EFFECT OF POSTPARTUM UTERINE DISEASES ON MILK YIELD, MILK COMPONENTS, AND REPRODUCTION IN LACTATING DAIRY COWS UNDER CERTIFIED ORGANIC MANAGEMENT

THESIS

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By
Juan M. Piñeiro, DVM
Graduate Program in Comparative and Veterinary Medicine

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Thesis Committee:
Dr. Gustavo M. Schuenemann, Advisor
Dr. William P. Weiss
Dr. Hans Bothe
Dr. Santiago Bas
Abstract

The objective of the present study was to assess the effect of postpartum uterine diseases on milk yield (kg/day), and milk components (percent fat and protein, and SCC). Lactating cows (n = 3,217) from 2 dairy herds were screened for retained placenta (RP; >24 hours after parturition), metritis (MET) within 20 days in milk (DIM), and clinical endometritis (CE) at 26 ± 3 DIM. Milk yield and components from the DHIA test-days up to 305 DIM and reproductive performance at first service were collected. Weekly, a list of cows was obtained using on-farm computer records screened for RP, MET, and CE. Parity (lactations 1, 2 and ≥3) of cows was accounted for milk yield, milk components, and reproduction. The statistical analyses were performed using MIXED (milk yield and components), GLIMMIX (risk factors associated with uterine diseases and reproduction), and CORR (correlation of risk factors with uterine diseases) procedures of SAS. Cows diagnosed with MET or CE had a negative effect on milk yield, increased milk fat percentage and SCC, and reduced reproductive performance at first service. Regardless of parity, lactating cows diagnosed with MET and CE had significantly reduced milk yield (by 2 to 3 kg/cow/day) for at least one of the first 4 DHIA tests (P < 0.05), but no difference was observed in subsequent tests. For the first 2 DHIA tests relative to calving, lactating cows diagnosed with MET and CE had significantly higher SCC and fat content compared with cows without MET and CE (P <
Milk protein content was not different between cows with or without uterine diseases. Cows with MET and CE had increased ($P < 0.05$) DIM to first service and significantly lower ($P < 0.05$) pregnancies to first service compared with cows without MET and CE, regardless of parity. In conclusion, uterine diseases (MET and CE) decreased milk yield and altered milk components (primarily SCC and fat) early in lactation; Furthermore, MET and CE were substantial risk factors for reduced reproductive performance in lactating dairy cows under certified organic management.
This thesis is dedicated to my family in Argentina.

Especially to my nephew, Joaquin, and my grandparents.
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Vita

June 11, 1988

Born – Viedma, Rio Negro, Argentina.

November 21, 2012

DVM; University of La Plata, Argentina.

2013 to present

Graduate Students (MS combined program),

Department of Veterinary Preventive Medicine,

The Ohio State University.

Peer-reviewed abstracts and proceedings


Peer-review publications


Fields of Study

Major Field: Comparative and Veterinary Medicine
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Chapter 1

Introduction

The transition period, often defined as three weeks prior to and after parturition (Grummer, 1995; Drackley, 1999; Galvão, 2013), is characterized by hormonal, metabolic, behavioral, immunological, and physiological changes that occur to adapt the cow from late pregnancy to parturition, and to initiate lactation (Kehrli and Goff, 1989; Grummer, 1995; Drackley, 2001; Huzzey et al., 2007). Some of these changes have been associated with decreased dry matter intake (DMI) that, coupled with and increased demand of nutrients from the mammary gland for colostrum and milk production, lead to a substantial drop in energy (NEB) and minerals, especially calcium (Goff and Horst, 1997; Grummer et al., 2004). Although the underlying cause in the drop of feed intake during the transition period is under investigation, several studies have already shown that the decreased blood calcium concentration and higher circulating concentration of plasma non-esterified fatty acids (NEFA) and β-Hydroxybutyrate (BHBA) lead to an impaired immune response, particularly affecting neutrophils and lymphocytes function (Hoeben et al. 1997; Kimura et al., 2006; Ster et al., 2012). Hypocalcemia and ketosis combined with reduced immune response predispose cows to metabolic and infectious diseases in early lactation, which is the period when most diseases are observed (Kehrli and Goff, 1989; Drackley, 1999).
Transition cow diseases have a negative impact on farm profitability by decreasing milk production, altering milk composition, reducing reproductive performance, and increasing treatment costs and culling rate (Rajala and Gröhn, 1998; Fourichon et al., 1999; Groenendaal et al., 2004; Meadows et al., 2005; Dubuc et al., 2011). Postpartum uterine diseases such as retained placenta (RP), metritis (MET) and clinical endometritis (CE), have been associated with reduced milk yield (Rajala and Gröhn, 1998; Fourichon et al., 1999; Dubuc et al., 2011), decreased reproductive performance (Gröhn and Rajala, 2000; Fourichon et al., 2000; Gilbert et al., 2005) and increased culling (Gröhn et al., 1998; Leblanc et al., 2002; Dubuc et al., 2011). The prevalence of RP, MET and CE in conventional dairies has been reported in previous studies (van Werven et al., 1992; Kelton et al., 1998; Rajala and Gröhn, 1998). However, scant information is available in the literature regarding the prevalence of uterine diseases and their association with on milk yield, milk composition, and reproductive performance in lactating cows under certified organic management. The National Organic Program (NOP) for dairy and livestock requires that cattle are actively grazing and at least 30% of the total dry matter intake (on a daily basis) be provided from grazing pasture for a minimum of 120 days during the growing season. Furthermore, the use of antimicrobials and synthetic substances are prohibited by the NOP. Therefore, the association between uterine diseases and milk yield as well as components was assessed on lactating dairy cows under certified organic management.
2.1. Transition Period

The transition period has been defined as the frame of time that comprehends approximately 3 weeks before and 3 weeks after parturition (Grummer, 1995; Drackley, 1999; Galvão, 2013). This definition may vary among different authors who may consider that the transition period starts at dry-off (mammary involution process) and ends at 30 or 60 days in milk (DIM). Regardless, this is the time when most physiological changes occur to adapt the cow from a non-milk producing pregnant status to parturition and initiation of calostrogenesis and lactation (Grummer, 1995). Immediately following parturition, the requirements for glucose of dairy cows increase by two or three times (Drackley et al., 2001); and most transition health problems occur (Drackley, 1999). Additionally, most lactating dairy cows go through immunosuppression characterized by a reduced neutrophil and lymphocyte functions that last until the second or third week after parturition (Kehrli and Goff, 1989).

Regardless of time frame different authors may use to define the transition period, the understanding of metabolic and immune changes during the periparturient period are paramount to prevent health problems (e.g., metabolic and infectious diseases). One factor leading to the immunosuppression in the periparturient cow is the high nutrient
requirements and decreased dry matter intake (DMI) that leads to deficiencies in blood glucose concentrations, which leads to an exacerbated body fat mobilization and increased plasmatic concentrations of non-esterified fatty acids (NEFA) and ketone bodies (Ster et al., 2012). In addition, as lactation is initiated, calcium is secreted by the mammary gland leading to clinical and subclinical hypocalcemia (Goff and Horst, 1997; Grummer et al., 2004).

Clinical ketosis is defined as a metabolic disease that occurs when cows are in NEB, usually few weeks before parturition or in early lactation, and is characterized by with plasma concentrations of β-Hydroxybutyrate (BHBA) higher than 3,000 µmol/L, decreased appetite, hypoglycemia, weight loss, decreased milk yield, dullness and in some cases neurological signs (Baird, 1982; Oetzel, 2004) Subclinical ketosis is defined as a metabolic disease with plasma concentrations of BHBA higher than 1200 µmol/L without clinical signs (Oetzel, 2004; Ospina et al., 2013). Although the proportion (cut-off point) of lactating cows experiencing subclinical ketosis at a herd level is not well defined, some authors suggested an alarm level of 10% of the cows having a concentration of blood BHBA higher than 1,200 µmol/L as a benchmark (Oetzel, 2004; Ospina et al., 2013).

Similarly, new evidences suggest a higher threshold for total blood calcium (Ca) as indicator of subclinical hypocalcemia. Subclinical hypocalcemia is a metabolic disease defined as low blood serum calcium concentration without the presence of clinical signs
(Reinhardt et al., 2011). Some authors suggest a cut-off point of 8.0 mg/dL (Reinhardt et al., 2011), while other authors suggest 8.5 mg/dL (Goff, 2008) or 8.59 mg/dL (Martinez et al., 2012). Milk fever (clinical hypocalcemia) is defined as a metabolic disease characterized by severe hypocalcemia, anorexia, tetany, recumbence and, if untreated, coma and dead (Horst et al., 1997). Also, it is not well defined which proportion of cows would indicate a problem of hypocalcemia at a herd level, some authors have suggested a cut-off point of ≥30% for parturient hypocalcemia (cows with blood Ca <8.0 mg/dL with or without clinical signs of hypocalcemia) ≥8% for clinical hypocalcemia in multiparous Holstein cows (Oetzel, 2004).

Both metabolic diseases, ketosis and hypocalcemia, blunt the immune system and predispose to infectious diseases (Hoeben et al., 1997; Kimura et al., 2006; Ospina et al., 2013). Increased concentrations of BHBA in plasma reduce the activity of neutrophils predisposing dairy cows to infections in the early postpartum period (Hoeben et al., 1997). Low plasmatic Ca leads to a depletion in the storage of intracellular Ca, which is necessary for signaling activation, and therefore blunts the immune response in peripheral blood mononuclear cells (Kimura et al., 2006).

