential relationship between parity and the effectiveness of mastitis treatment (Pinzón-Sánchez and Ruegg, 2011)

In our data, the incidence of mastitis in the subsequent lactation was greater in in Mult-Mult compared to Prim-Mult cows ($P < 0.001$; 17.2% vs. 8.6% ± 4.8%; Table 3). Cows in their third or subsequent lactations are up to 15.4 times more likely to have a recurrence of mastitis (Pinzón-Sánchez and Ruegg, 2011). The increased incidence of mastitis in cows with higher parity has been attributed to factors such as the weakening of the sphincter, increased openness of the teat canal, and the relaxation of median ligaments supporting the teat, making the udder more susceptible to the introduction of pathogens (Bhat et al., 2017).

In our data, hyperketonemia and hot seasons were associated with a risk of mastitis by 1.66 and 1.36, respectively ($P< 0.001$ and $P=0.008$; Table 8). As previously discussed, infectious diseases and hyperketonemia are interrelated where it is difficult to determine if one is causative of the other. Since mastitis decreases dry matter intake, cows may be susceptible to mastitis from reduced energy availability for the immune system; conversely, cows with infection have depressed intake which could lead to negative energy balance and hyperketonemia (Sepúlveda-Varas et al., 2016). Some pathogens such as *Streptococcus uberis* and *Escherichia coli* where found to be more prevalent in mastitis cases occurring during the summer (Olde Riekerink et al., 2007) which could be the case of or increased risk for mastitis during the summer. Another risk

factor for mastitis is high milk production; cows producing more than 10,600 kg are 1.5 times more susceptible to mastitis compared to cows producing less than 8,600 kg (Gröhn, 2000), although our data is observational and we do not have the production of the animals across lactation, our elevated risk for recurrence could be related to the high production of some of the evaluated animals.

Most genes associated with mastitis resistance are related to the immune response, highlighting the potential connection between health traits and mastitis recurrence. It has been shown that there is a correlation between innate immune responses and genetics (Sugimoto et al., 2006; Chen et al., 2015), forebrain embryonic zinc finger-like, was linked to lower transcriptional activity and a corresponding reduced expression of immune response genes such as TNF-α and IL-8, following mastitis infection (Sugimoto et al., 2006). Furthermore, a meta-analysis of differentially expressed genes in mammary tissue post-bacterial infection and genome-wide association studies related to somatic cell count, that are key pathways involved in the bovine mammary gland's response to infection, such as granulocyte adhesion and diapedesis, and leukocyte activity regulation pathways where corelted with mastitis resistane (Chen et al., 2015) . The adaptive immune responses are also influenced by genetics, an extensive analysis of immune phenotypes in twins revealed that a significant proportion of immune traits (76%) are heritable (Mangino et al., 2017). Selecting for strong immunity could be an effective strategy, potentially improving resistance to other infectious diseases as well (Thompson-Crispi and Atalla, 2014). Cows with stronger antibody responses were 1.6 to 2.5 times less susceptible to clinical mastitis compared to their peers (Thompson-Crispi et al., 2012). Novel strategies, such as genetic selection for effective antibody and cell-mediated adaptive immune responses, have been shown to have higher heritability than the resistance for mastitis, with heritability ranging from 0.25 to 0.35

(Thompson-Crispi et al., 2012) when compared to the 0.02 and 0.10 found for mastitis resistance (Bloemhof et al., 2009; Koeck et al., 2012).

2.5 Conclusion

A higher risk for recurrence has been observed in all diseases and conditions discussed, including dystocia, twinning, stillbirth, induced calving, retained placenta, metritis, displaced abomasum, hypocalcemia, hyperketonemia, and mastitis. History of these conditions significantly increases the odds of their occurrence in subsequent lactations. A selection again these diseases and metabolic condition is not an easy task due to the low heritability. However, genomics and data gathering could help selecting more robust animals with a stronger immune system, a well-regulated inflammatory response, and a high capacity for metabolic adaptation, such as fat and calcium mobilization. Simultaneously being diligent in the management of the herd and treatment of the Individual may lower the recurrences risk specially in more susceptible animals.

