AUTOMATED MONITORING OF DAIRY COW HEALTH AND IMPACT OF POSTPARTUM VOLUNTARY WAITING PERIOD DURATION ON DAIRY COW PHYSIOLOGY AND HERD PERFORMANCE

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ABSTRACT

The objectives of the studies presented in Section I were to evaluate: the performance of an automated health-monitoring system (**AHMS**) that combines rumination time and physical activity into an alert system (i.e., health index score, **HIS**) for identification of cows with health disorders; the timing of alerts in relationship to clinical diagnosis (**CD**) of disorders; and the patterns of rumination time, physical activity, and HIS around CD of disease. The objectives of the experiments presented in Section II were: evaluating the reproductive performance, herd exit dynamics, lactation performance, and profitability of dairy cows managed with different duration of the voluntary waiting period and methods of submission for first service.

The studies presented in Section I demonstrated that the sensitivity of the HIS to identify cows with health issues was high for metabolic and digestive disorders (93%) and moderate for cases of clinical mastitis (55%) and metritis (55%). Among mastitis cases, HIS was effective for identifying cows with mastitis caused by *Escherichia coli* (80.7%), but it was less effective in identifying cows with mastitis caused by other pathogens (45 to 48%). Cows diagnosed with metritis and flagged based on HIS had substantial alterations of their rumination, activity, and HIS patterns around CD, suggesting that the AHMS was effective for identifying cows with severe cases of metritis, but less effective for identifying cows with mild cases of metritis. The overall accuracy of the AHMS alerts combining rumination and activity indicated that it could be a useful tool for identifying cows with metabolic and digestive disorders, and more severe cases

of mastitis and metritis.

Experiments presented in Section II demonstrated that extending duration of VWP from 60 to 88 days increased pregnancies per artificial insemination to first service, but delayed time to pregnancy during lactation and increased the risk of leaving the herd, particularly for multiparous cows. This shift in pregnancy timing led to an extension of the lactation length, which resulted in greater total milk yield per lactation. Extending the duration of the VWP may increase profitability of primiparous cows and reduce profitability of multiparous cows, primarily due to differences in herd replacement dynamics and milk production efficiency. Therefore, farms may benefit from extending the duration of the VWP beyond 60 d for primiparous cows but not for multiparous cows. Finally, we also observed that reproductive management strategies that led to similar average days to the first service (~60 d) through a combination of inseminations at estrus with timed-artificial insemination (**TAI**) or all TAI resulted in reduced time to pregnancy after calving when compared with an all TAI program with a longer VWP.

BIOGRAPHICAL SKETCH

Matias Stangaferro was born on February 28, 1984 in Carlos Pellegrini, Santa Fe, Argentina. He graduated as a Doctor of Veterinary Medicine from the Universidad Nacional del Litoral (Argentina) in 2009 where he received the academic excellence award for the highest GPA in his class. Thereafter, he worked as a Professor of Theriogenology in the College of Veterinary Medicine, Universidad Nacional del Litoral from 2009 to 2013. During that time, he obtained a Master of Science degree in Dairy Production Medicine. He arrived to Cornell University in 2013 as a Fulbright grantee to conduct research in the Dairy Cattle Biology and Management Laboratory in the Department of Animal Science under the guidance of Dr. Julio Giordano. His research activities focused on dairy cattle reproductive physiology and management, use of automated health monitoring system in dairy farms, and the effects of nutritional management on the reproductive and productive performance of dairy cattle.

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TABLE OF CONTENTS

SECTION I

AUTOMATED MONITORING OF DAIRY COW HEALTH

SECTION I

GENERAL CONCLUSION

LIST OF FIGURES

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER VI

CHAPTER VII

CHAPTER VIII

LIST OF TABLES

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER VI

CHAPTER VII

CHAPTER VIII

SECTION I

AUTOMATED MONITORING OF DAIRY COW HEALTH

CHAPTER I

EARLY POSTPARTUM HEALTH DISORDERS AND THE POTENTIAL USE OF AUTOMATED HEALTH MONITORING SYSTEMS FOR DISEASE DETECTION

1. General introduction

Early postpartum health disorders are a major cause of poor dairy cow welfare (Dechow and Goodling, 2008; Von Keyserlingk et al., 2009) and reduced farm profitability (De Vries, 2006; St-Pierre and Eastridge, 2013). In spite of recent advances in disease prevention, a substantial proportion of dairy cows may have a health disorder during lactation, with highest incidence during the first month after calving (Mulligan and Doherty, 2008; LeBlanc, 2010). Several diseases commonly affect dairy cows during lactation; however, the most prevalent include retained placenta, ketosis, displaced abomasum (**DA**), indigestion, metritis, mastitis, lameness, and pneumonia (Ingvartsen et al., 2003; Ingvartsen, 2006; Mulligan and Doherty, 2008). The consequences for cow well-being and performance vary with the nature and severity of the disorder but, to some extent, all of them negatively affect productive and reproductive performance, and increase the risk of culling during lactation (Grohn et al., 1998; Bell and Roberts, 2007; Schukken et al., 2009; Ribeiro et al., 2013).

Most commercial dairy farms implement a health monitoring program to detect and treat sick cows in an effort to reduce the consequence of poor transition cow health on performance and well-being (Espadamala et al., 2016). Unfortunately, these programs are usually time-consuming, labor intensive, and most evaluations carried out by farm personnel inherently subjective. During clinical examination cows are typically restrained in self-locking gates or palpation rails which may reduce the time they spend expressing other natural behaviors, such as resting and eating.

Automated, sensor-based monitoring of behavioral, physiological, and productive parameters can be an alternative to conventional health examinations, reducing cow disruption and the burden of traditional health monitoring programs for dairy farms. Using these technologies, farm personnel can focus on cows that may be suffering health disorders while the rest of the cows are not disrupted. Furthermore, there is a possibility that subtle changes in parameters continuously monitored by sensors may be beneficial for prompt disease detection, even before the development of evident clinical signs. Subsequently, earlier, more accurate disease detection may benefit cows by preventing the progression of the disorder, enhancing the response to treatments, and improving cow well-being, all of which can help improve public perception of animal care and welfare on dairy farms. Nevertheless, limited data are available about the performance of automated health monitoring systems (**AHMS**) on farms and the pattern of change in behavioral, physiological, and production parameters around the time of clinical diagnosis has yet to be fully characterized.

This chapter aims to describe basic metabolic changes and health disorders that cows experience around parturition, the burden associated with intensive health monitoring programs, and current knowledge about the potential use of AHMS to identify cows with health disorders.

2. Transition period in dairy cattle

2.1. Major metabolic adaptations around parturition

The complex metabolic adaptations that cows experience during the transition period have been extensively reviewed in the literature (Bauman and Currie, 1980; Grummer, 1993; Bell, 1995; Grummer, 1995; Bell and Bauman, 1997; Horst et al., 1997; Drackley, 1999; Goff, 2000; Drackley et al., 2001; Overton and Waldron, 2004; Ingvartsen, 2006; Sundrum, 2015). Although several definitions have been proposed, most define the transition period for dairy cows by its temporal relationship with parturition, predominantly the period from approximately 3 weeks before until approximately 3 weeks after calving (Grummer, 1995; Drackley, 1999). This period is characterized by a reduction in DMI at a time when nutrient requirements increase abruptly, leading to some degree of negative energy balance [(**NEB**); Grummer et al., 2004; Ingvartsen, 2006; Esposito et al., 2014]. Consequently, cows undergo dramatic metabolic changes that require coordinated actions from different tissues to support their challenging physiological state (Bauman and Currie, 1980; Overton and Waldron, 2004; Ingvartsen and Moyes, 2013).

Nutrient demands [especially glucose and amino acids (**AA**)] increase considerably in late gestation as a result of rapid fetal growth (Ingvartsen, 2006; Ingvartsen and Moyes, 2013). After parturition the requirements for glucose, AA, and fatty acids increase so dramatically that their demand cannot be met by dietary intake (Bell, 1995). For instance, after parturition the demand for glucose, AA, and fatty acids by the mammary gland is 2.7, 2, and 4.5 times greater than those of the gravid uterus during late pregnancy (Bell, 1995). Additionally, calcium (**Ca**) requirements increase four fold on the day of calving (Horst et al., 1997; Overton and Waldron, 2004). Therefore, successful metabolic adaptation during the transition period to avoid physiological imbalances, is dependent on the homeorhetic control of nutrient partitioning (Bauman and Currie, 1980; Overton and Waldron, 2004; Ingvartsen, 2006). For example, in order to support the increased glucose demand, the cow can metabolically adapt by increasing hepatic gluconeogenesis along with reducing glucose oxidation by peripheral tissues (Bauman and Elliot, 1983; Reynolds et al., 2003).

Growth hormone (**GH**) concentrations increase at parturition stimulating milk production, gluconeogenesis, and lipolysis (Bauman and Currie, 1980). Despite the significant increase in gluconeogenesis at this time, most glucose is used by the mammary gland to synthetize lactose, reducing circulating levels of glucose and consequently, insulin (Ingvartsen, 2006; Sundrum, 2015). Low insulin concentrations in blood uncouple the GH-insulin like growth factor I (**IGF-I**) axis by down-regulation of the GH 1A receptor in the liver (Butler, 2003). Thus, high circulating levels of GH, combined with low levels of glucose, insulin and IGF-I, lead to massive lipolysis which provides energy-rich non-esterified fatty acids (**NEFA**) and glycerol to meet overall energy requirements during the period of negative energy balance (Lucy, 2004; Ospina et al., 2010b; Mann et al., 2015; Sundrum, 2015).

Circulating concentrations of NEFA are increased substantially during early lactation (Ospina et al., 2010b; Mann et al., 2015). Once in circulation, NEFA can be taken up by the mammary gland to contribute to milk fat production, be used as fuel by skeletal muscle and tissues with a limited glucose supply, be used by the liver as fuel through partial or complete oxidation, or be re-esterified to produce triglyceride [**TAG**; (Bell, 1995; Herdt, 2000; Adewuyi et al., 2005)]. Although the liver can take up NEFA, this organ has a limited capacity to catabolize or export NEFA as TAG (Emery et al., 1992; Reynolds et al., 2003; Overton and Waldron, 2004). Therefore, TAG accumulate in the liver of almost all high-yielding dairy cows after calving, which may negatively impact the gluconeogenic and ureagenic capacity of the liver (Cadórniga-Valiño et al., 1997; Strang et al., 1998; Overton and Waldron, 2004).

Another important metabolic adaptation of cows during the transition period is maintaining Ca homeostasis immediately after calving. Circulating concentrations of Ca remain under the strict control of parathyroid hormone, 1,25-dyhydroxyvitamin D3, and calcitonin (Reinhardt et al., 1988; Horst et al., 1994; Goff, 2000). As the Ca demand increases dramatically at the onset of lactation to support of colostrum and milk synthesis (Ramberg et al., 1970; Horst et al., 1997), concentrations of Ca in blood reach a nadir at about 12 to 24 h after parturition (Goff, 2008). In response to low levels of blood Ca, parathyroid hormone and vitamin D stimulate Ca bone resorption, renal reabsorption, and intestinal absorption in an effort to restore normal Ca levels (Horst et al., 1994; Overton and Waldron, 2004). This mechanism must be rapidly activated after calving to avoid hypocalcemia and its negative consequence on health, milk production, and reproduction (Chapinal et al., 2012; Martinez et al., 2012; Roberts et al., 2012).

In summary, the transition from a non-lactating to a lactating state in dairy cows is characterized by dramatic metabolic changes controlled by homeorhetic mechanisms that coordinate the action of different hormones, tissues, and organs to support lactation. Successful metabolic adaptations during the transition period are crucial to avoid nutrient imbalances and, consequently, reduce the risk of developing health disorders around parturition. Because some cows fail to maintain this balance, they develop health disorders that affect their well-being, performance, and profitability. Therefore, health monitoring programs consisting of routine clinical examination and complementary diagnostic tools are essential to quickly identify and treat cows with health disorders.

2.2. Health disorders during the transition period

Most health disorders in dairy cattle occur during the transition period (Drackley, 1999; Ingvartsen, 2006; Ingvartsen and Moyes, 2013), with up to 75% of diseases recorded during the first 30 DIM (LeBlanc et al., 2006). The risk of metabolic and infectious disorders is particularly high during this time (Goff and Horst, 1997; Hammon et al., 2006; McArt et al., 2013), and it has been reported that 30 to 50% of dairy cows develop at least one event of a disease around parturition (LeBlanc et al., 2006). Numerous health disorders can affect lactating dairy cows; however, the most prevalent include retained placenta, metritis, mastitis, ketosis, DA, indigestion, lameness, and pneumonia (Ingvartsen et al., 2003; Ingvartsen, 2006; Mulligan and Doherty, 2008). Collectively, these illnesses are usually referred to as production disorders, reflecting the inability of some cows to adapt to the demands after calving (Mulligan and Doherty, 2008).

The origin of health disorders in early lacation are varied. Nevertheless, excessive NEB due to the abrupt increase in nutrient requirements to support milk production and a concurrent depression in dry matter intake (Grummer et al., 2004; Ingvartsen, 2006; Esposito et al., 2014) has been linked to certain disorders and/or may increase the risk for others. For example, metabolic problems such as ketosis and DA have been linked to massive lipid mobilization in response to excessive NEB (Drackley, 1999). Poor metabolic status and severe NEB can also increase the risk of infectious diseases due to an impaired immune response (Loiselle et al., 2009; Esposito et al., 2014; Sundrum, 2015). High circulating NEFA concentrations can exert a direct inhibitory effect on PMN function (Scalia et al., 2006; Ster et al., 2012) such as neutrophil killing ability (Hammon et al., 2006). Also, when adipose tissue is mobilized, pro-inflammatory cytokines like interleukin-6 (**IL-6**) and tumor necrosis factor α (**TNF-α**) are released. These cytokines can stimulate the release of acute phase proteins that affect neutrophil chemotaxis, diapedesis, and migration (LeBlanc, 2014), and can also contribute to more insulin resistance, exacerbating the release of NEFA (Ingvartsen and Moyes, 2013; Sordillo and Raphael, 2013). Similarly, excessive formation of reactive oxygen species (**ROS**) due to the increased metabolic rate (oxidative stress) may increase inflammation by activating pro-inflammatory (NF-κB and TNFα) pathways (Sordillo et al., 2009; LeBlanc, 2014). Collectively, evidence from numerous research studies suggest that severe NEB and oxidative stress may contribute to an impaired inflammatory response after calving in dairy cows, which is a major risk factor for the development of infectious and non-infectious health disorders.