2.1.1. Dry matter Intake and Metabolic Status

The reduction in dry matter intake (DMI) coupled with increased nutrient and energy requirements in the transition period leads to nutrient deficiencies, NEB and in
some cases metabolic disorders in dairy cows; which results in more diseases, less milk production and less profitability (Drackley, 1999). Required and consumed net energy of a Holstein dairy cow at 4 days in milk (DIM) are approximately 33 Mcal/day and 24 Mcal/day, respectively (Bell, 1995; Drackley, 1999). This difference of 9 Mcal/day, results in a NEB in the first weeks of lactation of dairy cows and leads to body fat mobilization. Approximately 23 of the 33 Mcal/day required, are destined to mammary use, suggesting that most of the energy (approximately 70%) is destined to milk production (Bell, 1995; Drackley, 1999). Moreover, metabolizable protein (MP) consumption is also much lower than the MP requirements, 1.7 and 2.25 kg/d respectively; and 1.4 kg/d of MP goes to milk production (Bell, 1995; Drackley, 1999).

Dry matter intake is regulated by physical, metabolic and hormonal factors (Conrad et al., 1964; Grummer et al., 1990; Goff and Horst, 1997; Grummer et al., 2004). A study that evaluated DMI and digestibility in 114 trials showed that in cows eating a diet with low digestibility physical factors (e.g., gut fill) regulated DMI, while in animals fed diets with a higher digestibility chemostatic factors regulated DMI (Conrad et al., 1964). The increase in the size of the uterus in the late pregnancy and consequent reduction in the volume of the rumen by one-third may be another factor affecting DMI (Goff and Horst, 1997). However, major fetal development occurs during the last trimester of gestation, and the observed reduction in DMI is greater (30% less) 2 days before and 2 days after parturition, suggesting that factors other than a space constraint
and reduced rumen fill might be affecting DMI in the periparturient cow (Goff and Horst, 1997, Grummer et al., 2004). Changes in steroid hormones can be another factor influencing DMI, some authors have suggested that the increased concentrations of estradiol decrease feed intake in periparturient cows (Grummer et al., 1990; Douglas et al., 1998). Additionally, due to the increased body fat mobilization in periparturient cows, the liver is not capable of metabolizing all the non-esterified fatty acids (NEFA) through the tricarboxylic cycle; thus, leading to production of ketone bodies (beta-hydroxybutyrate, BHBA) or storage of these fatty acids in the liver as triglycerides (TG). Excessive increased concentrations of BHBA and TGs can result in clinical or subclinical ketosis and fatty liver, respectively (Goff and Horst, 1997; Grummer, 2004). According to the hepatic oxidation theory (HOT), which states that the hepatic oxidations of fuels (lipids, carbohydrates and amino acids) regulate DMI, the higher mobilization of NEFA and the increased hepatic metabolism of lipids would reduce the firing rate of hepatic vagal afferent nerves and inhibit the hypothalamic feeding centers (Allen et al, 2009). The reduction in the firing rate of the vagus nerve might be due to an increased concentration of ATP in the hepatocyte, which leads to an increased activity of the Na/K-ATPase and K channels, resulting in hyperpolarization of the membranes and consequently reduction of the firing rate of the vagus (Allen et al., 2009).

In addition to the reduction of feed intake, the variability of DMI between cows is considerably higher during the transition period (30 to 40% at 7 DIM) compared with
cows at the peak of lactation (6 to 10%; Drackley, 1999). This variation is usually associated with a higher incidence of diseases in cows in the periparturient period, and the drop in DMI could be the cause or the consequence of the health disorders cows experience during this period. Many authors have found an association between reduction of the feed intake and health disorders. Cows that presented any health disorder in the periparturient period had reduced DMI in the first 20 DIM (13.9 kg/d) compared with the DMI of cows that did not have any health disorder (17.8 kg/d; Wallace et al., 1996). The reduction in DMI started some days before the onset of the health event, suggesting that a reduced supply of propionate and consequent metabolic disorders might have been the cause (Drackley, 1999). Propionate, or propionic acid, is a metabolite produced in the rumen that helps preventing metabolic disorders since it is the principal source for glucose synthesis in the liver through gluconeogenesis; and glucose stimulates the release of insulin, which reduces body fat mobilization and high plasma NEFA concentrations (Drackley, 1999).

Reduction of DMI on periparturient cows seems to be multifactorial, (i.e., gut fill, metabolites, estrogen, corticosteroids, insulin, and leptin) and further research is needed for a better understanding of the factors controlling DMI (Ingvartsen and Andersen, 2000; Grummer et al., 2004). The peripheral signals that regulate feed intake are integrated in the brain, mainly in the arcuate nucleus (ARC), which has first order neurons that will activate second order neurons in the lateral hypothalamic area (LHA)
and paraventricular nucleus (PVN). The ARC contains neurons that release orexigenic neurotransmitters (neuropeptide Y and Agouti-related protein); or anorexigenic neurotransmitters (α-melanocyte stimulating hormone); and the LHA contains neurons that release melanin-concentrating hormone and orexin, which stimulate appetite. These neurons are affected by different peripheral signals such as metabolites (e.g., glucose, VFA, aminoacids), nerve impulses from the digestive tract (e.g., from the vagus due to oxidation of fuels in the liver), hormones (e.g., leptin, insulin), and inflammatory cytokines (Schwartz et al., 2000; Sartin et al., 2011). Controlling DMI is critical to prevent fatty liver and ketosis; however, maximizing DMI in the prefresh period might not be the solution to prevent these metabolic diseases. Some studies have suggested that a feed restricted diet, whether by feeding more fiber or restricting the amount fed, reduces metabolic disorders in periparturient cows (Grum et al., 1996, Douglas et al., 1998, Grummer, 2004), indicating that preventing great changes in DMI close to parturition would reduce body fat mobilization in periparturient cows, and thus prevent metabolic and infectious diseases and fatty liver (Grummer, 2004).

2.1.2. Immune System

During the transition period the functioning of the innate and adaptive immune system is impaired (Mallard et al., 1998). Studies that measured the activity of leukocytes in periparturient cows, showed a reduction in the respiratory burst activity of
polymorphonuclear leukocytes (PMNL) (23% less superoxide anion production), iodination reaction (47% less), and oxidative metabolic capacity (28% less; Kehrli and Goff, 1989), when comparing 2 weeks before and 1 week after parturition.

Lymphocytes function is also affected during the transition period. A reduction in bovine lymphocyte blastogenesis occurs one week after calving (Kehrli and Goff, 1989). In a study that measured the subpopulations of lymphocytes in periparturient cows, it was shown that a decreased in some T-cell populations (e.g., CD3, and CD4) occurs before parturition, reaches a nadir one day after calving, and returned to normal levels at 14 DIM (Kimura et al., 1999). Furthermore, the study suggested that the decrease in the immune cell function during the transition period of dairy cows can be related to the reduction of some T-cell populations, because T-cells play an important role in the immune system (Kimura et al., 1999). Typically, T-helper cells recognize antigens, stimulate the chemotaxis of PMNLs by producing cytokines, activate macrophages and PMNLs, and modulate the humoral and cell-mediated immune response; while T-cytotoxic cells can act as effector cells killing microorganisms and tumor cells (Kimura et al., 1999).

One of the reasons leading to an impaired immune system function can be the increase of plasma cortisol cows experienced at parturition. It has been shown that high concentrations of cortisol affect PMNL function and lymphocytes blastogenesis (Roth et al., 1982). However, the immunosuppression begins many days before calving,
suggesting that other factors rather than the increase of plasma cortisol may be affecting the immune function (Kehrli and Goff, 1989). Nevertheless, cortisol may still affect the magnitude and duration of immunosuppression after parturition (Kehrli and Goff, 1989). High concentration of blood estrogens have also been associated with impaired immune function, specifically estrone, which its concentration increases 1 week before parturition (Kehrli and Goff, 1989). As mentioned before, with the reduction of DMI close to calving, the cause of the immunosuppression in the transition period might be also multifactorial, and changes in plasma concentrations of different hormones (e.g., estrogen, progesterone, prolactin, and somatotropin) might play a role in the immunosuppression of periparturient cows (Kehrli and Goff, 1989).

Additionally, other factors that can be involved in the immunosuppression of periparturient cows are high plasma concentrations of BHBA and NEFA. In vitro studies have shown that concentrations of BHBA of 1 mM and 2.5 mM decrease the respiratory burst of PMNL by 10% and 25%, compared to normal levels of BHBA (0.01 to 1 mM) (Hoeben et al. 1997). However, another study did not found substantial evidence to associate high plasma concentrations of BHBA with an impaired immune function, but did found an association between high plasmatic concentrations of NEFA and decreased production of INF-γ and replication of peripheral blood mononuclear cells (Ster et al., 2012). It is important to note that blood elevated concentrations of BHBA (clinical or subclinical ketosis) within 3-15 DIM in post-partum cows were associated with increased
risk for metritis and displaced abomasum, reduced reproductive performance and increased culling early in lactation (Ospina et al., 2010; McArt et al., 2012). Furthermore, lactating dairy cows with increased serum concentration of NEFA and BHBA post-partum had decreased serum calcium concentrations within the first 7 DIM (Martinez et al., 2012).

Finally, low plasma calcium concentration is another factor leading to immunosuppression during the transition period of dairy cows (Kimura et al., 2006). There is an association between extracellular and intracellular storage of calcium (Kimura et al., 2006). Intracellular calcium is key for activation of immune cells (Grafton and Thwaite, 2001), it seems reasonable that cows that present hypocalcemia are at a greater risk of developing infectious diseases such as metritis (Curtis et al., 1983). There is a drop in dry matter intake around parturition (Drackley et al., 2001); thus, increased NEFA blood concentrations with the subsequent risk for ketosis early in lactation (reviewed by Grummer, 2004). This increased risk for ketosis (BHBA) early in lactation combined with low calcium concentrations lead to increased prevalence of metabolic (e.g., hypocalcemia) and infectious diseases (e.g., metritis) due to increased oxidative stress and impaired immune functions (Martinez et al., 2012).
2.2. Uterine Involution

Uterine involution is a process that occurs during the postpartum period of the cow and last around 40 days (Sheldon, 2004). To become pregnant again, the cow has to go through a process of uterine involution, regeneration and restructuration of the endometrium, elimination of bacteria from the uterus lumen and return to ovarian cyclicity (Sheldon, 2004).