These data can help the producer to make educated decision about keeping animals or discard them, and it can also help the producer in the herd selection, utilizing sire with strong health traits specially in recurrent animals. Furthermore, parity significantly increases the risk of hyperketonemia, while also being a protective factor against metritis. Hypocalcemia is identified as a risk factor that significantly increases the likelihood of hyperketonemia and metritis Additionally, certain health challenges like dystocia, while not statistically significant, are suggested to possibly increase the risk of hypocalcemia. The risk of displaced abomasum is dramatically increased by hyperketonemia, indicating that metabolic disturbances could be more likely due to a bidirectional relationship, where one condition worsens the other. Seasonal variations also appear to influence health, with hot weather increasing the risks of both metritis and mastitis, potentially due to heat stress and its effects on cow immunity and metabolism.

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Table 1. Descriptive statistics according to parity group and farm

	Primiparous				Multiparous			
Item	Herd A	Herd B	Herd C	Herd D	Herd A	Herd B	Herd C	Herd D
Location	FL	FL	CA	CA	FL	FL	CA	CA
Cow-lactations, n	260	716	963	1,488	510	2,059	2,817	4,948
Dystocia, %	18.5	53.6	53.3	28.7	12.4	35.2	25.1	11.8
Twins, %	1.5	0.6	3.2	2.3	2.2	4.8	7.6	7.3
Stillbirth, %	9.2	11.9	10.0	9.3	4.9	2.9	3.5	3.1
Induced calving, %	3.5	12.2	---	---	7.1	9.8	---	---
Calving problem, %	25.0	60.5	34.1	32.4	22.0	42.9	22.0	11.3
Hypocalcemia, %	0.0	0.0	---	---	4.1	2.0	---	---
Hyperketonemia, %	12.3	3.6	---	---	34.7	11.4	---	---
Displaced abomasum, %	1.2	0.7	0.0	0.1	4.5	1.6	0.1	0.0
Retained placenta, %	0.8	5.2	10.9	6.9	3.9	7.3	6.5	6.7
Metritis, %	30.0	14.8	41.0	54.4	20.4	7.9	16.5	12.3
Mastitis, %	18.8	5.6	3.8	2.1	28.0	4.5	6.9	8.5

[1] Cows were classified as having calving problems when they had at least one of the following conditions: dystocia, stillbirth, presence of twins, induction of parturition, and/or abortion.

Table 2. Summary of Disease Incidence in Current and Subsequent Lactations

Item	Current Lactation % (n/n)	Subsequent Lactation % (n/n)
Dystocia	30.2% (1988/6575)	23.5% (795/3379)
Twins	4.5% (344/7617)	6.3% (479/7622)
Stillbirth	6.0% (460/7617)	3.4% (262/7622)
Induced Calving	7.6% (173/2263)	10.3% (232/2263)
Calving Problems	31.0% (2369/7631)	19.1% (1455/7631)
Hypocalcemia	0.8% (17/2263)	2.9% (65/2263)
Ketosis	8.3% (188/2263)	18.6% (421/2263)
Displaced Abomasum	0.5% (39/7631)	0.7% (54/7631)
Retained Placenta	6.6% (507/7631)	6.7% (515/7631)
Metritis	24.6% (1874/7631)	13.4% (1021/7631)
Mastitis	5.8% (443/7631)	9.4% (714/7631)

Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$

Table 3. Incidence of diseases in the subsequent lactation during the postpartum period in Holstein cows that had or not had that same disease in the previous lactation.