Production diseases in dairy cows are usually inter-related because of common risk factors (e.g., excessive NEB and impaired immune response). For instance, over-conditioned dry cows mobilize more adipose tissue after calving, which increases the risk of developing ketosis, fatty liver, and DA (Mulligan and Doherty, 2008; Ospina et al., 2010a; Sundrum, 2015), and can impair immune function (Ingvartsen et al., 2003; Mulligan and Doherty, 2008). In addition, over-conditioned cows during the dry period are more likely to suffer hypocalcemia at parturition, which reduces immune response and may lead to dystocia and retained placenta (Houe et al., 2001; Mulligan and Doherty, 2008). It is evident that an impaired inflammatory response is a common factor in the pathogenesis of uterine disorders (Galvão et al., 2010; LeBlanc, 2012). The association between subclinical ketosis and mastitis have been extensively reported (Dohoo and Martin, 1984; Gröhn et al., 1989; Oltenacu et al., 1990), whereas retained placenta, metritis, ketosis, and hypocalcemia have been proposed as risk factors for the development of DA (Massey et al., 1993; Shaver, 1997; LeBlanc et al., 2005). Therefore, individual production diseases should not be considered in isolation, because they may be inter-connected by common risk factors during the transition period and the occurrence of one of them can increase the likelihood of developing other disorders (Peeler et al., 1994; Mulligan and Doherty, 2008). For that reason, prevention and early diagnosis of health disorders is essential to minimize the occurrence and/or progression of multiple disorders.

3. Burden of health monitoring in commercial dairy farms

Early identification of dairy cows suffering health disorders is pivotal for not only successful treatment, but also to prevent disease progression and ensure cow well-being. Therefore, dairy farms design and implement intensive health monitoring programs to identify, treat, and care for sick cows (Guterbock, 2004; LeBlanc, 2010; Espadamala et al., 2016). Although the extent and intensity of health monitoring programs vary widely among farms, routine clinical examinations of all or most cows during the first weeks of lactation is common practice (LeBlanc, 2010; Risco and Melendez, 2011). For instance, a recent survey conducted in 45 dairy farms in California reported that 78% of the herds surveyed performed fresh cow examinations at least once daily, 20% examined cows 2 to 6 times a week, and 2% did not perform routine clinical examinations (Espadamala et al., 2016). Similarly, Scott (2016) reported that on average, cows were monitored 2.4 times a day during the first 14 DIM in a group of ~55 New York dairies.

Clinical examinations require qualified and experienced workers to evaluate cow attitude, appetite, locomotion, rectal temperature, and use diagnostic aids such as auscultation, palpation, and collection of bodily fluids for cow-side or laboratory testing (Guterbock, 2004; LeBlanc, 2010; Risco and Melendez, 2011). For example, to detect cows with clinical mastitis, health monitoring programs include the evaluation of milk characteristics, signs of udder inflammation, and systemic signs of illness (Nash et al., 2002; Wenz et al., 2006). On the other hand, the diagnosis of metritis is primarily based on the evaluation of the uterus (e.g., size and tone), uterine discharge, and rectal temperature (Földi et al., 2006; Sheldon et al., 2006; Benzaquen et al., 2007). Ketosis diagnosis is usually complemented with cow-side tests to measure ketone bodies in blood, urine, or milk, whereas auscultation is used for the identification of cows with DA or pneumonia (LeBlanc, 2010; Risco and Melendez, 2011). Of note, these complex health examinations, including complementary tests, are generally performed by farm personnel and not by veterinarians or veterinary technicians (Guterbock, 2004; Espadamala et al., 2016). For that reason, skilled labor, re-trained on a regular basis, and under regular supervision is essential for accurate and consistent disease diagnosis and health monitoring.

Performing clinical examinations and diagnostics tests for a large number of cows can be time consuming, labor intensive, and inherently subjective, particularly as herd size increases. For instance, Espadamala et al. (2016) reported that large herds (between 2,000 and 9,500 lactating cows) typically employ two or more workers to perform fresh cow evaluations, which also introduces variability to the diagnosis. In addition, this study found a positive correlation between the number of cows in the fresh pen and the duration of exams which may translate into impaired time budgets and disrupted natural behaviors because cows sorted from herd mates are examined while restrained in self-locking head gates or in palpation rails (Guterbock, 2004; Espadamala et al., 2016). Cows may spend substantial time restrained preventing them from resting, eating, and expressing other important natural behaviors.

In summary, a substantial amount of time and resources are dedicated by dairy operations to conduct health-monitoring programs. Implementation of these programs is demanding and may become even more challenging in large dairy herds with decreased availability of qualified personnel.

4. Automated health monitoring systems

Although the use of sensors to monitor different cow parameters is not a new idea (Frost et al., 1997; Edwards and Tozer, 2004), there has been a recent explosion in the development and adoption of sensor-based AHMS for dairy farms.

Sensors used in dairy management can be grouped by the type of parameter monitored. There are sensors that monitor behavior (e.g., physical activity, rumination time, feeding time), physiological parameters (e.g., body temperature, rumen pH), or productive parameters (e.g., milk yield, electrical conductivity, milk components). Rutten et al. (2013) classified sensors according to their position relative to cows in two categories: attached and nonattached. Attached sensors are those fitted on different parts of the cow body (e.g., neck, leg, ear) or inside the cow (e.g., rumen, vagina). In contrast, nonattached sensors are those not in contact with the cow. The latter are usually located in a specific area where cows or their products (e.g., milk) pass by, over, or through (Rutten et al., 2013). Examples of nonattached sensors include milk meters that collect milk weight or electrical conductivity, walk-in scales, and environmental temperature and humidity sensors.

Numerous AHMS based on single or multiple sensors have been developed and are commercially available (Ferrero et al., 2014; Barkema et al., 2015; Borchers and Bewley, 2015). For instance, changes in physical activity can be monitored by different commercially available sensors, such as Heatime (SCR Engineers Ltd., Netanya, Israel), DeLaval Activity Meter System (DeLaval International AB, Tumba, Sweden), and CowManager SensOor system (Agis, Harmelen, Netherlands). Similarly, sensors attached to the cow leg, such as AfiAct II (Afimilk, Kibbutz Afikim, Israel) and CowScout Leg (GEA Farm Technologies GmbH, Bönen, Germany) can monitor not only physical activity, but also standing and lying behavior. Some systems consist of multiple sensors for monitoring different parameters. Examples are the Heatime HR System (SCR Dairy, Netanya, Israel), and the Afimilk Silent Herdsman Neck Collar (Afimilk, Kibbutz Afikim, Israel) which integrate rumination time and cow activity. Several other parameters can be monitored by different sensors, including milk yield, milk components and electrical conductivity, body or milk temperature, body weight, and body condition score, among others (Borchers and Bewley, 2015). For example, AfiLab (Afimilk, Kibbutz Afikim, Israel) measures not only milk weights but also fat, protein, and lactose concentration, and alerts the presence of blood. Because of the large number of commercially available technologies and systems, dairy farmers have the opportunity to explore multiple AHMS options and select the one that better meets the demands of their herd.

4.1. Potential of automated health monitoring systems in the dairy industry

Over the past years, the structure of the dairy industry has changed with fewer dairy operations and larger herd sizes (Wolf, 2003; Von Keyserlingk et al., 2013; Barkema et al., 2015). Concurrently, there has been a reduction of qualified labor (LeBlanc, 2010; Mottram, 2016). These factors, along with increased interest on improving animal welfare (Von Keyserlingk et al., 2009; Ventura et al., 2015) and an increase in the number of technologies available for dairy farms have become major drivers for the automation of different farm activities.

Accurate AHMS have the ability to positively change herd health management in many ways. For example, continuous monitoring of health status may allow earlier and more objective disease detection than when using non-automated health monitoring. Indeed, previous studies demonstrated that cows which developed subclinical ketosis in early lactation had reduced rumination time before disease diagnosis or even before calving (Gaspardy et al., 2014; Kaufman et al., 2016; Schirmann et al., 2016), suggesting this parameter could be used for earlier identification of sick cows. Early detection may enable treatments to be more effective, prevent the progression of the disorder, and hinder the development of secondary illnesses.

Another potential benefit of AHMS is the opportunity to improve cow welfare and performance by optimizing cow time budgets. In most herds, cows are examined while restrained in self-locking head gates or in palpation rails after sorting from herd mates. Cows that spend considerable amounts of time locked up have their normal behavior disrupted (Grant and Albright, 1995; Bolinger et al., 1997; Grant and Albright, 2001). Thus, using AHMS to detect potentially sick cows may allow farm personnel in charge of health examinations to focus on and perform exhaustive clinical evaluations only on cows that may be suffering from a health disorder while not disrupting the rest of the cows. As a result, AHMS may help decrease human interventions on cows with no health issues during the transition period avoiding unnecessary disruptions in cow behavior and improving time budget of the herd.

Increased labor cost is another major thrust for automation. In this regard, AHMS could allow larger herds to be managed with fewer personnel and smaller farms to allocate limited labor resources to other tasks (Rutten et al., 2013). For example, performing clinical examination only to cows identified by the AHMS instead of all cows in the first weeks postpartum may reduce not only the time spent on these exams but also the number of people involved.

Accordingly, labor resources and cow care (clinical examinations and treatments) can be targeted to cows that truly need attention, reducing the time spent in evaluating health status of the herd and improving labor productivity.

Finally, AHMS may provide an indication of overall cow health without collection of tissues or bodily fluids (e.g., blood, milk, and urine) or the need for human interventions (e.g., clinical examination). For example, diagnosis of uterine diseases usually requires handling and restraining cows in self-locking headgates to perform rectal or vaginal manipulation (Sheldon et al., 2006; Benzaquen et al., 2007; LeBlanc, 2008). These interventions disrupt natural behavior and induce stress to some extent, which may deteriorate cow welfare (Waiblinger et al., 2004; Kovács et al., 2014). Conversely, accurate AHMS that correctly identify sick and healthy cows may prevent unnecessary manipulation of the latter, reducing stress associated with these interventions and improving public perception of animal welfare on dairy farms (Rutten et al., 2013).

4.2. Behavior, physiological, and productive parameters as signs of illness

Changes in cow behavioral, physiological, and productive parameters have been widely used for the diagnosis of health disorders (Guterbock, 2004; Radostits et al., 2006; Risco and Melendez, 2011). For example, many clinical signs, such as depression, lethargy, lack of appetite, or a sudden reduction in milk production, are normally used to identify sick cows by veterinarians and farm personnel in charge of health monitoring programs. However, if not measured objectively, most of these signs are subjective, depend on the experience of the observer, and disease diagnosis is susceptible to poor reliability (Weary et al., 2009).

Nowadays, several parameters can be continuously monitored objectively by different sensors providing valuable information for detection of animals that deviate from normal. For example, rumination time can by monitored constantly by devices attached to the cow, such as neck collars or ear tags, and milk yield and components can be assessed at each milking by sensors located in milking units. Changes in the pattern of these parameters (i.e., increments or reductions in comparison with other cows or with themselves in previous days) have been associated with the presence of different health disorders (Edwards and Tozer, 2004; Liboreiro et al., 2015; Schirmann et al., 2016). However, the degree, timing, and duration of the changes around the day of clinical diagnosis for different health disorders are less clear.

In this regard, Weary et al. (2009) classified changes in different cow parameters in relation to a health issue as positive or negative. Positive indicators are those that increase in magnitude or frequency when the animal is sick [e.g., body temperature, somatic cell counts (**SCC**), milk fat to protein ratio]. For instance, positive deviations from normal values for milk SCC and/or electrical conductivity has been extensively used to detect cows with clinical and subclinical mastitis (Norberg et al., 2004; Hovinen and Pyörälä, 2011; Kamphuis et al., 2013; Lien et al., 2016; Sørensen et al., 2016). In contrast, negative indicators are those that decrease in magnitude or frequency in the presence of disease (e.g., milk yield, feeding time, rumination time). As an example, King et al., (2017) using data from a limited number of cows reported a significant reduction (negative deviation from normal) in milk production for cows that developed DA (4.4 kg/d from days -4 to -1 relative to clinical diagnosis), pneumonia (4.1 kg/d from days -4 to -1 relative to clinical diagnosis), and subclinical ketosis (1.2 kg/d from days -5 to -1 relative to clinical diagnosis). Moreover, reduced daily rumination time in cows with DA (~45 min/d from days -8 to -1 relative to clinical diagnosis), pneumonia (~50 min/d from days -5 to -1 relative to clinical diagnosis), and subclinical ketosis (~26 min/d from days -6 to -1 relative to clinical diagnosis) was also observed. In summary, changes in these indicators (positive or negative) could provide valuable information about alterations in organ function (e.g., SCC or electrical conductivity in milk) or the general health status of the animal (e.g., feeding time), which collectively may be useful to identify cows suffering from health disorders.

4.3. Changes in parameters monitored by sensors in cows with metabolic and digestive disorders

Multiple studies with dairy cows have established associations between changes in behavioral, physiological, or productive parameters and the development of metabolic or digestive disorders. One of the first studies that characterized changes in physical activity (measured as number of steps per unit of time) and milk production in cows that developed metabolic and digestive disorders was conducted by Edwards and Tozer (2004). In this study, cows with ketosis, DA, and digestive disorders had increased walking activity and reduced milk production compared with healthy cows. In addition, changes in pattern for the parameters of interest started earlier (~8 to 9 for activity, and ~5 to 7 days for milk production) than the day of clinical diagnosis.

Feeding behavior has also been related to metabolic disorders in dairy cows. By using computerized feeders, Gonzalez et al. (2008) reported that cows with ketosis presented a rapid decrease in feed intake, feeding time, and feeding rate and the changes were observed on average ~4 days before diagnosis. However, only eight cows with ketosis were evaluated in this study. Similarly, Goldhawk et al. (2009), comparing 10 cows with subclinical ketosis and 10 healthy cows, reported that one week before and two weeks after calving cows with subclinical ketosis had reduced feed intake, fewer visits to the feeder, and spent less time at the feeder than healthy cows.

Another behavioral parameter that changes in cows with metabolic disorders is rumination time. A study conducted in Israel showed that rumination time and body weight are reduced in the days around diagnosis of subclinical ketosis (Gaspardy et al., 2014). Likewise, Schirmann et al. (2016) reported that cows suffering subclinical ketosis (n = 9) in early lactation had lower rumination time prepartum and lower dry matter intake pre- and postpartum than healthy cows (n = 20). In addition to those findings, Kaufman et al. (2016) observed that multiparous cows that developed subclinical ketosis had lower rumination time than healthy cows during the week before and the two weeks after calving.