After the expulsion of the fetus (birth of the calf), the third phase of parturition is characterized by the release of fetal membranes (van Werven et al., 1992). This process can take up to 24 hours and if takes longer is consider retained placenta (Eiler, 1997; Kelton et al., 1998).

One factor leading to the expulsion of the fetal membranes is the contractions of the myometrium, induced by oxytocin, which continue for many days after parturition facilitating the elimination of fluid and lochia from the uterus (Noakes et al., 2001). The oxytocin is also released during the process milk let-down; thus, this stimulation is important in the expulsion of the fetal membranes (Noakes et al., 2001). An inflammatory reaction in the endometrium is another factor that facilitates the expulsion of the fetal membranes. Anti-inflammatory drugs such as dexamethasone increase the risk for retained fetal membranes by approximately 53% (Lewing et al., 1985). Moreover, it has been observed that neutrophils from cows with retained placenta have less killing ability and a lower chemotactic activity, measured by myeloperoxidase activity assays and
chemotaxis assays toward cotyledon preparations, respectively (Kimura et al., 2002). Low plasma concentrations of interleukin-8 before calving have been associated with a reduced chemotactic activity of neutrophils and low collagenase secretion to separate cotyledons from the caruncles, and the impaired function of neutrophils was observed before and after calving, suggesting that it is one of the factors leading to retained placenta (Luo et al., 2000, Kimura et al., 2002). Metabolic disorders that blunt the immune system, such as hypocalcemia or ketosis, are also associated with retained fetal membranes (Risco et al., 1994, Ospina et al., 2010). Previous research showed the cows with a more pronounced NEB (measured by higher NEFA serum concentrations) presented 80% more probabilities of presenting RP. Additionally, regardless of the NEFA serum concentrations, dairy cows with lower concentration of vitamin E presented higher concentration of retained placenta (LeBlanc et al., 2004).

After the release of the fetal membranes, the uterus goes through a process of shrinkage, elimination of lochia and bacteria, and restructuring of the endometrium. The uterus shrinks substantially during the first week after parturition: from 2 to 5 DIM, weight is reduced from 10 kg to 4.5 kg, and diameter from 40 cm to 20 cm approximately (Gier and Marion, 1968). Later, it continues to shrink more slowly until it has a weight of 2 kg and a diameter of 10 cm at 15 DIM, and 1 kg and 5 cm at 25 DIM, after this day changes are more difficult to detect (Gier and Marion, 1968). Similarly, the diameter of the cervix reduces very fast during the first days after calving, from 15 cm at 2 DIM to 10
cm at 10 DIM, and more slowly after that until it reaches a diameter of approximately 7 cm at 30 DIM (Gier and Marion, 1968; Maquivar et al., 2015). Under normal conditions, the elimination of lochia, which comes from fetal fluids and necrotic tissue of the caruncles, is completed around 18 DIM, and the regeneration of the endometrium is complete around 42 and 56 DIM (Sheldon, 2004).

All lactating dairy cows have uterine bacterial contamination (e.g., primarily E. coli) during parturition (Sheldon and Dobson, 2004; Sheldon et al., 2008). However, some lactating cows might develop metritis that, along with other metabolic disorders such as clinical ketosis, lead to a delay in the process of uterine involution (Sheldon, 2004) and in some conditions to more severe and life threatening diseases such as puerperal or toxic metritis (Olson et al., 1986).

2.3. Defining Uterine Diseases

Defining uterine diseases is paramount for accurate diagnosis, treatment and record-keeping of calving related events in dairy herds. Retained placenta (RP) can be defined as the presence of the fetal membranes observed outside the vulva or vagina 24 hours after parturition (Kelton et al., 1998). However, this definition may vary according to the author. One study with 1010 cows claimed that RP definition may depend on the age of the cow and should be considered after 6 hours, because at least for cows in their fourth or greater lactation they found increased days to first service (17 d) and increased
days open (26 d; van Werven et al., 1992). This study found that the risk of retained placenta increases with parity and suggested that, if considering RP 6 hours after calving, the incidence could be at least the double (34%) of what we would expect if we just considered a period of time of 24 hours (15%; van Werven et al., 1992). However, 24 hours after calving is the time period most accepted definition of RP by most authors (Kimura et al., 2002; LeBlanc, 2008; Dubuc et al., 2011). Moreover, 95% of cows that have RP for 12 hours after calving will still present RP after 24 hours (van Werven et al., 1992). Lactating dairy cows with RP have an increased risk of developing uterine diseases such as metritis and clinical endometritis (Dubuc et al., 2010a).

Puerperal metritis is the inflammation of all layer of the uterine wall originated from an bacterial infection within the first 21 DIM characterized by a red-brownish, watery and fetid uterine discharge (i.e., enlarged uterus) with systemic signs of illness reduced milked yield and elevated body temperature ($\geq 39.5 \,^\circ C$; Sheldon et al., 2006). If the cow does not present systemic signs, but still present an enlarged uterus and a purulent discharge within 21 DIM, is classified as having metritis (MET, Sheldon et al., 2006). It is interesting to note that metritis compromise all the layers of the uterine wall, whilst endometritis is just the inflammation of the endometrium of the uterus (Bondurant, 1999).

Clinical endometritis (CE) is defined as the inflammation of the endometrial lining of the uterus characterized clinically by mucopurulent or purulent vaginal
discharge, or cytologically by endometrial inflammation occurring 21 to 40 DIM (LeBlanc et al., 2002; Sheldon et al., 2006). Subclinical endometritis is defined as the inflammation of the endometrial lining characterized by the presence of >18% neutrophils in uterine cytology within 34-60 DIM (Gilbert et al., 2005; Sheldon et al., 2006). However, a recent study proposed the term purulent vaginal discharge (PVD) to replace CE; because it has been shown that only 41.7% of the cows that had a purulent vaginal discharge presented cytological endometritis (Dubuc et al., 2010a). In this study, cytological endometritis was defined as the presence of >6% PMNL in endometrial cytology at 35 DIM, and they also found that only 32.1% of cows with cytological endometritis had PVD (Dubuc et al., 2010a). These results suggest that PVD might be a more accurate term than CE, and that the purulent discharge could be a result of a cervicitis or vaginitis and not necessarily the presence of endometritis (Dubuc et al., 2010b). Furthermore, PVD and cytological endometritis should be considered different manifestations of uterine disease. On one hand they share common risk factors (e.g., serum haptoglobin ≥0.8 g/L in the first week postpartum), but hand they differ in some others (e.g., twinning, dystocia and metritis for are risk factors for PVD; while hyperketonemia and low BCS at parturition are risk factors for cytological endometritis). Additionally, there is an additive effect on reproductive performance for both conditions (Dubuc et al., 2010b).
Finally, pyometra is defined as the accumulation of purulent exudate in the lumen of the uterus with the presence of a persistent corpus luteum (CL) and closed cervix (Sheldon et al., 2006). However, the closure of the cervix is not always complete and some uterine discharge is visible within the vagina in cows with pyometra (Sheldon et al., 2006).

2.4. Risk Factors for Uterine Diseases

In recent years, there has been an increased interest from researchers in investigating risk factors leading to uterine diseases. In an epidemiologic study, the authors found that reproductive diseases are interrelated, for instance, abortion and dystocia are risk factors for retained placenta (OR of 4.1 and 16.8, respectively), and RP (along with abortion and dystocia) is also risk factor for MET (OR of 4.4, 3.7, and 3.2, respectively; Gröhn et al., 1990).

In addition to abortion and dystocia, the risk of developing RP also increases with parity (older cows have higher prevalence; Gröhn et al., 1990). Some authors have found an association between hypocalcemia and RP (Risco et al., 1994), suggesting that decreased concentrations of serum calcium before parturition can be another risk factor for retained fetal membranes. Other authors found that hypocalcemia was one of the factors impairing the immune system and leading to the neutrophil dysfunction (Kimura 2006), and that neutrophil dysfunction and lack of interleukin-8 are risk factors for RP.
(Kimura et al., 2002). Therefore, impaired immune function due to low plasma calcium might be one important factor leading to RP. Additionally, increased concentrations of NEFA prepartum also blunt the immune system (Ster et al., 2012), and thus predispose to RP. Some studies have shown that serum concentration of NEFAs higher than 0.37 mEq/L two weeks before parturition is a risk factor for RP (Ospina et al., 2010). As mentioned before in this review, several studies have shown that the use of anti-inflammatory drugs such as dexamethasone (used to synchronize parturition; Lewing et al., 1985) or flunixin meglumine (Duffield et al., 2009) considerably increase the incidence of RP, indicating the important role that the inflammatory reaction has in the expulsion of the fetal membranes.

With regards to MET, risk factors include dystocia, RP, serum concentrations of NEFAs higher than 0.6 mmol/L one week before parturition, serum concentrations of haptoglobin higher than 0.8 g/L within the first 7 DIM (Dubuc et al., 2010b) and hygiene of the perineum at parturition (Schuenemann et al., 2011). It is interesting to note, that the occurrence of dystocia, RP, MET and cystic ovary are also interrelated (Gröhn et al., 1990). For instance, dystocia is a risk factor for RP, MET and cystic ovary while RP is a risk factor for MET, and MET is a risk factor for cystic ovary (Gröhn et al., 1990). More recent studies have shown that giving birth to a dead calf was a risk factor for MET, and that increased concentrations of insulin-like growth factor-1 decreased the risk of retained fetal membranes (Giuliodori et al., 2013a).
Some studies, suggested that the risk factors for CE are twinning, RP, MET, dystocia, high concentrations of prepartum NEFA (>0.45 mM) and postpartum BHBA (LeBlanc et al., 2002; Giuliodori et al., 2013b). Lactating dairy cows experiencing dystocia, births of twins, MET, and serum concentrations of haptoglobin higher than 0.8 g/L within the first 7 DIM, have increased risk for CE or PVD (Dubuc et al., 2010b). With regards to cytological endometritis, the risk factors include reduced BCS at calving, high serum concentrations of BHBA (≥1,100 μmol/L), and haptoglobin (≥0.8 g/L) within the first 7 DIM. (Dubuc et al., 2010b).