	Prim-Mult		Mult-Mult			*P*-value		
Item	Disease	NoDisease	Disease	NoDisease	SEM	Disease	Parity	Disease x Parity
Dystocia	26.8	19.3	24.3	18.3	6.7	< 0.001	0.30	0.73
Twins	9.5	3.5	8.9	6.4	2.1	< 0.001	0.20	0.10
Stillbirth	4.9	2.8	5.1	3.7	0.8	0.07	0.52	0.62
Induced calving	17.6	7.6	16.2	9.5	3.3	< 0.001	0.71	0.41
Calving problem	20.0	16.0	23.2	20.1	6.5	< 0.001	< 0.001	0.49
Hypocalcemia	---	0.6	41.2	4.1	5.3	<0.001	<0.001	---
Hyperketonemia	23.9	14.3	42.4	27.8	13.9	<0.001	<0.001	0.97
Displaced abomasum	---	0.4	13.3	0.9	1.1	0.02	0.008	---
Retained placenta	9.2	5.2	9.7	6.9	1.4	0.002	0.26	0.47
Metritis	16.7	10.4	21.4	14.2	2.8	<0.001	<0.001	0.75
Mastitis	12.0	6.1	23.2	12.5	5.1	<0.001	<0.001	0.97

Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$

Table 4. Adjusted Odds Ratio and incidence of diseases and conditions in current and subsequent lactation

Item	Level	% (n/n)	Adjusted OR (95% CI)	P-value
Dystocia	No	18.9% (1238/6575)	Referent	-
	Yes	25.5% (750/3379)	1.48 (1.21-1.80)	<0.001
Twins	No	4.8% (365/7617)	Referent	-
	Yes	9.2% (42/7622)	2.02 (1.33-3.05)	<0.001
Stillbirth	No	3.2% (230/7617)	Referent	-
	Yes	5.0% (52/7622)	1.58 (0.96-2.59)	0.07
Induced Calving	No	8.5% (192/2263)	Referent	-
	Yes	16.9% (40/2263)	2.19 (1.45-3.33)	<0.001
Calving Problems	No	18.0% (630/3512)	Referent	-
	Yes	21.5% (150/698)	1.25 (1.10-1.43)	<0.001
Hypocalcemia	No	1.8% (40/2263)	Referent	-
	Yes	19.7% (18/91)	13.32 (4.72-37.59)	<0.001
Ketosis	No	8.3% (188/2263)	Referent	-
	Yes	18.6% (421/2263)	1.90 (1.30-2.79)	<0.001
Displaced Abomasum	No	0.4% (20/5000)	Referent	-
	Yes	1.4% (30/2131)	3.68 (1.21-11.20)	0.0215
Retained Placenta	No	6.0% (470/7876)	Referent	-
	Yes	9.5% (45/474)	1.63 (1.20-2.22)	0.002
Metritis	No	12.2% (555/4555)	Referent	-
	Yes	19.0% (75/394)	1.68 (1.44-1.97)	<0.001
Mastitis	No	8.8% (444/5031)	Referent	-
	Yes	16.8% (110/654)	2.10 (1.57-2.80)	<0.001

Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$

Table 5. Risk factors for hypocalcemia

Item	Level	% (n/n)	Univariable		Multivariable	
			OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Parity	Prim	0 (0/965)	Referent		---	---
	Mult	2.5 (63/2,555)	---	---	---	---
Season	Cold	2.0 (44/2,173)	Referent		---	---
	Hot	1.4 (19/1,347)	0.69 (0.39 – 1.17)	0.18	---	---
Dystocia	No	1.5 (35/2,312)	Referent		Referent	
	Yes	1.5 (28/1,208)	1.54 (0.93 – 2.55)	0.09	1.60 (0.96 – 2.64)	0.07
Twins	No	1.8 (61/3,404)	Referent		---	---
	Yes	1.7 (2/116)	0.96 (0.16 – 3.13)	0.96	---	---
Stillbirth	No	1.8 (61/3,327)	Referent		---	---
	Yes	1.0 (2/193)	0.56 (0.09 – 1.81)	0.42	---	---
Induced parturition	No	2.0 (62/3,187)	Referent		Referent	
	Yes	0.3 (1/333)	0.15 (0.01 – 0.69)	0.06	0.14 (0.01 – 0.66)	0.06

[1] Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$.