More recently, a longitudinal pilot study using a limited number of cows managed with an automatic milk system reported that cows with DA (n = 5) had decreased rumination time, milk yield, and physical activity, but longer lying time and bouts when compared to themselves in the period of non-disease (King et al., 2017). Similarly, a reduction in rumination time, milk yield, activity, and body weight was reported for cows that developed subclinical ketosis (n = 19; (King et al., 2017).

Collectively, these studies suggest that the use of sensors for monitoring different behavioral, physiological, and performance parameters could be useful for the identification of cows suffering metabolic or digestive disorders.

4.4. Changes in parameters monitored by sensors in cows with mastitis

The severity mastitis cases can may range from mild changes in milk appearance to being very systemically sick (Sargeant et al., 1998; Bradley and Green, 2001; Nikolić et al., 2003). For this reason AHMS to detect cows with mastitis can be divided in two major groups according to type of information provided: (1) systems that monitor changes in milk production or milk attributes (e.g., daily milk weights, milk composition, electrical conductivity, SCC), and (2) systems that offer information about the general health status of the animal (e.g., rumination time, physical activity, feeding behavior).

Many AHMS that monitor milk have been tested and are available to detect mastitis through changes in milk yield and milk characteristics. For example, in-line measurement of electrical conductivity is one of the most frequently used sensors for automated mastitis detection in dairy cows (Norberg et al., 2004; Hovinen and Pyörälä, 2011; Ferrero et al., 2014; Lien et al., 2016). Likewise, milk yield and temperature are also commonly employed as screening methods to detect cows with mastitis, especially in automatic milking systems (Maatje et al., 1992; de Mol and Ouweltjes, 2001; Hogeveen and Ouweltjes, 2003). In this regard, integration of these parameters (milk yield, electrical conductivity, and milk temperature) through complex algorithms improved detection of subclinical (de Mol et al., 1997; de Mol et al., 1999) and clinical (de Mol et al., 1997; de Mol et al., 1999; De Mol et al., 2001) mastitis.

Somatic cell count in milk is another parameter widely used for mastitis detection (Sears et al., 1993; Sargeant et al., 2001; Middleton et al., 2004). Recent incorporation of sensors to monitor SCC in-line improved sensitivity and specificity for subclinical and clinical mastitis detection, especially in automatic milking systems where no visual evaluation of the udder and milk is performed by farm personnel (Kamphuis et al., 2008b; Kamphuis et al., 2013; Kamphuis et al., 2016; Sørensen et al., 2016). Additional sensor collected data, such as milk color (Kamphuis et al., 2008a; Brandt et al., 2010; Hovinen and Pyörälä, 2011), presence of ions (Legin et al., 1999; Mottram et al., 2007), protease activity, (Koop et al., 2015); and the combination of milk production rate, milk flow rate, and electrical conductivity (Cavero et al., 2006; Cavero et al., 2008) have been suggested for use in mastitis detection.

On the contrary, scarce information is available about changes in behavior to detect cows with clinical mastitis. Siivonen et al., (2011) studied the behavior of six cows with acute mastitis after infusion of endotoxin. On the day of mastitis induction cows had longer eating time, spent less time lying, and walked more steps than the prior day. In addition, this study showed lying and rumination time was reduced when the udder was severely swollen and cows had fever. Similarly, Fogsgaard et al., (2012) described changes in behavior after experimentally inducing *E. Coli* mastitis in 20 primiparous cows. In the first 24 h after induction of mastitis, cows had lower feeding time, lower frequency of self-grooming behavior and turning the head against the udder, lower rumination time, and increased duration of standing idle compared with previous days.

A case-control study compared cows that developed spontaneous (not induced), mild cases of clinical mastitis against healthy control cows (Medrano-Galarza et al., 2012). Two days after diagnosis, cows with mastitis had reduced lying time compared with their controls. In addition, the frequency of kicks and lifts during milking, as well as the frequency of steps was greater for the days immediately after mastitis diagnosis compared to 10 days later for the same cow (Medrano-Galarza et al., 2012). Likewise, Fogsgaard et al. (2015) showed that cows with naturally occurring cases of clinical mastitis had reduced feed intake from 10 days before until 5 days after clinical diagnosis compared with control cows. Moreover, cows with mastitis had greater kicking frequency during milking from 2 days before to 10 days after diagnosis, and they spent less time lying in the first 5 days after diagnosis compared to controls cows (Fogsgaard et al., 2015). More recently, Sepúlveda-Varas et al. (2016) reported that cows suffering a naturally occurring case of clinical mastitis had decreased feed intake over the five days before diagnosis, mostly determined by a reduction in feeding rate. However, after mastitis diagnosis and treatment, feed intake, feeding time and competitive replacements at the feeder increased compared to the day of clinical diagnosis (Sepúlveda-Varas et al., 2016).

In summary, AHMS to detect cows with mastitis by evaluating changes in milk production or its attributes have been extensively studied and tested under farm conditions. Consequently, these programs have proved to be more pertinent than using behavior monitoring as the sole method or to complement other methods of mastitis detection. Systems that monitor behavior may provide additional insights about overall cow health not provided by sensor systems that only monitor milk.

4.5. Changes in parameters monitored by sensors in cows with metritis

Metritis diagnosis in dairy cows is generally based on the characteristics of the uterus and uterine discharge (Földi et al., 2006; Sheldon et al., 2006; Sheldon et al., 2009). Depending on the severity of the case, signs of systemic illness such as anorexia, fever, depression, and dehydration are also common in cows with metritis (Benzaquen et al., 2007; Sheldon et al., 2009; Risco and Melendez, 2011). Thus, these signs can be monitored by sensors to help idetify cows suffering metritis. For example, feeding time and dry matter intake around parturition were extensively associated with occurrence of metritis. Urton et al. (2005) reported that cows with metritis in early lactation had reduced feeding time pre- and postpartum compared to healthy cows. Moroever, using a threshold of 75 min/d of average daily feeding time, a sensitivity of 89% and a specifity of 62% was obtained to detect cows with severe puerperal metritis (reddish brown, watery, foul smelling vaginal discharge plus fever). Additionally, Huzzey et al. (2007)

reported that cows suffering severe puerperal metritis spent less time feeding and had lower feed intake than healthy cows from 2 weeks before to 3 weeks after calving, with the greatest difference on the week before parturition. Similarly, Schirmann et al. (2016) found that cows with metritis had lower dry matter intake than healthy cows during the first two weeks postpartum.

Rumination time, physical activity, and lying behavior may also have value for the detection of metritis. For instance, Titler (2013) documented that cows with metritis spent more time standing, presented fewer steps, and had fewer lying bouts from 1 to 3 days before clinical diagnosis than healthy cows. Liboreiro et al. (2015) reported lower rumination and activity after calving for cows with metritis compared to healthy cows, although the temporal relationship between the day of metritis diagnosis and the changes in behavior was not explored and/or reported.

More recently, King et al. (2017) reported that from 4 days before until the day of metritis diagnosis, body weight declined by 13.5 kg/d, activity tended to decline by 20%, and milk temperature increased by 0.17 °C/d in cows that developed metritis. Cows with metritis also had longer lying time and longer lying bout duration during the week before clinical diagnosis (King et al., 2017). Another study conducted in Israel evaluating the use of automated swinging cow brushes by dairy cows after parturition (i.e., the proportion of cows using the brush and the duration of usage), found that between 8 and 21 DIM, a lower proportion of cows that developed metritis used the brush compared with healthy cows. In addition, the duration of the brush usage was reduced by half in cows with metritis (Mandel et al., 2017).

Overall, these previous studies suggested that some parameters monitored by AHMS could be used to identify cows with metritis, although additional research is needed to support their commercial use in dairy farms. Additionally, sensors that continuously monitor different behavior parameters may provide additional insights about the overall health status of cows not provided by clinical evaluation of the uterus and its content.

5. Issues with existing automated health monitoring systems

Over the last decades, there has been an explosion of commercially available technologies for automating different management tasks on dairy farms. Systems to detect and flag cows in estrus, automated calf feeders, and automated milking units have been extensively studied and successfully adopted by the dairy industry (Rossing and Hogewerf, 1997; Hepola, 2003; Jónsson et al., 2011; Jacobs and Siegford, 2012; Mottram, 2016). Conversely, scientific data about the on-farm use of new technologies for monitoring behavior and/or physiological parameters for the identification of cows suffering health disorders is not as readily available.

Rutten et al. (2013) categorized the degree of information provided by sensors systems to dairy farm operators into four different categories. The first level corresponds to the ability of the sensor to measure a parameter about the cow (e.g., rumination time). The second level refers to the system's capability of using and summarizing sensor data in order to provide useful information about the status of the cow (e.g., rumination time reduction is probably associated with disease). The third level denotes the aptitude of the system to integrate sensor and non-sensor data (e.g., replacement cost) in a decision support model to generate advice for dairy producers (e.g., whether to sell or not to sell a cow). Lastly, the fourth level corresponds to the decision-making strategy. That is whether the system makes the decision autonomously or the farm operator makes the decision based on the system advice. Interestingly, most studies reviewed by Rutten et al. (2013) reported that sensor systems worked at levels one and two but no studies have reported systems working at the third or fourth levels. Furthermore, most the work in mastitis and fertility has been conducted at level two, but for locomotion or metabolic disorders, most of the work was done just at the first level (Rutten et al., 2013).

In summary, most of the studies evaluating parameters monitored by sensors to identify cows with heath disorders have documented different trends and changes in the patterns of parameters monitored and only one or few disorders were evaluated at the same time. Moreover, most of these studies focused on the period around parturition, but little is known about the patterns of these parameters around the day clinical diagnosis for different health disorders. Although data for identification of sick cows is promising, few research studies have evaluated the on-farm performance of AHMS based primarily on sensors. Therefore, more data is needed to demonstrate the accuracy and practicality of AHMS in dairy herds, and to describe the relationship (degree, timing, and duration) between changes in the parameters monitored by sensors and changes in cow health status and performance.

6. Summary

The literature reviewed for this section discussed the major metabolic adaptations that dairy cows experience during their transition period, the common health disorders that cows suffer during this period as consequence of poor adaptation, and the burden associated with the implementation of health monitoring programs in dairy herds. In addition, it summarizes current research regarding changes in behavioral, physiological, and productive parameters associated with the presence of different health disorders, in particular metabolic and digestive issues, mastitis, and metritis.

Health disorders during the transition period negatively affect dairy cow welfare and farm profitability. Although there have been important advances in disease prevention during the last decades, a considerable proportion of cows continue to develop health disorders in early lactation. The magnitude of the impact on cow well-being and performance vary with the nature of the disorder and its severity, but all reduce productive performance, cow fertility, and survivability to some extent. Consequently, dairy operations spend a significant amount of time and resources to identify, treat, and care for sick cows. For example, daily evaluation of health status is typically performed to all cows in early lactation by farm personnel. Unfortunately, clinical examinations are time-consuming, labor intensive, and inherently subjective. Additionally, cows are typically restrained in self-locking gates or palpation rails during clinical examination, which may reduce the time they spend expressing other natural behaviors, such as resting and eating.

Automated, sensor-based monitoring of behavioral, physiological, and production parameters can be an alternative to more cow-disruptive examinations and may also help reduce the burden of traditional health monitoring programs for dairy farms. However, sensor data are only useful if interpreted and used efficiently in decision-making. Most commercially available AHMS have not been thoroughly tested on farms, and more data about the pattern of changes in behavioral, physiological, and productive parameters around the time of clinical diagnosis of health disorders is needed to improve performance and adoption of these technologies.

Therefore, we conducted observational prospective cohort studies to test the performance of a commercially available AHMS that integrates rumination and activity data through custom-designed algorithms for identification of cows suffering health disorders. Accordingly, the studies presented in Chapter II, III, and IV of this section had the primary objective of evaluating the performance of an automated rumination and physical activity monitoring system to identify cows with metabolic and digestive disorders, mastitis, and metritis, respectively.

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CHAPTER II

USE OF RUMINATION AND ACTIVITY MONITORING FOR THE IDENTIFICATION OF DAIRY COWS WITH HEALTH DISORDERS. PART I. METABOLIC AND DIGESTIVE DISORDERS

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ABSTRACT

The objectives of this study were to evaluate (1) the performance of an automated health-monitoring system (**AHMS**) to identify cows with metabolic and digestive disorders - including displaced abomasum, ketosis, and indigestion- based on an alert system (health index score, **HIS**) that combines rumination time and physical activity; (2) the number of days between the first HIS alert and clinical diagnosis (**CD**) of the disorders by farm personnel; and (3) the daily rumination time, physical activity, and HIS patterns around CD. Holstein cattle (n = 1,121; 451 nulliparous and 670 multiparous) were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags, SCR Dairy, Netanya, Israel) from at least −21 to 80 d in milk (DIM). Raw data collected in 2-h periods were summarized per 24 h as daily rumination and activity. A HIS (0 to 100 arbitrary units) was calculated daily for individual cows with an algorithm that used rumination and activity. A positive HIS outcome was defined as a HIS of <86 during at least 1 d from −5 to 2 d after CD. Blood concentrations of nonesterified fatty acids, β-hydroxybutyrate, total calcium, and haptoglobin were determined in a subgroup of cows (n = 459) at −11 ± 3, −4 ± 3, 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 DIM. The sensitivity of the HIS was

98% [95% confidence interval (CI): 93, 100] for displaced abomasum (n = 41); 91% (95% CI: 83, 99) for ketosis (n = 54); 89% (95% CI: 68, 100) for indigestion (n = 9); and 93% (95% CI: 89, 98) for all metabolic and digestive disorders combined (n = 104). Days (mean and 95% CI) from the first positive HIS <86 and CD were −3 (−3.7, −2.3), −1.6 (−2.3, −1.0), −0.5 (−1.5, 0.5), and −2.1 (−2.5, −1.6) for displaced abomasum, ketosis, indigestion, and all metabolic and digestive disorders, respectively. The patterns of rumination, activity, and HIS for cows flagged by the AHMS were characterized by lower levels than for cows without a health disorder and cows not flagged by the AHMS from −5 to 5 d after CD, depending on the disorder and parameter. Differences between cows without health disorders and those flagged by the AHMS for blood markers of metabolic and health status confirmed the observations of the CD and AHMS alerts. The overall sensitivity and timing of the AHMS alerts for cows with metabolic and digestive disorders indicated that AHMS that combine rumination and activity could be a useful tool for identifying cows with metabolic and digestive disorders.