The risk factors for pyometra are not well understood. The retention of an active CL and the subsequent failure of luteolysis may be the cause for pyometra with temporary anestrus (reviewed in Sheldon et al. 2006). The progesterone secreted by the CL induces the closure of the cervix and accumulation of purulent exudates within the uterus (Sheldon et al., 2006), which is predominantly caused by T. pyogenes, F. necrophorum and Bacteroides melaninogenicus. (Olson et al., 1984). Since increased concentrations of progesterone predispose to a higher bacterial proliferation in the uterus, another risk factor for pyometra could be an early first ovulation after calving in the presence of a contaminated uterus (Olson et al., 1984). In agreement with this, other researchers found that more lactating cows that have their first ovulation before 25 DIM had prolonged luteal function (18 of 73 cows) and this likely increased the risk for pyometra compared to cows that ovulate between 25 and 45 DIM (0 of 34 cows; Ball and
McEwan, 1998). A delay in uterine involution and uterine infections might affect the production or transportation of PGF$_{2\alpha}$ leading to a persistent CL (reviewed by Wiltbank, 2002). A previous study shown that contamination of the uterus with recognized pathogens is associated with ovarian dysfunction (smaller follicles and CL diameters with reduced plasma progesterone concentration; Williams et al., 2007) and increased risk of developing cysts (follicular and luteal) and CE in postpartum dairy cows (Brick et al., 2012; Maquivar et al., 2015). Therefore, the presence of a persistent and active CL (secreting progesterone) combined with CE lead to the development of pyometra in lactating dairy cows (reviewed by Sheldon et al., 2006).

2.5. Association of Uterine Diseases with Milk Yield and Components

The association of uterine diseases with milk yield and milk components varies considerably in the literature. Perhaps, some of this variation might be due to different definitions of uterine diseases among studies, different adjustments utilized when comparing milk yield (e.g., parity, by milk production in the previous lactation, diseases), the use of different statistical methods to analyze the data, and different time frames to compare milk yield between diseased and control groups (e.g., 100 DIM, 305 DIM, and lactation length).

A study conducted in Finland with 37,776 Ayrshire dairy cows in 2,337 herds analyzed the association of dystocia and uterine diseases for different lactations (1, 2, 3
and ≥4 lactations) and different milk yield levels (Rajala and Ghrön, 1998). In this study, the authors did not find any association of uterine diseases on milk yield when evaluating up to 305 DIM. However, when evaluating by monthly test days, cows with RP produced significantly less milk and it varied, across parity and production level, between 1.7 to 3.5 kg/d less for the first 2 to 6 weeks in lactation (Rajala and Ghrön, 1998). A later review of 35 studies to assess the effect of milk losses due to different health disorders found that in 8 of 13 studies there was not association between RP and milk yield, 2 studies found a small short-term effect (cows with RP produced 12-15 kg/d less), whilst only 3 studies found long-term effects (cows with RP produced 200-250 kg less on average per lactation). Furthermore, 4 studies suggested that lactating cows with RP produced more than control cows when averaging production through all of the lactation. Additionally, in 2 studies these differences might be confound by the lower reproductive performance of lactating cows with RP and likely MET, and subsequently increased milk yield during lactation due to reduced reproductive performance (reviewed by Fourichon et al, 1999).

The definition of metritis from different studies presents a considerable variation that makes the assessment of uterine diseases on milk yield difficult. For instance, in one study the authors did not found any statistical difference when evaluating metritis and CE combined (Rajala and Ghrön, 1998). However, when evaluating these diseases separately, cows diagnosed with vaginal discharge ≥28 DIM did not have any statistical difference on milk yield compared to control cows (Rajala and Ghrön, 1998).
Conversely, lactating cows diagnosed with early metritis (≤28 DIM) produced less milk (between 1.4 to 3.6 kg/d) for the first 2 weeks after calving (Rajala and Ghrön, 1998). This decrease in milk yield was sometimes before the day that the disease was diagnosed, possibly due to effects of other health disorders that occurred previously such as dystocia or RP (Rajala and Ghrön, 1998). On the other hand, the authors did not find differences in milk yield in cows with or without metritis in 8 out of 10 studies (reviewed by Fourichon et al., 1999). However, these results might be confounded by higher culling of cows with metritis or different definitions of uterine diseases (reviewed by Fourichon et al., 1999). Conversely, two studies found a negative association of the occurrence of metritis and milk yield (entire lactation) with reductions of 100 kg for lactating cows diagnosed with early and late metritis combined, and 270 kg for cows diagnosed with early metritis (reviewed by Fourichon et al, 1999).

A more recent study assessed 2,178 Holstein cows in 6 herds enrolled in the Dairy Herd Improvement Association program (DHIA) to determine the effect of uterine diseases on milk yield for the first 4 DHIA tests (Dubuc et al., 2011). The authors did not find any association of uterine diseases with milk yield in primiparous cows (Dubuc et al., 2011). However, multiparous cows diagnosed with metritis produced significantly less milk than control cows for the first DHIA test (3.7 kg/d), but had no significant effect in later tests (Dubuc et al., 2011). The authors included RP in the model to account for the effect of this disease, since it was a confounder. With regards to the effect of RP,
multiparous cows with RP produced significantly less milk (2.6 kg/d) for the first 4 DHIA tests (Dubuc et al., 2011). Additionally, the authors also found a significant reduction in milk yield when evaluating the entire lactation (projected 305 DIM) of cows with RP and metritis (Dubuc et al., 2011). Furthermore, milk yield did not differ in lactating cows diagnosed with or without endometritis at each DHIA tests or projected 305-d milk yield (Dubuc et al., 2011).

Regarding milk components (milk fat and protein), lactating dairy cows diagnosed with metritis had increased milk fat percentage and clinical mastitis (Toni et al., 2011). The ratio of milk fat-to-protein early in lactation is a valuable indicator of the negative energy status of post-partum cows (Toni et al., 2011). Furthermore, there is an association between severe NEB (body weight loss) and increased SCC early in lactation (van Straten et al., 2009). Considering the fact that most lactating dairy cows will likely experience NEB early in lactation, they are more likely to develop metabolic (e.g., ketosis and hypocalcemia) and infectious disorders (e.g., clinical mastitis; Martinez et al., 2012). Therefore, cases of clinical mastitis will likely result in increased milk SCC during early lactation (van Straten et al., 2009).

2.6. Association of Uterine Diseases with Reproductive Performance

The association of uterine diseases with reproductive performance reveals great variation, likely due to different definitions used. Uterine health disorders such as MET
(Dubuc et al., 2010a) and CE (Brick et al., 2012) have been associated with decreased conception to first service and increased pregnancy losses (Brick et al., 2012; Maquivar et al., 2015). In a meta-analysis of seventy studies published between 1987 and 1999, the authors found that lactating cows with RP (defined as failure to expel fetal membranes by 24 hours after parturition) had a delay of 3.5 days to first service and a reduction of conception at first service of 10.3% (Fourichon et al., 2000). In this meta-analysis the authors also found that cows with MET had a delay of 8.2 to 12.4 days to first service and a CR at first service of 21.5% to 28.7% lower compared to cows without MET (Fourichon et al., 2000). Similarly, another study found a reduction on CR of 14% on cows that presented RP and 15% on cows with MET (Gröhn and Rajala, 2000). Additionally, an observational study from the Netherlands with 21 dairy farms found that lactating cows (>3 lactations) with RP had reduced reproductive performance (it took 13 days longer to first service, and conception to first service was 7.3% lower) compared to cows first lactation cows (it took 3 days longer to first service and conception to first service was 6.5% lower; van Werven et al., 1992).

Regarding the effect of CE or PVD on reproductive performance, the outcomes vary depending on the definition used. In a study that defined CE as the presence of >5% neutrophils (low volume uterine lavage between 40-60 DIM), cows with CE had a delay to their first service of 21 days and a conception rate at first service 25% lower compared to cows without CE (Gilbert et al., 2005). A previous study reported that lactating cows
with uterine infections early in lactation had altered ovarian dysfunction (smaller follicles and CL diameters with reduced plasma progesterone concentration; Williams et al., 2007). Furthermore, lactating dairy cows with CE had reduced proportion of CL (Maquivar et al., 2015) and increased proportion of cysts at 26±3 DIM (Brick et al., 2012; Maquivar et al., 2015) with the subsequent reduction of conception to first service and increased pregnancy losses (Brick et al., 2012; Maquivar et al., 2015).

2.7. Statement of the Problem and Rationale

Prevention of disease at the herd level requires a constant effort with effective coordination of the whole system (facilities, feed management, environment, and personnel). Optimum management of transition dairy cows is key to prevent health disorders in postpartum cows and survival with profound implications on profitability of dairy herds.