Table 6. Risk factors for hyperketonemia

Item	Level	% (n/n)	Univariable		Multivariable	
			OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Parity	Prim	6.0 (58/965)	Referent		Referent	
	Mult	16.1 (411/2,555)	3.00 (2.27 – 4.03)	< 0.001	3.67 (2.74 – 5.01)	< 0.001
Season	Cold	13.4 (290/2,173)	Referent		---	---
	Hot	13.3 (179,1,347)	0.99 (0.81 – 1.21)	0.96	---	---
Dystocia	No	14.0 (324/2,312)	Referent		Referent	
	Yes	12.0 (145/1,208)	0.84 (0.68 – 1.03)	0.10	0.77 (0.61 – 0.96)	0.02
Twins	No	13.2 (448/3,404)	Referent		---	---
	Yes	18.1 (21/116)	1.46 (0.88 – 2.32)	0.12	---	---
Stillbirth	No	13.5 (449/3,327)	Referent		---	---
	Yes	10.4 (20/193)	0.74 (0.45 – 1.16)	0.22	---	---
Induced parturition	No	12.5 (399/3,187)	Referent		Referent	
	Yes	21.0 (70/333)	1.86 (1.39 – 2.46)	< 0.001	1.71 (1.25 – 2.31)	< 0.001
Hypocalcemia	No	12.9 (447/3,457)	Referent		Referent	
	Yes	34.9 (22/63)	3.61 (2.10 – 6.06)	< 0.001	2.81 (1.58 – 4.87)	< 0.001
Retained placenta	No	12.6 (419/3,320)	Referent		Referent	
	Yes	25.0 (50/200)	2.31 (1.64 – 3.21)	< 0.001	0.96 (0.64 – 1.40)	0.83
Metritis	No	10.6 (325/3,077)	Referent		Referent	
	Yes	32.5 (144/443)	4.08 (3.24 – 5.13)	< 0.001	5.10 (3.97 – 6.53)	< 0.001

[1] Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$.

Table 7. Risk factors for displaced abomasum

			Univariable		Multivariable	
Item	Level	% (n/n)	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Parity	Prim	0.8 (8/965)	Referent		Referent	
	Mult	2.2 (56/2,555)	2.68 (1.35 – 6.11)	0.009	1.07 (0.51 – 2.53)	0.87
Season	Cold	1.9 (41/2,173)	Referent		---	---
	Hot	1.7 (23/1,347)	0.90 (0.53 – 1.50)	0.70	---	---
Dystocia	No	1.9 (43/2,312)	Referent		---	---
	Yes	1.7 (21/1,208)	0.93 (0.54 – 1.56)	0.80	---	---
Twins	No	1.8 (62/3,404)	Referent		---	---
	Yes	1.7 (2/116)	0.95 (0.15 – 3.08)	0.94	---	---
Stillbirth	No	1.8 (61/3,327)	Referent		---	---
	Yes	1.6 (3/193)	0.85 (0.20 – 2.31)	0.78	---	---
Induced parturition	No	1.9 (59/3,187)	Referent		---	---
	Yes	1.5 (5/333)	0.81 (0.28 – 1.84)	0.65	---	---
Hypocalcemia	No	1.7 (59/3,457)	Referent		Referent	
	Yes	7.9 (5/63)	4.97 (1.69 – 11.72)	< 0.001	2.11 (0.76 – 5.89)	0.15
Hyperketonemia	No	0.2 (7/3,051)	Referent		Referent	
	Yes	12.2 (57/469)	60.2 (29.2 – 145.6)	< 0.001	60.2 (29.2 – 145.6)	< 0.001
Retained placenta	No	1.8 (58/3,320)	Referent		---	---
	Yes	3.0 (6/200)	1.74 (0.66 – 3.77)	0.20	---	---
Metritis	No	1.6 (50/3,077)	Referent		Referent	
	Yes	3.2 (14/443)	1.98 (1.04 – 3.51)	0.03	0.68 (0.35 - 1.25)	0.23

[1] Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$.