Keywords: rumination, activity, metabolic disorder, digestive disorder

INTRODUCTION

Health disorders in the early postpartum period affect a substantial proportion of lactating dairy cows, with negative results for their health, welfare, and performance (Ingvartsen, 2006). Metabolic and digestive disorders such as ketosis, displaced abomasum (**DA**), and indigestion are detrimental to cow well-being and farm profitability because they cause losses in milk production (Gröhn et al., 1998; Bareille et al., 2003; Edwards and Tozer, 2004), increase the risk of culling and death (Gröhn et al., 1998; Pinedo et al., 2010; Seifi et al., 2011), increase treatment costs (Kaneene and Hurd, 1990; Bartlett et al., 1995), and impair reproductive performance (Raizman and Santos, 2002; Ribeiro et al., 2013; Vercouteren et al., 2015).

For dairy operations, the burden of metabolic and digestive diseases is exacerbated by the additional effort and costs associated with implementing the monitoring necessary to identify cows with these disorders (McArt et al., 2015). Although the intensity of health-monitoring programs varies widely among farms, protocols that include a systematic evaluation of cow health status once or twice per day within the first 1 to 3 wk after calving are common (Risco and Melendez Ratamal, 2011; Espadamala et al., 2015). Such protocols may include evaluation of attitude, appetite, locomotion, and rectal temperature, as well as auscultation, palpation, and collection of bodily fluids for cow-side or laboratory testing (LeBlanc, 2010; Risco and Melendez Ratamal, 2011). Performing these evaluations and diagnostic tests for a large number of cows can be time-consuming and labor-intensive. Moreover, cow behavior and time budgets are disrupted, because in free-stall herds, cows are examined while restrained in self-locking head gates or in palpation rails after they have been sorted from their herd mates. In this regard, automated monitoring of cow behavior and physiological parameters using non-invasive sensors may help reduce the burden of health-monitoring programs. Sensor-generated data could be used alone or with traditional health-monitoring protocols to identify cows with health disorders (Rutten et al., 2013; Lukas et al., 2015). Furthermore, continuous monitoring of behavior and physiological parameters may allow for the detection of subtle changes before evident clinical signs appear. Earlier disease detection may benefit cows by preventing progression and improving response to treatment.

In recent years, multiple devices have been developed and implemented by the dairy industry to automatically monitor behavior and physiological parameters (Rutten et al., 2013;

Ferrero et al., 2014; Barkema et al., 2015). Physical activity levels and rumination time are 2 parameters that are currently available for monitoring cow health. Cows with health disorders would be expected to demonstrate alterations in their activity and rumination patterns of sufficient magnitude to be detected by specific algorithms or visual inspection of data. Indeed, previous studies have found that rumination time and activity were associated with clinical and subclinical health disorders (Soriani et al., 2012; Gaspardy et al., 2014; Liboreiro et al., 2015). These studies have documented general trends and changes in rumination and activity patterns for some disorders, but the performance of automated health-monitoring systems (**AHMS**) that use rumination and activity to detect cows with metabolic and digestive disorders has not been well documented. In addition, more information is needed about the patterns of rumination and activity around the timing of clinical diagnosis (**CD**) of metabolic and digestive disorders in dairy cows.

We hypothesized that an AHMS that continuously monitors rumination and activity would be able to identify cows with metabolic and digestive disorders. Also, we expected that changes in rumination and activity before evident clinical signs of disease would result in earlier identification of disease. The objectives of this study were to evaluate (1) the performance of an automated rumination and physical activity monitoring system to identify cows with metabolic and digestive disorders; (2) the interval between the AHMS alert based on a health index score (**HIS**) and the day of CD by farm personnel; and (3) the rumination, activity, and AHMS-generated alert pattern for cows with the disorders of interest. We also used markers of energy status [nonesterified fatty acids (**NEFA**) and BHB], mineral status (total plasma Ca), and systemic inflammation (haptoglobin) to complement the diagnosis of health disorders and the performance of the AHMS alert.

MATERIALS AND METHODS

Animals and Management

All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University. This study was conducted from November 2013 to October 2014 at a commercial dairy operation in Cayuga County, New York State. Holstein cows (n = 1,121; 451 nulliparous and 670 multiparous) were enrolled in the study at approximately 240 to 250 d of gestation. During the prepartum period, cows were grouped by parity (nulliparous vs. parous) and housed in a freestall barn with pens that had 3 rows of stalls. Cows were monitored for signs of calving every 45 min by farm personnel. At the first signs of calving (visualization of the allantoic sac through the vulva, restlessness, discomfort), cows were moved to a loose housing pen to evaluate and assist calving, or both. Immediately after calving, cows were moved to another loose housing pen with straw bedding for 1 d. Thereafter, cows were moved to a postpartum pen if farm personnel considered them healthy. Primiparous and multiparous cows were commingled in the postpartum pen for about the first 30 DIM. Thereafter, cows were moved to pens based on lactation number (first, second, third and fourth or more) for the rest of their lactation. Cows with health disorders that were treated with antibiotics and required milk withdrawal were placed in a separate pen, and their milk was discarded until it was saleable.

Cows were milked 3 times per day, and individual milk yield and conductivity were recorded at each milking (Afimilk, Kibbutz Afikim, Israel). The projected 305-d milk production for cows that calved during the study period was 13,036 kg. Cows had ad libitum access to feed and water and were fed a TMR once daily. A detailed description of the diets fed during the study is presented in Table 1.

Study Design

The study followed an observational prospective cohort design. Cows were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags; SCR Dairy, Netanya, Israel) approximately 4 wk before calving to monitor rumination time and physical activity from at least 21 d before expected calving until at least 80 d after calving (Figure 1). Rumination raw data were recorded in minutes per 2 h interval; activity raw data were also recorded every 2 h as an arbitrary number. Activity level was determined by a 3-axis accelerometer that recorded the speed and angle of head movements. Based on these 2 parameters, the system software generated an arbitrary number from 0 to 255. These data were summarized as an arbitrary number per unit of time (2 h or 24 h). Data from individual tags were transferred to the system software (Dataflow; SCR Dairy) automatically every 20 min via antennas in the barns. Based on rumination and activity data, the system software generated a HIS (0 to 100 arbitrary units) for each cow using a series of internal algorithms (proprietary to SCR Dairy). A HIS of 100 arbitrary units represents a cow with an ideal pattern of rumination and activity; a HIS value of <86 arbitrary units may be indicative of a health disorder. Thus, HIS is designed to serve as an alert system to help dairy farm personnel identify cows for further clinical examination. A HIS report was generated daily to include cows with <86 arbitrary units (as determined by SCR) and stored for evaluation by the research group. During the study, farm personnel did not have access to the HIS report or any information generated by the AHMS.

Clinical Examination and Case Definitions

Before initiation of the study, the health-monitoring program was defined and discussed by the research team, the herd veterinarian, and the farm personnel responsible for conducting daily health monitoring. All farm personnel (n = 5) conducted the clinical evaluation of cows on a daily basis (1 or 2 people per day) and all had 2 to 10 yr of experience monitoring cow health at the time the study was conducted.

After calving, cows were examined daily from 1 to 10 DIM. The clinical examination included direct observation (general appearance and attitude, presence of fetal membranes outside the vulva, evaluation of vaginal discharge, foot health, udder health, and manure consistency), rectal temperature, urine ketones, and rumen auscultation. For any cow not diagnosed with metritis before 8 DIM, transrectal massage of the uterus was conducted to obtain and evaluate uterine discharge. Further, the progression of milk production and deviations in daily milk weights (difference between recorded and expected milk) were used as an aid in the identification of cows with health disorders during the entire lactation. Each disease of interest was defined, and case definitions were provided to farm personnel. Specific health disorders monitored included (1) retained placenta: failure to expel fetal membranes within 24 h after calving; (2) milk fever: fresh cow with low body temperature, muscle tremors, inability to rise, and in some cases lying down in abnormal reclining position with the head stretched over the back; (3) ketosis: decreased appetite, test at or above “moderate” using a urine ketone strip test (KetoStix, Bayer Diagnostics, Tarrytown, NY), and altered pattern of milk production (reduction from expected); (4) DA: movement of the fourth compartment of the stomach to an abnormal position on the right or left side of the abdomen, detected by auscultation of a “ping” sound with finger percussion; (5) indigestion: scant manure and lack of appetite with rumen and intestinal stasis; (6) metritis: watery, pink/brown, and fetid uterine discharge with or without fever (defined as rectal temperature >39.5°C); (7) clinical mastitis: swelling or pain in the udder, milk with an abnormal appearance (milk was stripped onto the floor and observed for flakes or clots), signs of udder inflammation possibly accompanied by depressed attitude, anorexia, and fever; (8)

pneumonia: fever, increased respiratory rate and effort, nasal discharge, and malodorous breath; and (9) lameness: cows with locomotion score ≥3 (1 = normal locomotion and 5 = severely lame).

After 10 DIM, cows were monitored daily following the same criteria, except that urine ketones were evaluated only in cows suspected of having ketosis, uterine discharge in cows suspected of having metritis, and rectal temperature in cows suspected of having metritis, mastitis, or other infectious disorders. Cows with health disorders after 10 DIM were monitored daily (or more frequently as needed) until recovery. Two consecutive episodes of the same disorder were evaluated separately when they occurred at least 7 d apart.

Blood Collection and Laboratory Analyses

Blood samples were collected on d −11 ± 3 and −4 ± 3 prepartum, and 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 after calving (Figure 1) from a subgroup of cows (n = 459). Blood was collected via puncture of the coccygeal vein or artery using evacuated tubes containing sodium heparin (Vacutainer; Becton Dickinson, Franklin Lakes, NJ). Samples were immediately placed on ice or refrigerated at 4°C and then transported to the laboratory for further processing (centrifugation at 2,000 × g, 15 min). Samples were stored at −20°C until assayed. Plasma samples were analyzed on specific days relative to calving for NEFA (d −11, −4, 0, 3, 7, 14, 28), BHB (d 0, 3, 7, 14, 28), total Ca (d 0, 3, 7, 14), and haptoglobin (d −4, 0, 3, 7, 14, 28).

Plasma concentrations of NEFA were analyzed in triplicate by enzymatic analysis using a commercial kit (NEFA-HR2; Wako Diagnostic Inc., Richmond, VA). Plasma concentrations of BHB were analyzed in triplicate by enzymatic analysis (BHB dehydrogenase) following an adaptation of the Sigma kit 310-UV using enzyme 3-HBDH from Rhodopseudomonas sphaeroides (Roche Diagnostics Corp., Indianapolis, IN) and standards made from dl-βhydroxybutyric acid sodium salt (Sigma-Aldrich, Saint Louis, MO). All spectrophotometric measurements were done using a Spectramax 190 microplate reader (Molecular Devices, Sunnyvale, CA). Intra- and interassay CV for the NEFA assay were 2.6 and 9.5%, respectively, and for the BHB assay were 12.8 and 24.0%, respectively.

Plasma total Ca concentrations were analyzed by spectrophotometric method using an enzymatic assay and following standard procedures for the Roche/Hitachi Modular P Chemistry Analyzer (Roche Diagnostics, Rotkreuz, Switzerland) at the Animal Health Diagnostic Center of the Cornell University College of Veterinary Medicine (Ithaca, NY).

Haptoglobin concentrations were evaluated in duplicate using an enzymatic analysis that measures haptoglobin-hemoglobin complex by estimated differences in peroxidase activity as described in Bicalho et al. (2014). Estimations of haptoglobin concentrations were calculated against a standard curve from 0 to 2.5 mg/mL (Molecular Innovations, Novi, MI). Samples with initial haptoglobin concentrations of 2.5 mg/mL were reanalyzed after dilution 1:4 in distilled water. Measurements were done using a Spectramax 190 microplate reader (Molecular Devices). Intra- and interassay CV for the haptoglobin assay were 10.5 and 20.6%, respectively.

Statistical Analysis

System Performance. The main outcome of interest for this study was the ability of the HIS to correctly identify cows with a metabolic or digestive health disorder. Clinical diagnosis by farm personnel was used as the reference test. Because HIS does not confirm the occurrence of disease or indicate the type of disease, a positive outcome was defined as a HIS of <86 arbitrary units on at least 1 d during the 5 d before, the day of, or 2 d after the disease CD. The sensitivity and 95% CI of the HIS for each disease of interest (DA, ketosis, indigestion) was calculated using PROC FREQ in SAS (version 9.4; SAS Institute Inc., Cary, NC) and was defined as the ability of the HIS to correctly identify cows with a positive CD outcome. The overall sensitivity of HIS to identify all cows with metabolic and digestive disorders combined was also calculated. To evaluate the potential confounding effect of other health disorders (i.e., all disorders of interest, pneumonia, and lameness) on the sensitivity of HIS, we conducted 3 separate analyses. The first analysis included all cows diagnosed with DA, ketosis, or indigestion (regardless of the occurrence of another disorder during the range of interest around CD); a second analysis included cows diagnosed only with DA, ketosis, or indigestion during the range of interest around CD; and a third analysis included cows diagnosed with the disorder of interest and at least one other health disorder. Data for mastitis and metritis are presented in 2 companion manuscripts (Stangaferro et al., 2016a,b). We determined differences in the sensitivity of HIS between the subgroup of cows with only the disorder of interest and cows with the disorder of interest and at least another disorder by logistic regression using the events over trials option of PROC LOGISTIC in SAS. Retained placenta and milk fever were recorded, but these disorders were not included in the analysis because both are easily identified with a simple visual inspection of the cow. Pneumonia (n = 31) and lameness (n = 49) events were also recorded but were not included in the analysis because they were not of interest in the current study.

Interval between the First Positive HIS Outcome and Clinical Diagnosis. To determine if HIS was capable of identifying cows with metabolic and digestive disorders earlier than CD by farm personnel, the interval (in days) between the first positive HIS outcome for each health event during the period of interest around CD (5 d before to 2 d after) and the day of CD was evaluated. For this analysis, which included only cows flagged by the AHMS, we compared the mean number of days from the first HIS positive outcome and the day of CD with a paired t-test conducted with the PROC TTEST in SAS.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis. Daily rumination time (min/d), daily activity (arbitrary units/d), and HIS (arbitrary units) were evaluated from 5 d before to 5 d after CD (d 0) for the first event of each disorder of interest. Before statistical analysis, we assessed the normality of the data for rumination, activity, and HIS using the Shapiro-Wilk statistic and graphical methods (histogram and Q-Q plot) and using PROC UNIVARIATE in SAS. Based on this analysis, no data transformations were necessary. For cows with each disorder of interest, cows were grouped according to the following criteria: health disorder diagnosed and HIS-positive (**HI+**; HIS <86 arbitrary units at least 1 d within 5 d before, the day of, and 2 d after CD); health disorder diagnosed and HIS-negative (**HI−**; HIS ≥86 arbitrary units from 5 d before, the day of, and 2 d after CD); and CD negative (nondisease, **ND**; cows not diagnosed with a health disorder during the study period). For cows in the ND group, we considered the average DIM at CD for cows with health disorders to be "day 0" (calculated for each disorder). Data were analyzed by ANOVA with repeated measurements using PROC MIXED in SAS. Models for each outcome of interest (rumination, activity, and HIS) included group (HI+, HI−, ND), time, group-by-time interaction, parity, and group-by-parity interaction as explanatory variables. The occurrence of another health disorder (i.e., all disorders of interest, pneumonia, and lameness) during the −5 to 5 d period after CD was offered as a categorical variable (0 = no occurrence and 1 = occurrence) to the initial models to evaluate the potential effect of multiple disorders on the parameter of interest. The final model for each parameter of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Akaike's information criterion. Cow within group was included as a random effect in all models. Cow was the subject of repeated measurements, and all models were run using an autoregressive (AR-1) covariance structure. Group, time, and group-by-time interaction were forced in all models. When the main effect or interaction between explanatory variables was significant, we used the LSD post hoc mean separation test to determine specific differences between groups of means.