Metritis significantly reduced herd profitability through decreased milk yield, extended days open, and increased replacement costs (Rajala-Schultz and Gröhn, 1999; Groenendaal et al., 2004; Meadows et al., 2005). During the transition period, most pregnant first-calf heifers and multiparous cows go through a process of NEB and immunosuppression likely due to reduced DMI around parturition and increase energy demand to support lactation (Kehrli and Goff, 1989; Drackley et al., 2001). Previous studies have shown that high plasma concentrations of NEFA, hypocalcemia, and ketosis
significantly increase the risk for uterine diseases in early lactation (Kimura et al., 2002; Ospina et al., 2010; Martinez et al., 2012; Ster et al., 2012). Additionally, these uterine diseases have a negative impact in milk production of lactating dairy cows in conventional systems (Rajala and Ghrön, 1998; Fourichon et al, 1999; Dubuc et al., 2011). However, the association of uterine diseases with milk yield and milk components has not been extensively investigated in lactating dairy cows under certified organic management. Therefore, the objective of the present study was to assess the effect of postpartum uterine diseases (RP, MET, and CE) with milk yield and milk components (percent fat and protein as well as SCC) up to 305 DIM on lactating dairy cows under certified organic management. The hypothesis was that uterine diseases would be detrimental to milk yield and change milk components early in lactation.
Chapter 3

EFFECTS OF POSTPARTUM UTERINE DISEASES ON MILK YIELD, MILK COMPONENTS, AND REPRODUCTION IN DAIRY COWS UNDER CERTIFIED ORGANIC MANAGEMENT
3.1. Abstract

The objective of the present study was to assess the effect of postpartum uterine diseases on milk yield (kg), and milk components (percent fat and protein, and SCC). Lactating cows (n = 3,217) from 2 dairy herds were screened for retained placenta (RP; > 24 hours after parturition), metritis (MET) within 20 days in milk (DIM), and clinical endometritis (CE) at 26 ± 3 DIM. Milk yield and components from the DHIA test-days up to 305 DIM and reproductive performance at first service were collected. Weekly, a list of cows by DIM was obtained using on-farm computer records and screened for RP, MET, and CE. Parity (lactations 1, 2 and ≥ 3) of cows was considered for milk yield, milk components, and reproduction. The statistical analyses were performed using MIXED (milk yield and components), GLIMMIX (risk factors associated with uterine diseases and reproduction), and CORR (correlation of risk factors with uterine diseases) procedures of SAS. Cows with MET and CE had an additive effect on reduced milk yield, increased milk components (percent fat and SCC), and reduced reproductive performance at first service. Regardless of parity, lactating cows diagnosed with MET and CE had significantly reduced milk yield (by 2 to 3 kg/cow/day) for at least one of the first 4 DHIA tests ($P < 0.05$), but was not different at later tests. For the first 2 DHIA tests relative to calving, lactating cows diagnosed with MET and CE had significantly higher SCC and fat content compared with cows without MET and CE ($P < 0.05$). Milk protein content was not different between cows with or without uterine diseases. Cows
with MET and CE had increased \((P < 0.05)\) days to first service and significantly lower \((P < 0.05)\) pregnancy to first service compared with cows without MET and CE, regardless of parity. Uterine diseases decreased milk yield and changed milk components (primarily SCC and fat) early in lactation; and these diseases were a substantial risk factor for reduced reproductive performance.

**Keywords:** Dairy, Organic Management, Uterine Diseases, Milk Yield, Reproduction

### 3.2. Introduction

Optimum management of transition dairy cows (the weeks before and after calving) is critical to avoid metabolic (hypocalcemia and ketosis) and infectious diseases early in lactation and improve overall performance of the herd (LeBlanc et al., 2002; Dubuc et al., 2010). Lactating dairy cows with metritis (MET) and clinical endometritis (CE) had ovarian dysfunction (perturbed follicular growth, smaller CL diameter with reduced plasma progesterone concentration; Williams et al., 2007), increased days to first service, longer time to conception, and increased risk for culling early in lactation (Fourichon et al., 1999; LeBlanc et al., 2002; Dubuc et al., 2010b; Dubuc et al., 2011; Maquivar et al., 2015). Some studies reported a decrease in milk yield on lactating cows diagnosed with retained placenta (RP) compared with cows without RP (Rajala and Ghrön, 1998; Dubuc et al., 2011). However, others authors reported that from a review of
13 studies, in 8 they did not find differences in milk yield, 2 had short-term losses, and 3 had long-term losses on milk yield (reviewed by Fourichon et al., 1999). It is important to note that most studies did not find an association of CE with milk yield throughout lactation (reviewed by Fourichon et al., 1999; Dubuc et al., 2010b). Although it was not statistically significant, lactating dairy cows diagnosed with CE produce more milk than cows without CE (Rajala and Ghrön, 1998), probably due to an impaired reproduction performance or a delay in becoming pregnant.

Uterine diseases, in particular MET, have been associated with reduced feed intake prepartum (Huzzey et al., 2007) and significant losses on milk yield early in lactation (Rajala and Ghrön, 1998; Dubuc et al., 2011). Furthermore, it seems that milk yield losses observed early in lactation for cows with RP may be confined to those cases that progress to MET (LeBlanc, 2008). Additionally, lactating dairy cows diagnosed with MET had increased milk fat percentage and SCC (Toni et al., 2011) for at least the first two DHIA test relative to calving. In dairy herds under conventional management, it is well documented the effect of breed and parity (Schutz et al., 1990), season (Jennes 1985), stage of lactation and negative energy balance (NEB; Shutz et al., 1990; Duffield et al., 1997), nutrition (Armentano and Pereira, 1997), and mastitis (Kitchen, 1981) on milk yield and milk components. However, limited information is available in the literature about the association of uterine diseases with milk components (percent fat and protein, and SCC) of lactating dairy cows managed under certified organic management.
The National Organic Program (NOP) for dairy and livestock requires that cattle are actively grazing and at least 30% of the total dry matter intake (on a daily basis) be provided from grazing pasture for a minimum of 120 days during the growing season. Furthermore, the use of antimicrobials and synthetic substances are prohibited by the NOP. Therefore, the objective of the present study was to assess the effect of postpartum uterine diseases (RP, MET, and CE) on milk yield and milk components (percent fat and protein as well as SCC) up to 305 DIM. The hypothesis was that uterine diseases would be detrimental to milk yield and change milk components early in lactation.

3.3. Materials and Methods

3.3.1. Animals, Facilities, and Feeding Management

Data was obtained from two large certified organic dairies in Colorado that shared the same maternity facility. The herds were enrolled on the national Dairy Herd Improvement Association (DHIA) milk recording program. Data was gathered from January 2012 through August 2013 from 3,227 lactating dairy cows (95% Holstein and 5% Jersey and Holstein cross), where 1,616 were primiparous cows and 1,611 were multiparous cows. Cows were housed in freestall barns with access to a contiguous dry lot, were milked three times a day, and were fed twice a day a total mixed ration (TMR; Table 1) with pushed up of the feed every 2 hours. From May to September they were
allowed to graze 8 hours per day in a mixed pasture (mixture of alfalfa, orchardgrass, and ryegrass, as well as a mixed pasture of sorghum, wheat, and triticale; Table 1).

3.3.2. Calving Related Events and Uterine Diseases

Weekly, a list of cows was obtained based on their calving dates using on-farm computer records (PCDART, Dairy Record Management Systems, Raleigh, NC). Data regarding calving events (e.g., stillbirth, calving difficulty, sex of calf, birth of twins, retain fetal membranes) were obtained from computer records. At 10±3 and 26±3 DIM cows were restrained using headlocks within the pen immediately after milking for diagnosis of MET and CE, respectively (Maquivar et al., 2015). Calving related events and uterine diseases were assessed by farm personnel (calving ease, birth of twins, sex of the calves, stillbirth, RP, MET 10±3 DIM) and veterinarians (CE at 26±3 DIM, Maquivar et al., 2015). All dairy cows had their body condition score (BCS) recorded at 26±3 DIM using a 5-point scale with 0.25 unit increments (Ferguson et al., 1994).

After parturition, the calving ease of cows (assistance provided at birth) was recorded using a 1 to 4 scale (1 = no assistance provided; 2 = light assistance by one person without the use of mechanical traction; 3 = mechanical extraction of the calf with an obstetric calf-puller; and 4 = severe dystocia: surgery or fetotomy needed; Schuenemann et al., 2011). Dystocia was defined as a cow experiencing difficult birth with a calving ease score of ≥2. Additionally, after parturition the birth of single and twin
calves were recorded. Stillbirth was defined as a calf born dead or died within 24 h after birth (from normal gestation length). RP was defined as the presence of the fetal membranes observed externally through the vulva 24 hours after parturition (Kelton et al., 1998). MET was defined as a foul-smelling, brown-red, watery vaginal discharge within the first 21 DIM with or without fever (e.g., rectal temperature ≥39.5 °C, Sheldon et al., 2006). CE was defined as any cow presenting a score of 2 or 3 (mucopurulent or worse vaginal discharge) at examination (Sheldon et al., 2006). The vaginal discharge was scored using a 0-3 scale (0 = normal uterine discharge, 1 = flakes of purulent exudates in the uterine discharge, 2 = >50% of the uterine discharge is made up of purulent exudates, 3 = hemorrhagic uterine discharge mixed with purulent exudates, adapted from Williams et al., 2005; Sheldon et al., 2006).

3.3.3. Milk Yield and Components

Milk yield (kg), somatic cell count (SCC), and percent milk fat and protein content were obtained for each DHIA test day relative to calving up to 305 DIM for all lactating dairy cows. DHIA tests were conducted approximately every 30 days intervals. Data pertaining milk yield and components were obtained from farm records and exported into excel for analyses. To assess the effect of season on milk yield and components, four seasons were defined according to calving date: Winter (calving from
December 21 to March 20), spring (calving from March 21 to June 20), summer (calving from June 21 to September 20), and fall (calving from September 21 to December 20).

3.3.4. Breeding Management

The reported voluntary waiting period was 45 days. The breeding program was based on estrus detection (using tail chalk plus visual observation) twice daily, and all animals presenting signs of standing estrous behavior were inseminated (AI). Cows that did not display estrous behavior or were not pregnant after 5 consecutive AI were turned into a bull pen for natural service. Additionally, open cows at the time of pregnancy diagnosis were reenrolled in the estrus detection program as described previously. All bulls were subjected to a breeding soundness examination once per year, and only bulls with a satisfactory score remained in the herd. Pregnancy diagnosis was performed at 40±3 d post AI via transrectal palpation.