Table 8. Risk factors for mastitis

			Univariable		Multivariable	
Item	Level	% (n/n)	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Parity	Prim	9.2 (89/965)	Referent		---	---
	Mult	9.2 (235/2,555)	1.00 (0.77 – 1.29)	0.98	---	---
Season	Cold	8.2 (178/2,173)	Referent		Referent	
	Hot	10.8 (146/1,347)	1.36 (1.08 – 1.71)	0.008	1.32 (1.05 – 1.67)	0.02
Dystocia	No	10.3 (238/2,312)	Referent		Referent	
	Yes	7.1 (86/1,208)	0.67 (0.51 – 0.86)	0.002	0.66 (0.50 – 0.85)	0.002
Twins	No	9.2 (314/3,404)	Referent		---	---
	Yes	8.6 (10/116)	0.93 (0.45 – 1.71)	0.83	---	---
Stillbirth	No	9.4 (314/3,327)	Referent		Referent	
	Yes	5.2 (10/193)	0.52 (0.26 – 0.95)	0.05	0.58 (0.28 – 1.08)	0.11
Induced parturition	No	9.3 (296/3,187)	Referent		---	---
	Yes	8.4 (28/333)	0.90 (0.59 – 1.32)	0.60	---	---
Hypocalcemia	No	9.1 (314/3,457)	Referent		Referent	
	Yes	15.9 (10/63)	1.89 (0.90 – 3.59)	0.07	1.74 (0.81 – 3.35)	0.12
Hyperketonemia	No	8.6 (261/3,051)	Referent		Referent	
	Yes	13.4 (63/469)	1.66 (1.23 – 2.21)	< 0.001	1.49 (1.09 – 2.01)	0.01
Displaced abomasum	No	9.1 (316/3,456)	Referent		---	---
	Yes	12.5 (8/64)	1.42 (0.62 – 2.83)	0.36	---	---
Retained placenta	No	9.5 (314/3,320)	Referent		Referent	
	Yes	5.0 (10/200)	0.50 (0.25 – 0.91)	0.04	0.37 (0.18 – 0.69)	0.004
Metritis	No	8.6 (263/3,077)	Referent		Referent	
	Yes	13.8 (61/443)	1.71 (1.26 – 2.29)	< 0.001	1.88 (1.36 – 2.58)	< 0.001

[1] Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$.

Table 9. Risk factors for metritis

			Univariable		Multivariable	
Item	Level	% (n/n)	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Parity	Prim	18.7 (180/965)	Referent		Referent	
	Mult	10.3 (263/2,555)	0.50 (0.41 – 0.62)	< 0.001	0.30 (0.24 – 0.39)	< 0.001
Season	Cold	11.4 (247/2,173)	Referent		Referent	
	Hot	14.6 (196/1,347)	1.33 (1.08 – 1.62)	0.006	1.40 (1.12 – 1.75)	0.003
Dystocia	No	10.2 (235/2,312)	Referent		Referent	
	Yes	17.2 (208/1,208)	1.84 (1.50 – 2.25)	< 0.001	1.40 (1.11 – 1.76)	0.005
Twins	No	11.8 (401/3,404)	Referent		Referent	
	Yes	36.2 (42/116)	4.25 (2.85 – 6.26)	< 0.001	3.65 (2.28 – 5.80)	< 0.001
Stillbirth	No	11.8 (393/3,327)	Referent		Referent	
	Yes	25.9 (50/193)	2.61 (1.85 – 3.64)	< 0.001	1.65 (1.11 – 2.42)	0.01
Induced parturition	No	11.7 (372/3,187)	Referent		Referent	
	Yes	21.3 (71/333)	2.05 (1.54 – 2.71)	< 0.001	1.44 (1.03 – 2.00)	0.03
Hypocalcemia	No	12.4 (430/3,457)	Referent		Referent	
	Yes	20.6 (13/63)	1.83 (0.95 – 3.30)	0.06	2.02 (0.98 – 3.92)	0.05
Hyperketonemia	No	9.8 (299/3,051)	Referent		Referent	
	Yes	30.7 (144/469)	4.08 (3.24 – 5.13)	< 0.001	5.30 (4.07 – 6.90)	< 0.001
Retained placenta	No	10.3 (341/3,320)	Referent		Referent	
	Yes	51.0 (102/200)	9.09 (6.74 – 12.27)	< 0.001	8.61 (6.16 – 12.06)	< 0.001

[1] Multivariable model included fixed effects from univariable models that resulted in $P \leq 0.10$