Differences (in units of measurement) and percentage change between 5 d before CD and the day of the nadir for rumination, activity, HIS, and milk production for cows in the HI+, HI−, and ND groups were analyzed using PROC MIXED in SAS. The statistical models contained group (HI+, HI−, and ND), parity, and group-by-parity interaction as explanatory variables. The occurrence of another health disorder during the −5 to 5 d period after CD was also offered to the initial models. The final model for each variable of interest and the mean separation test were conducted as described.

Plasma Concentrations of NEFA, BHB, Calcium, and Haptoglobin. Depending on the parameter of interest, plasma samples were analyzed at different days relative to calving (NEFA: d −11, −4, 0, 3, 7, 14, and 28; BHB: d 0, 3, 7, 14, and 28; Ca: d 0, 3, 7, and 14; haptoglobin: d −4, 0, 3, 7, 14, and 28). Cows were grouped in the same way as for evaluation of rumination, activity, and HIS pattern changes over time. Data were analyzed by ANOVA with repeated measurements using PROC MIXED in SAS as described for the other repeated measurement analyses.

Data for proportions are presented as arithmetical means and 95% CI; quantitative data are presented as LSM ± SEM or 95% CI, unless otherwise stated. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Disease Incidence and System Performance

Of 1,121 cows enrolled in the study, 41 (3.7%) were removed from the data set due to tag malfunction or misplacement during data collection. Thus, 1,080 cows were included in the final data set for analysis. Of those, 42% (n = 451) had no health disorders during the observation period and were included in the ND group, whereas 58% (n = 629) had at least 1 health disorder event during the study. Of the cows with health disorders, 70% had only one event and 30% had more than one.

Table 2 summarizes the incidence of metabolic and digestive disorders by type of disorder, DIM at CD, the sensitivity of HIS to identify cows with metabolic and digestive disorders, and the mean interval between the first positive HIS outcome (positive outcomes only) and CD. Except for indigestion, the sensitivity of HIS to detect cows with DA, ketosis, and all metabolic disorders combined was greater than 90%, and cows were identified earlier ($P < 0.01$) based on HIS than through CD by farm personnel. We observed no differences in sensitivity for cows with only the metabolic and digestive disorder of interest compared with cows with more than 1 disorder during the range of interest around CD.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis

Daily rumination time, activity, and HIS patterns from −5 to 5 d after CD for each disorder of interest are shown in Figures 2 and 3. In all cases, parity and group-by-parity interaction were not significant. For cows diagnosed with DA (Figure 2A, B, C), we observed an interaction between group and day ($P < 0.01$) for rumination. Only 1 cow with DA was in the HI− group. Data from this cow are presented in figures but were not included in the statistical analysis. Rumination was lower for cows in the in HI+ group (n = 40) than in the ND group (n =

435) from −5 to 1 d relative to CD, reaching a nadir (~216 min/d) on d 0. Thereafter, rumination increased for cows in the HI+ group until it was similar to that of cows in the ND group from 2 to 5 d after CD. We detected an interaction between group and day ($P < 0.01$) for activity. Cows in the HI+ group had lower activity than cows in the ND group from −5 to 4 d relative to CD, with the lowest value (~360 arbitrary units/d) on d 0. We detected an interaction between group and day ($P < 0.01$) for HIS, because cows in the HI+ group had lower HIS than cows in the ND group from −5 to 2 d after CD, reaching the lowest value on d 1 (~75 units). For cows with DA, HIS was also affected ($P < 0.01$) by the occurrence of ketosis. Cows with ketosis had lower HIS than cows without ketosis during the period of interest around CD (86 ± 2 vs. 93 ± 2 arbitrary units, respectively).

For cows diagnosed with ketosis (Figure 2D, E, F), we observed an interaction between group and day ($P < 0.01$) for rumination. Cows in the HI+ (n = 44) group had lower rumination time than cows in the ND group (n = 435) during the entire period analyzed, reaching a nadir (~275 min/d) on d −1. Cows in the HI− group (n = 5) had higher rumination time than cows in the HI+ group from −3 to 5 d relative to CD, but we observed no differences with the ND group. We detected an interaction between group and day ($P = 0.01$) for activity in cows with ketosis. Daily activity was lower for cows in the HI+ group than in the ND group during the entire period analyzed, and the HI− group had similar activity to cows in the HI+ and ND groups. Daily activity for cows in the HI+ group reached its lowest value on d 1 (~380 arbitrary units/d) relative to CD. We observed an interaction between group and day ($P < 0.01$) for HIS. Cows with ketosis in the HI+ group had lower HIS than cows in the ND group from −3 to 5 d relative to CD and lower HIS than cows in the HI− group from −2 to 3 d relative to CD. The nadir for cows in the HI+ group was observed on d 0 and 1 (~74 units of HIS). For cows with ketosis, HIS was also affected by the occurrence of DA and indigestion, because cows with DA had lower HIS ($P = 0.01$) than cows without DA during the period of interest around CD (78 ± 3 vs. 85 ± 2 arbitrary units, respectively), and cows with indigestion had lower HIS ($P < 0.01$) than cows without indigestion during the period of interest around CD (71 ± 4 vs. 91 ± 1 arbitrary units, respectively).

For cows with indigestion (Figure 3A, B, C), we observed an effect of group ($P < 0.01$) and a tendency for an interaction between group and day ($P = 0.06$) on rumination. Only 1 cow with indigestion was in the HI− group. Data from this cow are presented in figures but were not included in the statistical analysis. Cows in the HI+ group (n = 8) had lower rumination than cows in the ND group (n = 435), reaching a nadir (~304 min/d) on d 0. We observed an effect of group ($P < 0.01$) for activity, because activity was lower for cows in the HI+ than the ND group, with the lowest value (~382 arbitrary units/d) on d 1. For HIS we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group had lower HIS than cows in the ND group from 0 to 4 d after CD, with the lowest values on d 1 and 2 (~74 units of HIS). For cows with indigestion, HIS was also affected ($P < 0.01$) by the occurrence of pneumonia. Cows with pneumonia had lower HIS than cows without pneumonia during the period of interest around CD (72 ± 5 vs. 94 ± 2 arbitrary units, respectively).

For all metabolic and digestive events combined (Figures 3D, 3E, and 3F), we observed an interaction between group and day ($P < 0.01$) for rumination. Daily rumination time was lower for cows in the HI+ group (n = 92) than in the ND group (n = 435) during the entire period analyzed, reaching a nadir (~262 min/d) on d 0. Cows in the HI− group (n = 7) had higher rumination time than cows in the HI+ group from −5 to 4 d relative to CD. Compared with the ND group, cows in the HI− group had lower rumination time on d −5 relative to CD. Rumination time for cows with metabolic and digestive disorders was also affected by the occurrence of pneumonia, because cows with pneumonia had reduced rumination time ($P < 0.01$) than cows without pneumonia during the period of interest around CD (325 ± 31 vs. 463 ± 6 min/d, respectively). Activity patterns were also affected by the interaction between group and day ($P < 0.01$). Daily activity was lower for cows in the HI+ group than for cows the HI− and ND groups during the entire period analyzed, with the lowest value (~377 arbitrary units/d) on d 0 relative to CD. Finally, we observed an interaction between group and day ($P < 0.01$) for HIS. Cows in the HI+ group had lower HIS than cows in the HI− and the ND groups from −5 to 4 d, after CD reaching a nadir (~74 units of HIS) on d 1. For cows with metabolic and digestive disorders, HIS was also affected by the occurrence of metritis, pneumonia, and lameness during the period of interest around CD. Cows with metritis had lower HIS ($P = 0.05$) than cows without metritis (80 ± 2 vs. 83 ± 3 arbitrary units, respectively). Cows with pneumonia had lower HIS ($P < 0.01$) than cows without pneumonia (73 ± 3 vs. 91 ± 2 arbitrary units, respectively). Cows with lameness had lower HIS ($P < 0.01$) than cows without lameness (77 ± 4 vs. 86 ± 1 arbitrary units, respectively).

Table 3 includes differences (in units of measurement) and percent change between 5 d before CD and the day of the nadir for rumination, activity, HIS, and milk production for all metabolic and digestive disorders combined.

Plasma Concentrations of NEFA, BHB, Calcium, and Haptoglobin

Plasma concentration of NEFA, BHB, Ca, and haptoglobin relative to the day of calving for cows that developed DA are presented in Figure 4. Only 1 cow had a DA in the HI− group. Data from this cow are presented in figures but were not included in the statistical analysis. For NEFA, we observed an interaction between group and day ($P < 0.01$). Plasma concentrations of

NEFA were greater for cows in the HI+ group than in the ND group from −11 to 14 d after calving. Concentrations of NEFA for cows in the HI+ group peaked 0 d (~663 μEq/L) and 7 d after calving (~673 μEq/L), with differences of ~257 μEq/L and ~332 μEq/L between the HI+ and ND groups 0 and 7 d after calving, respectively. In addition, NEFA concentrations were also affected ($P < 0.01$) by the occurrence of ketosis. Cows with ketosis had greater NEFA concentrations than cows without ketosis (675 ± 62 vs. 308 ± 5 μEq/L, respectively). For concentrations of BHB in plasma, we also observed an interaction between group and day ($P < 0.01$). Plasma BHB concentrations were greater for cows in the HI+ group than in the ND group from calving to 14 d after calving. Concentrations of BHB reached their highest value (~15 mg/dL) 14 d after calving. The greatest difference in BHB concentrations between the HI+ and ND groups was observed at 14 d after calving (7 mg/dL). For concentrations of total Ca in plasma, we observed an effect of group ($P < 0.01$), but no group-by-day interaction ($P = 0.12$). Cows in the HI+ group had lower total Ca concentrations than cows in the ND group during the entire sampling period, reaching nadir concentrations (~7.7 mg/dL) and the greatest difference compared to cows in the ND group (~1.0 mg/dL) 3 d after calving. Concentrations of haptoglobin in plasma were affected by the interaction between group and day ($P = 0.01$). Cows in the HI+ group had greater haptoglobin concentrations than cows in the ND group 4 d before calving and from 3 to 28 d after calving, reaching peak concentrations (~3.9 mg/mL) and the greatest difference compared to cows in the ND group (~2.0 mg/mL) at 3 d after calving.

Plasma concentration of NEFA, BHB, Ca, and haptoglobin relative to the day of calving for cows that developed ketosis are presented in Figure 5. No cows with ketosis and from the HI− group were sampled (or represented). For plasma NEFA concentrations, we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group had greater NEFA concentrations than cows in the ND group during the entire period analyzed. Peak concentrations for NEFA were observed 0 d (~907 μEq/L) and 7 d (~876 μEq/L) after calving, with differences between the HI+ and ND groups of ~509 μEq/L and ~543 μEq/L 0 and 7 d after calving, respectively. In addition, NEFA concentrations were affected by the occurrence of metritis and mastitis, because cows with metritis had greater (P = 0.03) NEFA concentrations than cows without metritis (287 ± 45 vs. 144 ± 79 μEq/L, respectively), and cows with mastitis had greater (P < 0.01) NEFA concentrations than cows without mastitis (407 ± 29 vs. 124 ± 99 μEq/L, respectively). For plasma BHB concentrations, we observed an interaction between group and day (P < 0.01). Cows in the HI+ group exhibited greater BHB concentrations than cows in the ND group from 0 to 28 d after calving, with concentrations of ~16 mg/dL at 14 d after calving. The greatest differences for BHB concentrations between the HI+ and ND groups were observed at 14 d (8.3 mg/dL) after calving. In addition, BHB concentrations were affected by the occurrence of metritis, mastitis, and indigestion. Cows with metritis had greater (P = 0.04) BHB concentrations than cows without metritis (10.5 ± 1.1 vs. 8.2 ± 1.7 mg/dL, respectively), cows with mastitis had greater (P < 0.01) BHB concentrations than cows without mastitis (12.1 ± 1.0 vs. 6.6 ± 2.0 mg/dL, respectively), and cows with indigestion had greater (P < 0.01) BHB concentrations than cows without indigestion (12.4 ± 2.0 vs. 6.3 ± 1.0 mg/dL, respectively). For plasma total Ca concentration, we observed a group-by-day interaction (P = 0.01). Total Ca concentrations were lower for cows in the HI+ group than cows in the ND group from 7 to 14 d after calving, with the greatest differences between the HI+ and ND groups 7 and 14 d (~0.6 mg/dL) after calving. Total Ca concentrations were also affected by the occurrence of indigestion, because cows with indigestion had lower (P < 0.01) Ca concentrations than cows without indigestion (7.8 ± 0.4 mg/dL vs. 8.9 ± 0.1, respectively) during the period of interest around CD of ketosis. For plasma haptoglobin concentrations, we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group had greater haptoglobin concentrations than cows in the ND group from 3 to 14 d after calving, with the highest concentration (~3.6 mg/mL) 3 d after calving. Haptoglobin concentrations were also affected by the occurrence of DA and pneumonia. Cows with a DA had greater ($P < 0.01$) haptoglobin concentrations than cows without DA (3.9 ± 0.6 vs. 2.4 ± 0.4 mg/mL, respectively), and cows with pneumonia had greater ($P < 0.01$) haptoglobin concentrations than cows without pneumonia (4.1 ± 0.8 vs. 2.3 ± 0.2 mg/mL, respectively).

Data for cows with indigestion are not presented, because they were available from only 3 cows in the HI+ group and 1 cow in the HI− group. Briefly, cows in the HI+ group had greater NEFA concentrations than cows in the ND group from 0 to 14 DIM ($P < 0.01$), greater BHB from 3 to 14 DIM ($P < 0.01$), lower total Ca concentrations ($P < 0.01$), and no differences in haptoglobin concentrations ($P = 0.20$).