3.3.5. Statistical Analyses

Data from individual lactating dairy cows (e.g., lactation number, DIM, milk yield, milk components, service number, pregnancy status) were exported from PCDART. Before data analyses, enrolled lactating dairy cows that met the exclusion criteria (cows that died or were sold before the first DHIA test day and cows that initiated lactation with an abortion) were removed from the analysis. The prevalence of calving
related events and uterine diseases (dystocia, birth of twins, stillbirth, RP, MET, and CE) were computed using the MEAN procedure of SAS (SAS Institute, 2011). Additionally, the association of cows with and without uterine diseases (RP, MET, and CE) with respect to dystocia, (yes or no), birth of twins (yes or no), stillbirth (yes or no), parity (1, 2, and ≥3), calving season (winter, spring, summer, and fall), and BCS at 26±3 DIM were analyzed using GLIMMIX procedure of SAS (SAS Institute, 2011). A model procedure that included uterine health status (RP, MET, or CE) and variables of interest such as dystocia, birth of twins, stillbirth, parity, calving season, BCS at 26±3 DIM was used to compare differences among groups of cows. Nonsignificant variables were eliminated from the logistic model one at a time using the Wald statistic backward selection criterion (P > 0.15). Herd was included as a random effect. Least squares means were reported. The Tukey-Kramer method was used to perform multiple comparisons of the least squares means. Additionally, data pertaining to days to first service and proportion of cows pregnant at first AI were analyzed using generalized linear mixed models (Proc GLIMMIX; SAS, 2011). A model procedure that included treatment (CON or DEX), parity (primiparous or multiparous), season (winter, spring, summer, and fall), uterine health status (RP, MET, and CE) was used to compare differences among groups of cows.

The FREQ or CORR procedures of SAS was used to assess the correlation (Polychoric and spearman rank correlation coefficients) of calving related events (birth of
twins, stillbirth, and dystocia), BCS at 26±3 DIM, parity, and season with uterine diseases (RP, MET, and CE). The MIXED procedure of SAS was used to assess the effect of uterine health status (RP, MET, and CE) on milk yield (kg/day; Figures 1-2) and milk components (percent fat and protein, and SCC) by parity group (primiparous and multiparous) up to 305 DIM. All first-calf heifers that went through parturition and initiate lactation were considered primiparous. All prepartum dairy cows (lactation 1 or greater) that went through parturition and initiate lactation were considered multiparous. A model procedure that included dystocia, birth of twins, stillbirth, parity, calving season, BCS at 26±3 DIM was used to compare differences among uterine health status (RP, MET, and CE). Nonsignificant variables were eliminated from the logistic model one at a time using the Wald statistic backward selection criterion ($P > 0.15$). Herd was included as a random effect. First order autoregressive, AR(1), covariance structure was used to account for the correlated data structure between the repeated DHIA measurements within animals. Least squares means (±SEM) were reported. The Tukey-Kramer method was used to perform multiple comparisons of the least squares means. A $P < 0.05$ was considered statistically significant and a $P \leq 0.10$ was considered a tendency to differ.

3.4. Results

The prevalence of uterine diseases (RP, MET, and CE) and calving related events by parity (primiparous and multiparous cows) are presented in Table 2.
3.4.1. Risk Factors Associated with Uterine Diseases

The prevalence and the risk factors associated with uterine diseases are presented in Table 3. There was a tendency for season \((P = 0.07)\) to be associated with MET in the summer (16.65\%) compared to the prevalence of MET in the rest of the seasons. On the other hand, stillbirth, birth of twins, and dystocia had a significant effect \((P < 0.05)\) on uterine diseases. Lactating dairy cows experiencing these risk factors had higher prevalence of RP, MET, and CE when compared to cows that did not present these calving related events (Table 3). Additionally, parity had a significant effect \((P < 0.05)\) on uterine diseases with a higher prevalence of RP and MET in lactating cows with \(\geq 3\) lactations compared to primiparous cows (Table 3). Conversely, a higher prevalence of CE was observed in primiparous cows (43.51\%) compared to cows with 2 and \(\geq 3\) lactations (Table 3). With regards to the effect of BCS at 26±3 DIM on uterine diseases, the prevalence of RP was significantly higher for lactating dairy cows with BCS \(\leq 2.5\) compared to cows with BCS of 2.75 or \(\geq 3.0\) (Table 3). Additionally, the prevalence of MET and CE was higher \((P < 0.05)\) in cows with BCS \(\leq 2.5\) compared to cows with 2.75 (Table 3), and the prevalence of both diseases was also higher \((P < 0.05)\) in cows with a BCS of 2.75 compared to \(\geq 3.0\) (Table 3).

The correlation matrix shows that calving related events are significantly associated with uterine diseases (Table 4). RP and MET, for instance, presented the
highest correlation \((r = 0.90)\), followed by dystocia with stillbirth \((r = 0.54)\) and MET with CE \((r = 0.45;\) Table 4). 

3.4.2. Association of Uterine Diseases with Milk Yield

RP did not have a significant effect \((P > 0.05)\) on milk yield. Regardless of parity group, there was a significant reduction \((P < 0.05)\) in milk yield in cows experiencing MET (approximately 2 to 3 kg/cow/day) for the first 4 DHIA test days, but no differences in subsequent tests (Figures 1-2). Additionally, lactating dairy cows experiencing CE had a significant reduction \((P < 0.05)\) in milk yield for the first DHIA test days in primiparous cows, and for the first three DHIA tests in multiparous cows, but there were no significant differences in later tests (Figure 2). Although it was not significant, at the end of lactation this relationship was inverted and lactating cows with CE produced more milk than cows without CE for the last 3 DHIA tests for primiparous and multiparous cows, respectively (Figures 1-2). 

3.4.3. Association of Uterine Diseases with SCC and Milk Fat and Protein

RP was not significantly associated \((P > 0.05)\) with SCC or milk fat and protein percentage, regardless of parity. However, lactating dairy cows with MET had significantly higher SCC \((P < 0.05)\) compared to cows without MET for the first and second DHIA tests relative to calving (Table 5). Similarly, lactating cows with CE had
significantly higher ($P < 0.05$) SCC at the first DHIA test compare to cows without CE (Table 5). No significant differences ($P > 0.05$) in percent milk fat were observed between lactating cows with or without MET (Table 5). However, lactating dairy cows with CE presented significantly higher ($P < 0.05$) percent milk fat for the first DHIA test and significantly lower ($P < 0.05$) percent milk fat for the last five DHIA tests (Table 5). There was no significant association of RP, MET, and CE with percentage milk protein.

3.4.4. Association of Uterine Diseases with Reproductive Performance

As opposed to cows that had RP, lactating dairy cows diagnosed with MET and CE had extended days to first service and reduced pregnancy per AI (PAI) at first service (Table 6). Cows with MET had a delay of 11.9 days more to first service and a reduction in CR of 5.4 percentage points compared to cows without MET (Table 6). Additionally, cows with CE had a delay of 15.3 days more to first service and a reduction in PAI of 12.28 percentage points compared to cows without CE (Table 6).

3.5. Discussion

The objective of the present study was to assess the association of uterine diseases (RP, MET, and CE) with milk yield, and milk components (percent fat and protein and SCC) in lactating dairy cows under certified organic management. The study showed that (1) cows with MET and CE had reduced milk yield for the first 2 or 3 DHIA test relative
to calving; (2) cows with MET and CE had increased percent milk fat and higher SCC for at least the first 1 DHIA test relative to calving; (3) cows with CE had reduced milk fat content during the last 5 DHIA test relative to calving; and (4) cows without MET and CE had increased days to first service and reduced pregnancy at first AI.

In the present study, the overall prevalence of RP, MET, and CE was 7%, 14.7%, and 35.1%, respectively. The prevalence of RP and MET observed in multiparous cows compared to primiparous cows, is similar to those findings reported in dairy cows under conventional management (van Werven et al., 1992; Kelton et al., 1998; Rajala and Gröhn, 1998). However, in the present study, the prevalence of CE was higher in primiparous cows compared to multiparous cows. Although BCS was not assessed at calving, we speculated that this finding might be due to greater body weight loss within 26 DIM in growing primiparous cows (Ettema and Santos, 2004). Additionally, lactating dairy cows with MET and CE had increased days to first service and reduced pregnancy to first service. These findings were also similar to those reported previously for lactating dairy cows under conventional management (Fourichon et al., 2000). In the present study, RP did not have an effect on days to first service or pregnancy to first service, but it was highly correlated with MET. Previous studies have shown that RP is a risk factor for the occurrence of MET (Gröhn and Rajala, 2000); which has been associated with impaired reproductive performance in lactating dairy cows (Dubuc et al., 2011).
Regarding the association of uterine diseases (RP, MET, and CE) with milk yield, in the present study cows with MET had reduced milk yield by 2 to 3 kg/cow/day compared to cows without MET. This finding is in alignment with results reported in previous studies (Rajala and Ghrön, 1998; Dubuc et al., 2011). This reduction in milk yield might be partially due to a reduction in DMI and increased NEB of lactating cows experiencing MET (Huzzey et al., 2007). Previous studies have shown that lactating dairy cows with severe MET consume 1.8 to 6 kg of DM per day less within 2 weeks from calving compared to cows without MET (Huzzey et al., 2007). Therefore, the increase of energy demands from the mammary gland to support lactation, coupled with a drop in DMI exacerbates the NEB early in lactation with the subsequent reduced neutrophil functions due to elevated BHBA and reduced calcium concentrations in blood, thus, predisposing cows to infectious diseases in the early lactation (Hoeben et al. 1997; Martinez et al., 2012). Furthermore, CE affected milk yield in primiparous (first DHIA test) and multiparous (first 3 DHIA tests) cows, when compared to cows without CE. Although previous studies reported a significant reduction in milk yield in cows with RP (Rajala and Gröhn, 1998; Dubuc et al., 2011), in the present study RP did not have a significant effect on milk yield throughout lactation.

Milk components (percent fat and protein, and SCC) are important to obtain top quality milk products and dairy producers are usually paid their milk based on these components. Lactating dairy cows with MET (first 2 DHIA test) and CE (first DHIA test)
had significantly higher SCC when compared to cows without uterine disease. SCC is an indicator of mammary inflammation (mastitis) and share common risk factors with MET and CE (e.g., low plasma calcium after calving and higher NEFA and BHBA pre and postpartum, respectively). Therefore, we speculate that this is partially due to cows with MET, CE and mastitis sharing common risk factors that blunt the immune system. A meta-analysis has shown that cows that consumed monensin had a decreased risk of clinical ketosis, displaced abomasum and mastitis, likely due to lower concentrations of NEFA and BHBA (Duffield et al., 2008).

Many factors affect milk components in Holstein dairy cows. For instance, the stage of lactation is significantly associated with milk components. Higher concentration of milk fat and protein is observed in early lactation, followed by a decrease to a nadir at 50-60 DIM and a steady increase until the end of lactation (Schutz et al., 1990). Additionally, nutrition management alters milk fat percentage because it is positively correlated with forage NDF consumption (Armentano and Pereira, 1997). Furthermore, there is a clear seasonal drop in milk fat content during summer, especially in lactating cows grazing (Jennes 1985). Finally, milk component are affected by genetic variation within and between breeds (Schutz et al., 1990). In the present study, lactating dairy cows with CE had increased milk fat percentage for the first DHIA test compared to cows without CE. Perhaps, this might be related to a more pronounced and prolonged NEB in cows that presented CE compared to healthy cows (Duffield et al., 1997; Toni et al.,
It is important to note that this relationship was inverted throughout lactation, and that CE cows presented a decrease in milk fat percentage for the last 5 DHIA tests relative to calving compared to cows without CE. The lower milk fat content in non-CE compared to CE cows later in lactation has not been reported in previous studies. Although BCS was not assessed later in lactation, the prevalence of MET and CE was significantly higher in lactating dairy cows that had lower body condition score in early lactation (BCS ≤ 2.75 at 26±3 DIM). Therefore, we speculate that milk fat percentage later in lactation may be lower in those lactating cows diagnosed with CE since they had a lower BCS compared to cows without CE. Additionally, although it was not statistically significant, CE cows had a higher milk yield for the last 4 DHIA tests which might contribute to decrease fat percentage as previous studies have already shown that milk fat percentage decreases as milk yield increases (Schutz et al., 1990). The higher milk fat percentage in CE cows for the first DHIA test does not imply higher milk fat yield (kg). Actually, milk fat for CE cows was numerically lower compared to the milk fat yield in non-CE cows.

In conclusion, RP was not significantly associated with milk yield and components, but it was the greatest risk factor for MET. MET and CE were significantly associated with a decrease on milk yield for the first four DHIA tests relative to calving, regardless of parity. Additionally, MET and CE were significantly associated with higher SCC for at least the first DHIA test relative to calving. Lactating dairy cows experiencing
CE had higher percent fat for at least the first DHIA test compared to non-CE cows while percent milk protein was unchanged for all uterine diseases investigated. Finally, MET and CE were detrimental to reproductive performance of lactating dairy cows by increasing days to first service and decreasing pregnancy to first service. The effect of uterine diseases on milk components, primarily milk fat and its composition of fatty acids, warrants further investigation accounting for the effect of diets, feed management, and season.

3.6. Acknowledgments

The authors would like to acknowledge the collaborating dairy farm and their staff for providing the animals used in this study and their assistance during the project. Also, the authors thank Drs. M.G. Maquivar and A.A. Barragan for their assistance with field data collection. This project was partially supported by OSU Veterinary Extension, the USDA-Animal Health Formula Fund (Washington, DC), and Aurora Organic Farms (Boulder, CO).
### 3.6. Tables and Figures

Table 1. Ingredients and nutrient composition of pre- and postpartum diets (DM basis).

<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>Prepartum</th>
<th>Early Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass hay</td>
<td>45.12</td>
<td>5.51</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>-</td>
<td>0.95</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>25.72</td>
<td>28.73</td>
</tr>
<tr>
<td>Corn silage</td>
<td>10</td>
<td>16.07</td>
</tr>
<tr>
<td>Grain mix</td>
<td>15.93</td>
<td>46.14</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.19</td>
<td>2.49</td>
</tr>
</tbody>
</table>

**Nutrient Profile**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Prepartum</th>
<th>Early Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE(_L), Mcal/kg</td>
<td>1.30</td>
<td>1.62</td>
</tr>
<tr>
<td>CP, %</td>
<td>11.78</td>
<td>18.8</td>
</tr>
<tr>
<td>NDF, %</td>
<td>45.79</td>
<td>28.39</td>
</tr>
<tr>
<td>ADF, %</td>
<td>30.78</td>
<td>21.23</td>
</tr>
<tr>
<td>Starch, %</td>
<td>13.79</td>
<td>26.69</td>
</tr>
<tr>
<td>Ca, %</td>
<td>1.43</td>
<td>1.33</td>
</tr>
<tr>
<td>P, %</td>
<td>0.34</td>
<td>0.39</td>
</tr>
<tr>
<td>Mg, %</td>
<td>0.42</td>
<td>0.36</td>
</tr>
<tr>
<td>K, %</td>
<td>1.47</td>
<td>1.28</td>
</tr>
<tr>
<td>Na, %</td>
<td>0.25</td>
<td>0.42</td>
</tr>
<tr>
<td>Cl, %</td>
<td>1.10</td>
<td>0.38</td>
</tr>
<tr>
<td>S, %</td>
<td>0.36</td>
<td>0.23</td>
</tr>
<tr>
<td>DACD, mEq/100g DM</td>
<td>-5.3</td>
<td>25.6</td>
</tr>
</tbody>
</table>

The dietary cation-anion difference (DCAD) was calculated as follows:

\[
\text{DCAD} = (\text{mEq of Na} + \text{mEq of K}) - (\text{mEq of S} + \text{mEq of Cl}).
\]
Table 2. Mean prevalence (%) of calving-related events and BCS at 26±3 DIM in primiparous and multiparous Holstein cows.

<table>
<thead>
<tr>
<th>Variable†</th>
<th>Primiparous (n = 1,612)</th>
<th>Multiparous (n = 1,605)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystocia1, %</td>
<td>7.38</td>
<td>10.7</td>
</tr>
<tr>
<td>Birth of twins2, %</td>
<td>0.5</td>
<td>2.75</td>
</tr>
<tr>
<td>Stillbirth3, %</td>
<td>5.02</td>
<td>4.74</td>
</tr>
<tr>
<td>Retained Placenta (RP)4, %</td>
<td>5.19</td>
<td>8.71</td>
</tr>
<tr>
<td>Metritis5, %</td>
<td>12.91</td>
<td>16.42</td>
</tr>
<tr>
<td>Clinical Endometritis (CE)6, %</td>
<td>43.34</td>
<td>26.92</td>
</tr>
<tr>
<td></td>
<td>&lt;2.75</td>
<td>315 (19.5)</td>
</tr>
<tr>
<td></td>
<td>≥3.0</td>
<td>543 (33.7)</td>
</tr>
<tr>
<td>BCS at 26±3 DIM7, n (%)</td>
<td>2.75</td>
<td>754 (46.8)</td>
</tr>
<tr>
<td></td>
<td>≥3.0</td>
<td>543 (33.7)</td>
</tr>
</tbody>
</table>

†The prevalence of dystocia, birth of twins, stillbirth, retained placenta, metritis and clinical endometritis were recorded for Holstein dairy cows (n = 3,217) from calving to 26±3 DIM.

1Dystocia was defined as a cow experiencing difficult birth with a calving ease score of ≥2. After parturition, the calving ease of cows (assistance provided at birth) was recorded using a 1 to 4 scale (Schuenemann et al., 2011).

2After parturition, the birth of single and twin calves were recorded.

3Stillbirth was defined as a calf born dead or died within 24 h after birth (from normal gestation length).

4Retained placenta was defined as the presence of the fetal membranes observed externally through the vulva 24 hours after parturition (Kelton et al., 1998).

5Metritis was defined as a foul-smelling, brown-red, watery vaginal discharge within the first 21 days in milk with or without fever.

6Clinical endometritis was defined as any cow presenting a score of 2 or 3 (mucopurulent or worse vaginal discharge) at examination. The vaginal discharge was scored using a 0-3 scale (adapted from Williams et al., 2005; Sheldon et al., 2006).

7Body condition scores (BCS) were recorded at 26±3 DIM using a 5-point scale (Ferguson et al., 1994).
Table 3. Prevalence (%) and risk factors associated with uterine diseases (RP, MET, and CE).