DISCUSSION

The timely detection of cows with health disorders during the early postpartum period requires consistent implementation of a comprehensive health-monitoring program. This observational cohort study was designed to evaluate the feasibility of using an automated rumination and physical activity monitoring system to identify lactating dairy cows with metabolic and digestive disorders. Using CD by farm personnel following specific criteria for defining health disorders and standard operating procedures as reference, we found the sensitivity of the system based on HIS to be high-ranging, from 89% to 98% for each individual disorder and 93% for all disorders combined. We observed the greatest sensitivity for cows that developed DA, intermediate sensitivity for cows with ketosis, and lowest sensitivity for cows with indigestion. Likely, the differences in sensitivities are a reflection of the severity of the disorder or the number of cows included in the study for some of the specific conditions. For example, an episode of DA is more disruptive to cow health than an episode of ketosis. In addition, it is possible that cows with a DA were also ketotic before the DA was diagnosed (Geishauser et al., 1997; LeBlanc et al., 2005) affecting all of the parameters evaluated for several days before CD. For indigestion, we speculate that the low number of cows with the disorder may have been the main contributor to the lower sensitivity, because only 1 cow out of 9 was not flagged based on HIS. Interestingly, we did not observe higher sensitivity of HIS for cows with more than 1 health disorder, in spite of the fact that the occurrence of more than 1 disorder at the same time caused changes to patterns of rumination, activity, and HIS. These observations provided additional evidence that the sole occurrence of the metabolic and digestive disorders evaluated caused alterations to rumination and activity of sufficient magnitude to trigger a HIS alert.

We also speculate that cows not flagged based on HIS had a less severe episode of the disorder. Although we did not establish a severity score, this notion is based on the daily pattern of the parameters evaluated during the 5 d before and 5 d after CD for the ND group and those with the disorders. For cows with ketosis and all cows with metabolic and digestive disorders combined, cows in the HI− group demonstrated patterns of rumination, activity, and HIS that were not different from cows in the ND group for most of the days evaluated. More importantly, the rumination and activity patterns used to calculate HIS did not show a dramatic reduction in the days before CD. This was particularly relevant to the AHMS used in this study, because it did not compare cows to their herd mates; instead, it used changes in pattern of rumination and activity for individual cows. For the single cow that had a DA and was not flagged by the

AHMS, rumination and activity showed a marked decline, resulting in a reduction in HIS. However, this cow was not flagged because the HIS was not below the 86-point cutoff on any day evaluated. Interestingly, the nadir for rumination, activity, and HIS for this cow was observed 1 d before CD, and rumination showed a dramatic rebound before surgical treatment.

The notion that the severity of the disorders was different for cows in the HI− and HI+ groups is also supported by the changes in rumination, activity, HIS, and milk production between 5 d before CD and the day of the nadir for each parameter. Except for activity, which was similar for cows in the HI− and HI+ groups, we observed minor reductions or slight increments in rumination, HIS, and milk production for cows in the HI− and ND groups. This is in sharp contrast to the dramatic reductions in rumination, activity, and HIS for cows in the HI+ group. Notably, milk production in the HI+ group decreased by 8 kg or 27% from 5 d before to the day of CD. Although these results may question the validity of the CD and the economic benefits of treating cows not flagged by the AHMS, there may have been potential benefits to cow health and welfare that should not be overlooked if the cows had truly developed the disorder. Our study does not provide evidence that cows in the HI− group did not have a reduction in milk production or overall performance beyond the days included in the analysis. In this regard, previous studies have documented reductions in milk production in cows with ketosis and DA earlier than 5 d from CD (Lucey et al., 1986; Rajala-Schultz et al., 1999; Edwards and Tozer, 2004).

Another major objective of our study was to determine the interval between the time of the first alert based on HIS and the day of CD. Cows in the HI+ group for each disorder showed differences in rumination and activity compared with cows in the ND group as early as 5 d before CD, suggesting that some cows began experiencing consequences of the disorder more than 5 d before CD. Whether this is the result of very early manifestation of the disorder or because these cows did not recover after calving is unknown. Remarkably, cows with DA and ketosis were flagged based on HIS before CD, whereas cows with indigestion were detected by HIS at a similar time to CD. Previous studies have also reported dramatic reductions in rumination (Gaspardy et al., 2014) and activity (Edwards and Tozer, 2004) 5 to 6 d before the occurrence of ketosis and DA in dairy cows. For activity, however, we observed a more consistent decline until the day of CD than previously reported (Edwards and Tozer, 2004). These differences could be explained, at least in part, by differences between studies for the timing and frequency of pen moves around the day of CD and differences between the activity recording systems. Unlike cows with DA and ketosis, the pattern of decline for HIS for cows with indigestion was not as substantial until the day of CD, when a sudden drop in mean HIS below 86 points was observed. This sudden, rather sustained, decline in HIS before CD was the most likely reason for the short time frame between identification by the AHMS and CD.

Earlier identification of cows with health disorders presents opportunities and challenges. Detecting a health disorder at an early stage and before the manifestation of clear clinical signs may benefit cows by improving overall treatment response and reducing the negative long-term consequences of disease on overall cow health and performance (Fourichon et al., 1999; Seifi et al., 2011). Nevertheless, detecting cows with a health disorder at its very early stages may create new challenges, because farm personnel must determine whether the cow truly has a disorder and what the disorder is in the absence of clear clinical signs. Under these circumstances, the selection of a treatment strategy may be limited or less specific than when clinical signs are evident. Additional tests to confirm the presence of subclinical disorders or underlying predisposing factors for clinical diseases may facilitate decision-making (LeBlanc et al., 2005;

McArt et al., 2011, 2012). Future research is warranted to establish criteria for differentiating and treating specific health disorders based on the information provided by the AHMS.

In our study, the pattern of rumination, activity, and HIS for each disorder of interest was useful not only to explore potential differences among cows that were or were not flagged based on HIS, but also to document the pattern for these parameters around the timing of CD. Interestingly, cows in the HI+ group showed the nadir for the parameters measured on the day of or the day after CD, except rumination for ketosis observed on d −1. In agreement, a recent study reported that cows with subclinical ketosis (case definition not reported) had reduced daily rumination time before and during an episode of the disease (accumulated over 6 d) followed by a substantial rebound of ~60 to 100 min per d after resolution of the disorder (Gaspardy et al., 2014). Liboreiro et al. (2015) also reported differences between cows without a health disorder and cows with subclinical ketosis (BHB in blood >1,000 μmol/L) at 8 and 11 DIM, which was within the range of DIM for cases of ketosis in our study and others (McArt et al., 2012).

For all parameters of interest, their respective nadir was followed by a rebound of different magnitude depending on the disorder and parameter beginning on the day of or the first 2 d after treatment. Likely, this is the reflection of an immediate response to treatment. At the moment, however, it is unknown if cows would have had a further reduction in the parameters monitored if left untreated, because every cow received some form of treatment. Interestingly, Gonzalez et al. (2008) observed a more prolonged nadir and slower feed intake recovery after CD for a non-treated than a treated cow with ketosis.

In spite of the substantial rebound and recovery to levels observed 5 d before CD for cows with ketosis, the mean for all parameters evaluated did not fully match that of cows in the ND group 5 d after treatment. This could be a reflection of incomplete recovery or the fact that some cows developed 2 disorders sequentially. Indeed, 43% of the cows diagnosed with ketosis in our study were also diagnosed with DA within 5.1 d after the diagnosis of ketosis. This is not surprising, because ketosis is a major risk factor for DA in lactating dairy cows (LeBlanc et al., 2005; Seifi et al., 2011). On the other hand, the pattern of rumination and activity after CD and treatment may be useful for evaluating the course of disease and response to treatment. A better understanding of the specific patterns of rumination, activity, or parameters created based on their combination (e.g., HIS) for cows that develop different health disorders in the early postpartum period may help in designing specific algorithms to predict the type of disorder the cow had before manifestation of clinical signs.

The association between markers of energy status (NEFA and BHB), mineral balance (Ca), and systemic inflammation (haptoglobin) with the occurrence of metabolic and digestive disorders in dairy cows has been well established (LeBlanc et al., 2005; Huzzey et al., 2011; Seifi et al., 2011; Chapinal et al., 2012). Therefore, the differences in circulating concentrations of NEFA, BHB, Ca, and haptoglobin observed for cows in the ND group compared to cows that developed metabolic and digestive disorders was expected. Because it was not possible to predict which cows would develop health disorders and when, blood samples were collected from a convenient sample of cows on specific days before and after calving. As a result, it was not possible to report the levels of the markers of interest around the timing of occurrence of each particular disorder. However, based on DIM at CD, the pattern of circulating concentrations of all markers was in agreement with the early and dramatic changes observed for the parameters monitored by the AHMS. Within 0 to 3 until 14 DIM, NEFA, BHB, Ca, and haptoglobin concentrations for cows in the HI+ group were different than in the ND group, supporting the notion that these cows were in a more severe plane of negative energy balance, were Ca deficient, and were undergoing more severe systemic inflammation than cows without a health disorder. For cows in the HI− group, the limited number of observations precluded drawing any relevant conclusions, but the notion that these cows were less severely affected by the observed health disorder was partially supported. Whether rumination and activity were lower because of the underlying condition affecting the cow and its effect on feeding behavior, or because of the altered levels of the markers measured in our study, is unknown. Similar associations between blood markers of metabolic and health status and rumination and activity have been reported for lactating dairy cows (Soriani et al., 2012; Liboreiro et al., 2015) but no cause-and-effect relationships were established. When all cows with metabolic and digestive disorders identified based on HIS were combined, we observed differences compared to cows in the ND group for NEFA and haptoglobin before calving. These observations were not surprising, because elevated levels of NEFA and haptoglobin in the immediate prepartum period have been identified as risk factors for DA and ketosis (LeBlanc et al., 2005; Ospina et al., 2010; Huzzey et al., 2011).

CONCLUSIONS

Our findings suggest that monitoring rumination time and physical activity could be useful for identifying cows with metabolic and digestive disorders in the early postpartum period. The HIS created based on rumination and activity by the AHMS showed high sensitivity for identifying cows with metabolic and digestive disorders, and it identified cows with ketosis and DA earlier than CD by farm personnel. The patterns of rumination, activity, and HIS from −5 to 5 d after CD of the disorder of interest were characterized by marked differences compared to cows in the ND group as early as 5 d before CD and showed a nadir around the timing of CD (−1 to 1 d after CD). We observed a considerable recovery for rumination, activity, and HIS after

CD and treatment. For cows with clinical disorders not identified based on HIS, their rumination, activity, and milk production patterns were very similar to that of cows in the ND group and different from cows diagnosed with the disorder but flagged based on HIS. We conclude that automated health-monitoring systems that use rumination and activity are promising tools for the identification of cows with metabolic and digestive disorders in dairy farms.

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Table 1. Summary of formulated feed ingredient amounts and formulated nutrient composition of the diets during the study

	Prepartum		Postpartum	
Feed ingredient or nutrient	Heifers	Cows	Fresh (< 30 DIM)	High (> 30 DIM)
Ingredients, % of DM				
Conventional corn silage	46.6	46.2	23.2	25.5
BMR corn silage	—	—	16.0	12.5
Alfalfa silage	—	—	10.1	13.6
Grass hay	14.5	14.2	—	—
Wheat straw	14.5	14.2	3.9	—
Corn grain finely ground	—	—	17.5	16.7
Citrus pulp (dry)	—	—	—	4.1
Soy Plus[1]	—	—	6.0	5.2
Canola meal (solvent)	—	—	4.0	5.2
Molasses	—	—	5.1	4.3
Acid whey (liquid)	2.2	2.1	0.9	0.7
Prefresh concentrate mix	22.1	21.9	—	—
Fresh concentrate mix	—	—	13.4	—
High concentrate mix	—	—	—	12.2
Anionic mineral supplement	—	1.3	—	—
Nutrient composition (DM basis)				
CP, %	14.3	13.9	16.2	17.2
Ether extract, %	3.8	3.2	5.6	5.3
NDF, %	42.8	42.6	28.3	27.1
Starch, %	17.7	18.6	28.4	27.2
NFC,[2] %	30.2	31.2	42.7	43.1
Ash, %	9.5	9.5	7.3	7.6
Ca, %	1.77	1.75	0.72	0.79
P, %	0.35	0.36	0.40	0.40
Mg, %	0.41	0.47	0.31	0.31
Na, %	0.08	0.06	0.38	0.47
K, %	1.27	1.27	1.48	1.48
Cl, %	0.44	0.59	0.64	0.56
S, %	0.43	0.48	0.24	0.24
DCAD,[3] mEq/kg	-34	-118	213	279

[1]West Central, Ralston, IA.
[2]Calculated as 100 − CP − ether extract − NDF − ash.
[3]Calculated as mEq [(Na + K) − (Cl + S)].

Table 2. Incidence of metabolic and digestive disorders, DIM at clinical diagnosis, sensitivity of health index score (HIS) to detect cows with disorders, and interval between the first HIS-positive outcome and clinical diagnosis (CD) of disorders by farm personnel.

	Cows	Incidence	DIM[1]	Sensitivity		HIS positive to CD[2]		
	(n)[1]	(%)	Mean ± SD	%	(95% CI)	Mean (d)	(95% CI)	*P*-value
DA[3]	41	3.8	14.9 ± 10.5	98 (40/41)	93,100	-3.0	-3.7,-2.3	< 0.01
DA only[4]	20	1.9	19.2 ± 13.1	100 (20/20)	83,100	-3.2	-4.1,-2.2	< 0.01
DA with other disorders[5]	21	1.9	10.9 ± 4.8	95 (20/21)	76,100	-2.8	-3.9,-1.7	< 0.01
Ketosis	54	5.0	9.3 ± 5.4	91 (49/54)	83,99	-1.6	-2.3,-1.0	< 0.01
Ketosis only	28	2.6	10.1 ± 6.7	89 (25/28)	72,98	-0.9	-1.8,0.1	0.06
Ketosis with other disorders	26	2.4	8.3 ± 3.4	92 (24/26)	75,99	-2.4	-3.3,-1.5	< 0.01
Indigestion	9	0.8	8.3 ± 6.9	89 (8/9)	68,100	-0.5	-1.5,0.5	0.28
Indigestion only	7	0.6	7.8 ± 6.1	86 (6/7)	60,100	-0.7	-2.1,-0.8	0.29
Indigestion with other disorders	2	0.2	6.0 ± 0.0	100 (2/2)	16,100	0.0	-	-
All metabolic-digestive[6]	104	9.6	11.4 ± 8.3	93 (97/104)	89,98	-2.1	-2.5,-1.6	< 0.01
Metabolic-digestive only	55	5.1	13.2 ± 10.5	93 (51/55)	82,98	-1.7	-2.4,-1.1	< 0.01
Metabolic-digestive with other disorders	49	4.5	9.3 ± 4.2	94 (46/49)	83,99	-2.5	-3.1,-1.8	< 0.01

[1] DIM = days in milk at event.
[2] HIS-positive to CD = interval in days between the first positive health index score (HIS) outcome (positive outcomes only) and clinical diagnosis (CD).
[2]n = number of events diagnosed.
[3]DA = displaced abomasum.
[4]Cows diagnosed with the disease of interest only from -5 to 2 d relative to CD.
[5]Cows diagnosed with the disease of interest and at least another health disorder from -5 to 2 d relative to CD.
[6]Metabolic-digestive = metabolic and digestive disorders combined (DA, ketosis and indigestion).