<table>
<thead>
<tr>
<th>Variable†</th>
<th>Level</th>
<th>Cows, n</th>
<th>RP, %</th>
<th>P-value</th>
<th>MET, %</th>
<th>P-value</th>
<th>CE, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dystocia</td>
<td>Yes</td>
<td>284</td>
<td>11.6a</td>
<td>0.003</td>
<td>29.33a</td>
<td>&lt;0.0001</td>
<td>59.21a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>2,848</td>
<td>7.36b</td>
<td>-</td>
<td>13.44b</td>
<td>-</td>
<td>32.96b</td>
<td>-</td>
</tr>
<tr>
<td>Birth of twins</td>
<td>Yes</td>
<td>53</td>
<td>15.09a</td>
<td>0.03</td>
<td>39.62a</td>
<td>&lt;0.0001</td>
<td>58.49a</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3,132</td>
<td>7.12b</td>
<td>-</td>
<td>14.43b</td>
<td>-</td>
<td>34.95b</td>
<td>-</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>Yes</td>
<td>155</td>
<td>13.54a</td>
<td>0.02</td>
<td>31.61a</td>
<td>&lt;0.0001</td>
<td>60.92a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>3,030</td>
<td>6.93b</td>
<td>-</td>
<td>13.99b</td>
<td>-</td>
<td>34.02b</td>
<td>-</td>
</tr>
<tr>
<td>Parity</td>
<td>1</td>
<td>1,612</td>
<td>5.64b</td>
<td>0.005</td>
<td>13.09b</td>
<td>0.007</td>
<td>43.51a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>≥3</td>
<td>754</td>
<td>10.08a</td>
<td>-</td>
<td>17.90a</td>
<td>-</td>
<td>26.12b</td>
<td>-</td>
</tr>
<tr>
<td>Calving season</td>
<td>Winter</td>
<td>646</td>
<td>8.2</td>
<td>0.74</td>
<td>12.22</td>
<td>0.07</td>
<td>34.97</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>1,153</td>
<td>7.19</td>
<td>-</td>
<td>14.39</td>
<td>-</td>
<td>33.77</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>1,063</td>
<td>6.77</td>
<td>-</td>
<td>16.65</td>
<td>-</td>
<td>36.5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fall</td>
<td>355</td>
<td>7.32</td>
<td>-</td>
<td>16.05</td>
<td>-</td>
<td>39.22</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&lt;2.75</td>
<td>638</td>
<td>9.56a</td>
<td>0.01</td>
<td>24.13a</td>
<td>&lt;0.0001</td>
<td>54.56a</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BCS at 26±3 DIM</td>
<td>2.75</td>
<td>1,420</td>
<td>7.39ab</td>
<td>-</td>
<td>14.36b</td>
<td>-</td>
<td>36.49b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>≥3.0</td>
<td>1,159</td>
<td>5.86b</td>
<td>-</td>
<td>10.44c</td>
<td>-</td>
<td>23.65c</td>
<td>-</td>
</tr>
</tbody>
</table>

†Lactating dairy cows (n = 3,217) were screened for retained placenta (RP), metritis (MET), and clinical endometritis (CE) within 26±3 DIM.
Table 4. Correlation matrix showing the association among variables (*P*-values in parenthesis).

<table>
<thead>
<tr>
<th>Item†</th>
<th>RP 1 (-)</th>
<th>MET 1 (-)</th>
<th>CE 1 (-)</th>
<th>Dystocia 1 (-)</th>
<th>Birth of twins 1 (-)</th>
<th>Stillbirth 1 (-)</th>
<th>Parity 1 (-)</th>
<th>Season 1 (-)</th>
<th>BCS at 26±3 DIM 1 (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP</td>
<td>0.906 (0.014)</td>
<td>0.304 (0.039)</td>
<td>0.153 (0.052)</td>
<td>0.176 (0.082)</td>
<td>0.179 (0.052)</td>
<td>0.146 (0.031)</td>
<td>-0.028 (0.031)</td>
<td>-0.050 (0.004)</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>0.452 (0.021)</td>
<td>0.294 (0.04)</td>
<td>0.315 (0.051)</td>
<td>0.286 (0.049)</td>
<td>0.055 (0.001)</td>
<td>0.064 (0.021)</td>
<td>-0.132 (0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE</td>
<td>0.332 (0.037)</td>
<td>0.236 (0.042)</td>
<td>0.311 (0.025)</td>
<td>-0.243 (0.12)</td>
<td>0.027 (0.012)</td>
<td>-0.229 (0.0001)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dystocia</td>
<td>0.501 (0.031)</td>
<td>0.543 (0.042)</td>
<td>0.108 (0.035)</td>
<td>-0.026 (0.031)</td>
<td>-0.026 (0.004)</td>
<td>-0.062 (0.0004)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth of twins</td>
<td>0.481 (0.070)</td>
<td>0.295 (0.051)</td>
<td>-0.016 (0.34)</td>
<td>-0.076 (0.0001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td>0.042 (0.013)</td>
<td>0.027 (0.03)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1 (-)</td>
<td>0.007 (0.11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td>1 (-)</td>
<td>-0.063 (0.0003)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Lactating dairy cows (n = 3,217) were screened for retained placenta (RP), metritis (MET), and clinical endometritis (CE) within 26±3 DIM.
Figure 1. Effect of metritis (MET) on milk yield (kg) in primiparous and multiparous Holstein lactating dairy cows.

*Values marked with asterisk within each DHIA test day differ significantly at $P < 0.05$. Multiparous lactating dairy cows ($n = 1,605$) were screened for metritis (MET) and milk yield (kg) were recorded at each DHIA test day relative to calving up to 305 DIM.
Figure 2. Effect of clinical endometritis (CE) on milk yield (kg) in primiparous and multiparous Holstein lactating dairy cows.

*Values marked with asterisk within each DHIA test day differ significantly at $P < 0.05$. Primiparous lactating dairy cows ($n = 1,612$) were screened for clinical endometritis (CE) and milk yield (kg) were recorded at each DHIA test day relative to calving up to 305 DIM.
Table 5. Effect of metritis (MET) and clinical endometritis (CE) on milk fat and somatic cell count (SCC) in primiparous and multiparous lactating dairy cows.

<table>
<thead>
<tr>
<th>Item</th>
<th>Status</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCC</td>
<td>Non-MET</td>
<td>195</td>
<td>115</td>
<td>105</td>
<td>108</td>
<td>124</td>
<td>123</td>
<td>130</td>
<td>144</td>
<td>154</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>±10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>±8</td>
<td>±7</td>
<td>±8</td>
<td>±10</td>
<td>±13</td>
<td>±10</td>
<td>±15</td>
<td>±15</td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>302</td>
<td>163</td>
<td>120</td>
<td>135</td>
<td>141</td>
<td>135</td>
<td>140</td>
<td>148</td>
<td>171</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>±25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>±19</td>
<td>±18</td>
<td>±21</td>
<td>±21</td>
<td>±26</td>
<td>±23</td>
<td>±30</td>
<td>±25</td>
</tr>
<tr>
<td>Fat</td>
<td>Non-MET</td>
<td>3.65</td>
<td>3.33</td>
<td>3.11</td>
<td>3.15</td>
<td>3.20</td>
<td>3.25</td>
<td>3.36</td>
<td>3.45</td>
<td>3.53</td>
<td>3.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.05</td>
<td>±0.03</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.01</td>
<td>±0.02</td>
</tr>
<tr>
<td></td>
<td>MET</td>
<td>3.79</td>
<td>3.32</td>
<td>3.11</td>
<td>3.16</td>
<td>3.19</td>
<td>3.25</td>
<td>3.33</td>
<td>3.43</td>
<td>3.47</td>
<td>3.57</td>
</tr>
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<td>±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a,b</sup>Values with different superscript letters within a row differ significantly at *P* < 0.05.

1Lactating dairy cows (*n* = 3,217) were screened for metritis (MET) and clinical endometritis (CE). All lactating cows had their SCC (*x*10³ cells/mL) and percent fat recorded at each DHIA test day relative to calving up to 305 DIM.
Table 6. Effect of uterine diseases (retained fetal membranes, metritis, and clinical endometritis) on reproductive performance of Holstein lactating dairy cows (all parities combined).

<table>
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<th>Variable†</th>
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<th>No</th>
<th>P-value</th>
<th>Yes</th>
<th>No</th>
<th>P-value</th>
<th>Yes</th>
<th>No</th>
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<td>DIM to first AI, d</td>
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<td>36.2</td>
<td>0.96</td>
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<td>41.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

<sup>a,b</sup>Values with different superscript letters within a row differ significantly at P < 0.05.

†The prevalence of retained placenta (RP), metritis (MET), and clinical endometritis (CE) were recorded for primiparous Holstein dairy cows (n = 3,217) from calving to 26±3 DIM. The effect of RP, MET, and CE on days in milk (DIM) at first AI and pregnancy at first AI were assessed.
Chapter 4

Summary and Conclusions

Milk sales are the main source of income for dairy operations. However, not only milk yield will determine the profitability of the farm, also milk components (fat and protein, and SCC) would be crucial, since dairy producers are paid based on SCC as well as milk fat and protein content. Although there is variation among different studies regarding the effect of postpartum uterine diseases in milk yield, most studies available in the literature found a strong association of MET with decreased milk yield for the firsts DHIA tests relative to calving (Rajala and Gröhn, 1998; Fourichon et al., 1999; Dubuc et al., 2011). Additionally, other studies have found that MET is associated with a decreased in DMI that starts 2 weeks before cows were diagnosed with the MET (Huzzey et al., 2007). The present study showed that uterine diseases (MET and CE) are significantly associated with decreased milk yield, increased fat percentage (but numerically lower milk fat yield) and increased SCC for at least one of the first four DHIA tests. Moreover, although RP did not have a significant effect on milk yield, milk components or reproductive performance, it was the greatest risk factor for MET. Conversely, MET and CE did have an negative impact on reproductive performance decreasing conception at first service and increasing DIM to first service. Overall, the prevalence of uterine diseases (RP, MET and CE) in cows under certified organic management did not differ
from the prevalence reported in previous studies for conventional systems (van Werven et al., 1992; Kelton et al., 1998; Rajala and Gröhn, 1998; LeBlanc et al., 2002). Lactating dairy cows can achieve acceptable performance (milk yield and reproduction) without using synthetics hormones or anionic salts in pre-partum rations (to prevent hypocalcemia). Additionally, further research measuring serum NEFA and BHBA concentrations and immune response (e.g., neutrophils concentration, phagocytosis, and activity burst) would be necessary to investigate which factor would explain the association between uterine diseases with milk yield, milk fat percentage and milk quality (SCC). Moreover, in view of the growing consumer interest in milk fat composition of lactating cows under certified organic management, the potential benefits on milk fat composition requires further studies by considering the performance of lactating cows (e.g., health and milk yield), season, and nutrition management (pre- and post-partum diets).
References