Table 3. Differences (in units of measurement) and percent change between 5 d before clinical diagnosis (CD) and the day of nadir for daily rumination time, daily activity, health index score, and milk production for cows in the ND, HI−, and HI+ groups[1] for all metabolic and digestive disorders combined.

Parameter	Change from 5 d before CD to nadir[2]				Percent change from 5 d before CD to nadir[3]			
	Non-disease	HI-	HI+	*P*-value	Non-disease	HI-	HI+	*P*-value
Rumination (min/day)	-5.5 ± 4.4[a]	30.1 ± 27.4[a]	-152.6 ± 12.9[b]	< 0.01	-0.5 ± 1.1[b]	15.9 ± 5.7[a]	-30.9 ± 2.3[c]	< 0.01
Activity (arbitrary units/day)	10.2 ± 3.8[a]	-70.0 ± 23.3[b]	-72.5 ± 10.7[b]	< 0.01	2.8 ± 0.8[a]	-6.8 ± 3.9[b]	-13.1 ± 1.6[b]	< 0.01
HIS (arbitrary units)	-1.3 ± 3.1[a]	-2.1 ± 0.6[a]	-16.7 ± 1.3[b]	< 0.01	-1.3 ± 3.5[a]	-2.0 ± 0.7[a]	-16.1 ± 1.5[b]	< 0.01
Milk (kg/day)	3.3 ± 0.3[a]	1.9 ± 2.7[a]	-7.9 ± 0.9[b]	< 0.01	11.1 ± 1.1[a]	3.9 ± 10.8[a]	-27.0 ± 3.9[b]	< 0.01

[a-b] Different superscripts within a row indicate differences ($P \leq 0.05$) between means based on mean separation with the LSD test.
[1] ND (n = 435) = cows not diagnosed with a health disorder during the study period. For cows in the nondisease group, the average DIM at CD for cows with metabolic disorder, digestive disorder, or both, was considered d 0; HI− (n = 7) = health index score ≥86 arbitrary units from 5 d before, the day of, and 2 d after CD; HI+ (n = 92) = health index score <86 arbitrary units at least 1 d within 5 d before, the day of, and 2 d after CD.[3]Non-disease (n = 435) = cows not diagnosed with a health disorder during the study period. For cows in the Non-disease group, the average DIM at CD for cows that suffered metabolic and/or digestive disorder was considered as "Day 0".
[2]Differences (LSM ± SEM in units of measurement) for rumination, activity, health index score, and milk production between 5 d before and the day of CD or the day of nadir for the parameter of interest (d 0 for rumination, activity, and milk production, and d 1 for health index score).
[5]Percentage change (LSM ± SEM) in rumination, activity, health index score, and milk production between 5 d before CD and the day of CD or the nadir for the parameter of interest.

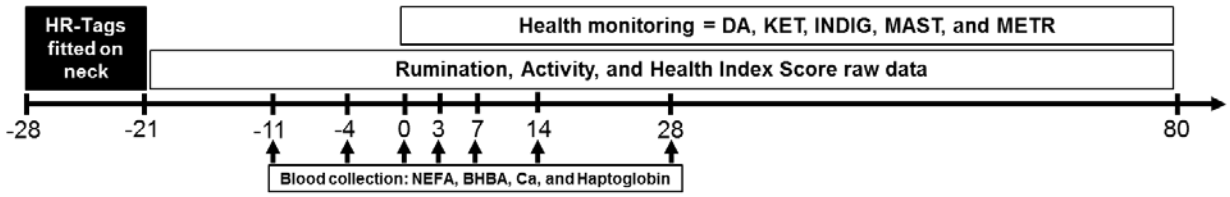

Figure 1. Graphical depiction of the study design. Cows were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags; SCR Dairy, Netanya, Israel) approximately 4 wk before calving to monitor rumination and activity from at least 21 d before calving until at least 80 DIM. Rumination and activity raw data were recorded every 2 h and transferred to the automated health-monitoring system (AHMS) software. The AHMS uses an alert system to flag cows that may have a health disorder. The alert is based on a health index score (HIS; 0 to 100 arbitrary units) created daily for individual cows by combining rumination and activity data. The AHMS performance was evaluated using clinical diagnosis of health disorders by farm personnel as the reference test. The patterns of rumination, activity, and HIS were evaluated around the clinical diagnosis of the health disorders of interest. Health disorders monitored included displaced abomasum, ketosis, indigestion, mastitis, and metritis. Data for displaced abomasum, ketosis, and indigestion are presented in this manuscript; data for mastitis and metritis are presented in 2 companion manuscripts (Stangaferro et al., 2016a,b). Blood samples were collected at −11 ± 3, −4 ± 3, 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 d relative to calving to determine circulating concentrations of nonesterified fatty acids (NEFA), BHB, total Ca, and haptoglobin. DA = displaced abomasum, KET = ketosis, INDIG = indigestion, MAST = mastitis, and METR = metritis.

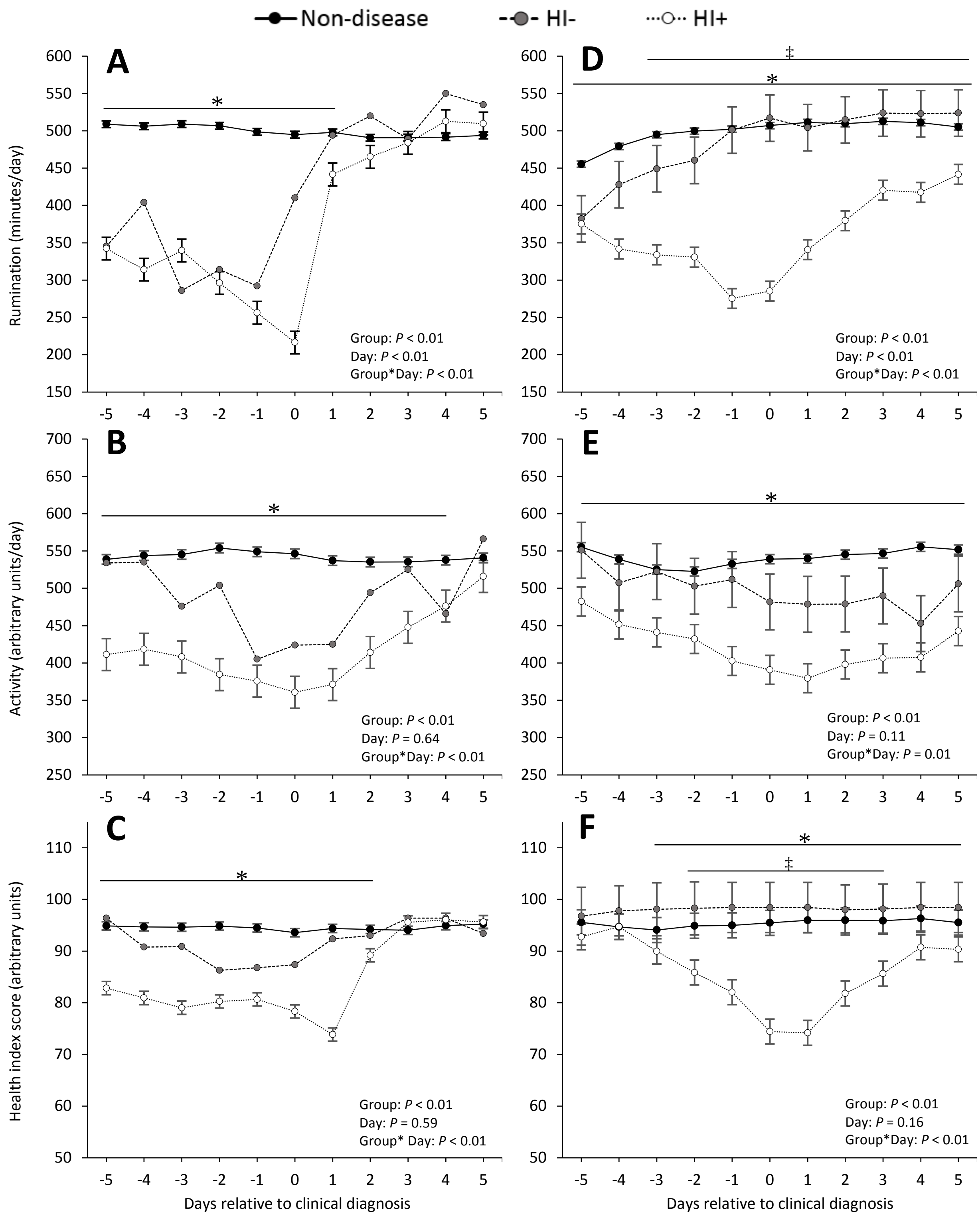

Figure 2. Daily rumination time (A), daily activity (B), and health index score (HIS; C) patterns from −5 to 5 d relative to clinical diagnosis (CD) for cows that developed a displaced abomasum

(DA) and cows in the nondisease (ND) group (n = 435; no health disorders during the study period). Cows with a DA were separated based on HIS patterns around the day of CD into HI+ (n = 40) and HI− (n = 1) groups. Cows were assigned to the HI+ or HI− group if they presented a HIS of <86 or ≥86 arbitrary units (AU), respectively, during the 5 d before, the day of, and 2 d after CD. Data from the single cow in the HI− group are presented in the figure but were not included in the statistical analysis. For cows in the ND group, the average DIM at CD for cows with DA was considered "day 0." Daily rumination time (D), daily activity (E), and HIS (F) patterns from −5 to 5 d relative to CD for cows that developed ketosis and cows in the ND group (n = 435). Cows with ketosis were assigned to the HI+ (n = 44) and HI− group (n = 5) following the same criteria as for cows with a DA. For cows in the ND group, the average DIM at CD for cows with ketosis was considered "day 0." Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; ‡HI+ vs. HI−.

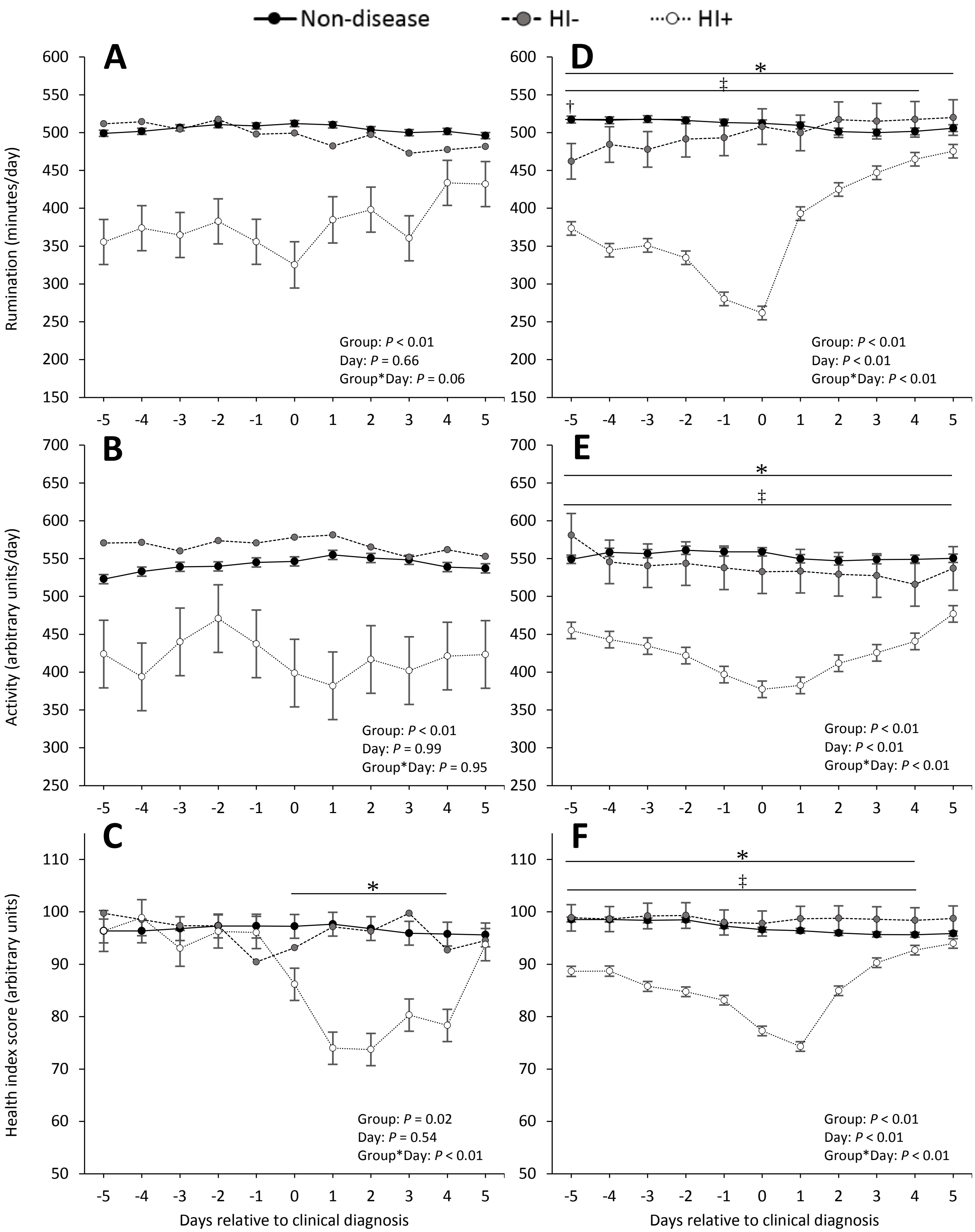

Figure 3. Daily rumination time (A), daily activity (B), and health index score (HIS; C) patterns from −5 to 5 d relative to clinical diagnosis (CD) for cows diagnosed with indigestion and cows

in the nondisease (ND) group (n = 435; no health disorders during the study period). Cows with indigestion were separated based on HIS patterns around the day of CD into HI+ (n = 8) and HI− (n = 1) groups. Cows were assigned to the HI+ or HI− group if they presented a HIS of <86 or ≥86 arbitrary units (AU), respectively, during the 5 d before, the day of, and 2 d after CD. Data from the single cow in the HI− group are presented in the figure but were not included in the statistical analysis. For cows in the ND group, the average DIM at CD for cows with indigestion was considered "day 0." Daily rumination time (D), daily activity (E), and HIS (F) patterns from −5 to 5 d relative to CD for cows that developed metabolic disorders, digestive disorders, or both, and cows in the ND group (n = 435). Cows with disorders were subdivided based on their health index score pattern around the day of CD into HI+ (n = 92) and HI− groups (n = 7) following the same criteria as for cows with indigestion. For cows in the ND group, the average DIM at CD for cows that had metabolic disorders, digestive disorders, or both was considered "day 0." Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; †control vs. HI−; ‡HI+ vs. HI−.

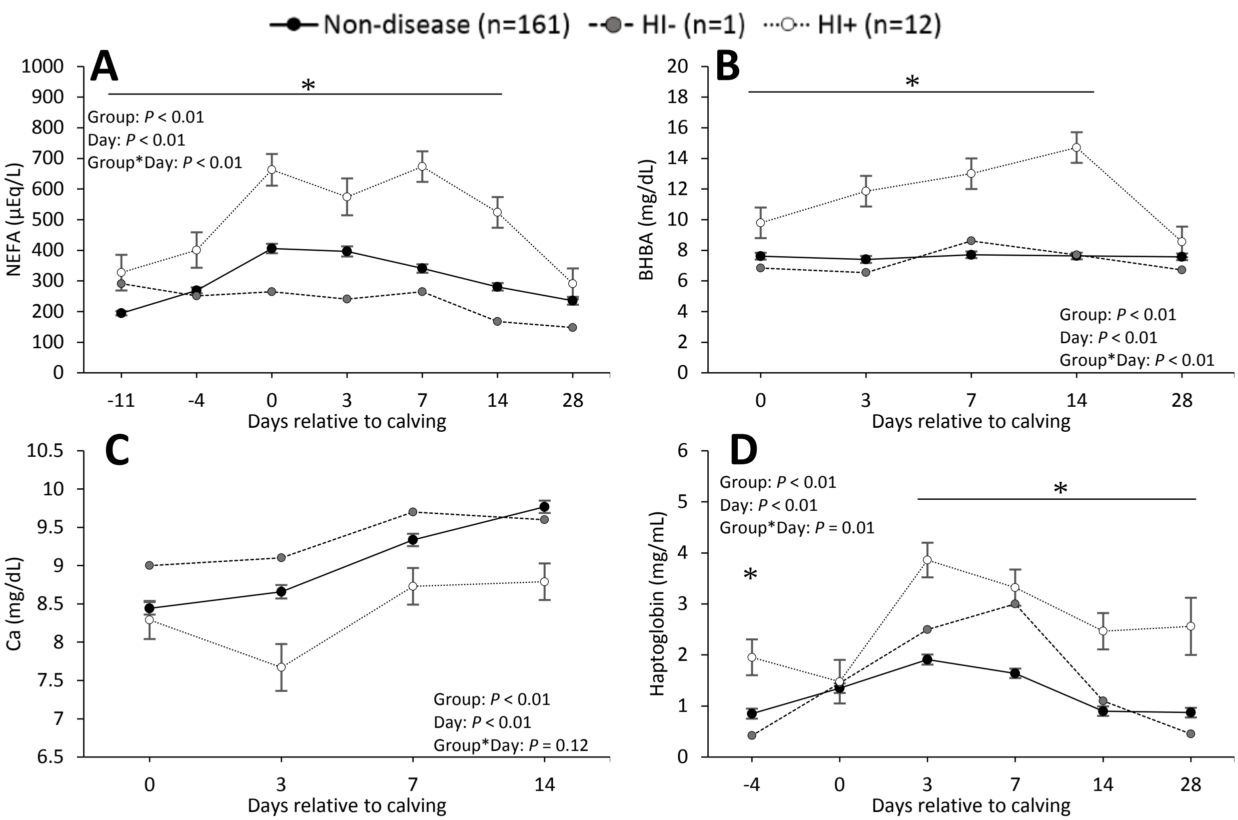

Figure 4. Plasma concentrations of nonesterified fatty acids (NEFA; A), BHB (B), total Ca (C), and haptoglobin (D) for cows that developed a displaced abomasum (DA) and cows in the ND group (n = 161; no health disorders during the study period). Cows with a DA were separated based on health index score (HIS) patterns around the day of clinical diagnosis (CD) into HI+ (n = 12) and HI− (n = 1) groups. Cows were assigned to the HI+ or HI− group if they presented a HIS of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after CD. Data from the single cow in the HI− group are presented in the figure but were not included in the statistical analysis. Values are presented as LSM ± SEM. Within a day, differences ($P \leq 0.05$) for pairwise comparisons between the ND and HI+ groups are represented by an asterisk (*).

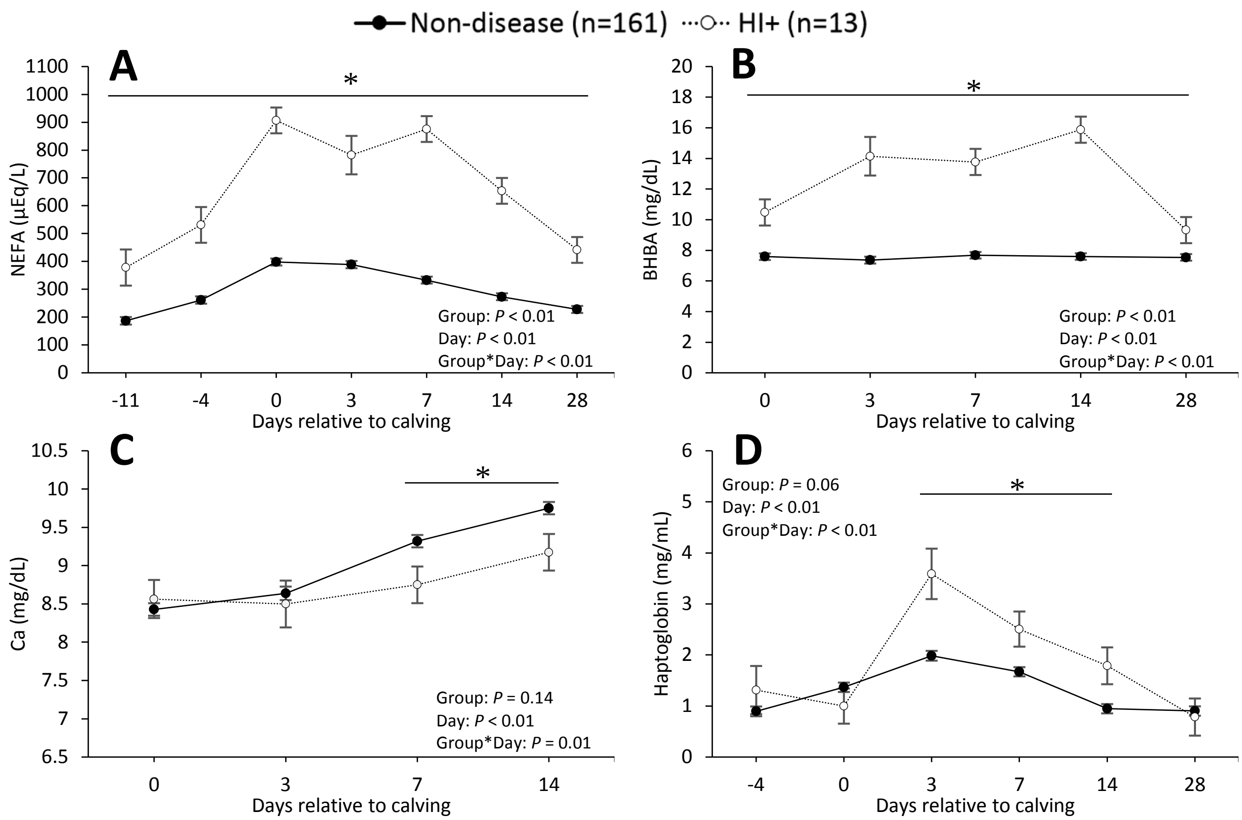

Figure 5. Plasma concentrations of nonesterified fatty acids (NEFA; A), BHB (B), total Ca (C), and haptoglobin (D) for cows that developed a case of ketosis (n = 13) and cows in the ND group (n = 161; no health disorders during the study period). All cows with ketosis were from the HI+ group. Cows were assigned to the HI+ or HI− group if they had a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after clinical diagnosis. Values are presented as LSM ± SEM. Within a day, pairwise differences ($P \leq 0.05$) between the ND and HI+ groups are represented by an asterisk (*).

CHAPTER III

USE OF RUMINATION AND ACTIVITY MONITORING FOR THE IDENTIFICATION OF DAIRY COWS WITH HEALTH DISORDERS: PART II. MASTITIS

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ABSTRACT

The objectives of this study were to evaluate (1) the performance of an automated health-monitoring system (**AHMS**) to identify cows with mastitis based on an alert system (health index score, **HIS**) that combines rumination time and physical activity; (2) the number of days between the first HIS alert and clinical diagnosis (**CD**) of mastitis by farm personnel; and (3) the daily rumination time, physical activity, and HIS patterns around CD. Holstein cows (n = 1,121; 451 nulliparous and 670 multiparous) were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags, SCR Dairy, Netanya, Israel.) from at least −21 to 80 d in milk (DIM). Raw data collected in 2-h periods were summarized per 24 h as daily rumination and activity. An HIS (0 to 100 arbitrary units) was calculated daily for individual cows with an algorithm that used rumination and activity. A positive HIS outcome was defined as an HIS of <86 units during at least 1 d from −5 to 2 d after CD. Blood concentrations of nonesterified fatty acids, β-hydroxybutyrate, total calcium, and haptoglobin were also determined in a subgroup of cows (n = 459) at −11 ± 3, −4 ± 3, 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 DIM. The sensitivity of the HIS was 58% [95% confidence interval (**CI**): 49, 67] for all cases of clinical mastitis (n = 123), and 55% (95% CI: 46, 64; n = 114) and 89% (95% CI: 68, 100; n = 9) for cases of mastitis alone or concurrent with other health disorders, respectively. Among clinical cases, sensitivity was 80.7% (95% CI: 67, 97) for cases caused by *Escherichia coli* (n = 31) and ranged from 45 to 48% for cases caused by gram-positive bacteria (n = 39; *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus* spp., *Staphylococcus* spp., and *Trueperella pyogenes*), *Staphylococcus aureus* (n = 11), or cases with no bacterial growth (n = 25). Days between the first HIS <86 and CD were −0.6 (95% CI: −1.1, −0.2) for all cases of mastitis. Cows diagnosed with mastitis had alterations of their rumination, activity, HIS patterns, and reduced milk production around CD depending on the type of mastitis case. Cows with mastitis also had some alterations of their calcium and haptoglobin concentrations around calving. The AHMS used in this study was effective for identifying cows with clinical cases of mastitis caused by *E. coli* and cows with another disease occurring during an event of mastitis, but it was less effective in identifying cows with mastitis not caused by *E. coli*.

Keywords: rumination, activity, mastitis, dairy cow

INTRODUCTION

Early postpartum health disorders negatively affect cow well-being and are associated with significant economic losses for dairy farms because of alterations to cow health, welfare, and performance (Bareille et al., 2003; Ingvartsen, 2006; Hailemariam et al., 2014). Clinical mastitis is one of the most prevalent disorders affecting cow health and performance (Kaneene and Hurd, 1990; Ingvartsen et al., 2003; Østerås et al., 2007). Mastitis leads to major milk losses (Gröhn et al., 2004; Bar et al., 2007; Schukken et al., 2009) and reduces reproductive performance (Santos et al., 2004; Ahmadzadeh et al., 2009; Hertl et al., 2010); some types of mastitis may severely compromise cow health, leading to increased culling or death (Gröhn et al., 2005; Whist et al., 2009; Hertl et al., 2011).

To detect cows with clinical mastitis, health-monitoring programs include the evaluation of milk characteristics, signs of udder inflammation, and systemic signs of illness (Nash et al., 2002; Wenz et al., 2006). Cases of mastitis caused by certain pathogens such as *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* are more commonly associated with mild changes in milk and udder inflammation (Todhunter et al., 1995; Keefe, 1997; Schukken et al., 2011); cases of mastitis caused by pathogens such as *Escherichia coli* and other gram-negative bacteria are characterized by a severe inflammatory response and systemic compromise (Harmon, 1994; Burvenich et al., 2007; Schukken et al., 2011). Thus, the severity of mastitis may range from mild changes in milk appearance to an important systemic compromise (Sargeant et al., 1998; Bradley and Green, 2001; Nikolić et al., 2003).

Multiple automated data collection systems based on sensors (e.g., daily milk weights, milk composition, electrical conductivity, somatic cell counts) have been tested and are available to detect mastitis through changes in milk production and its attributes (Kamphuis et al., 2008; Koop et al., 2015; Sørensen et al., 2016). Conversely, data about the use of automated rumination time and physical activity monitoring systems to detect cows with mastitis are scarce, because only a few studies have evaluated rumination time in cows with induced clinical mastitis or challenged with LPS (Siivonen et al., 2011; Fogsgaard et al., 2012; Fitzpatrick et al., 2013), and no studies have assessed a combination of both rumination and activity data to identify cows with mastitis. Beyond the potential of using rumination and activity monitoring alone or to complement other methods of mastitis detection, these 2 parameters may provide additional insights into overall cow health that are not provided by other sensor systems that monitor only milk or by clinical examination of cows and milk.

We hypothesized that an automated health-monitoring system (**AHMS**) that continuously monitors rumination and activity would be able to identify cows with mastitis. Also, we expected that changes in rumination and activity before evident clinical signs of disease would result in earlier identification of mastitis. The objectives of this study were to evaluate (1) the performance of an automated rumination and physical activity monitoring system to identify cows with mastitis; (2) the interval between the AHMS alert based on a health index score (**HIS**) and the day of clinical diagnosis (**CD**) by farm personnel; and (3) the rumination, activity, AHMS-generated alert, and milk production patterns for cows with mastitis. We also used markers of energy status [nonesterified fatty acids (**NEFA**) and BHB], mineral status (total calcium), and systemic inflammation (haptoglobin) were used to complement the diagnosis of mastitis and the performance of the AHMS alert.

MATERIALS AND METHODS

Animals and Study Design

All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University. The study followed an observational prospective cohort design. Details about the animals and study design are provided in a companion manuscript (Stangaferro et al., 2016). Briefly, Holstein cows (n = 1,121; 451 nulliparous and 670 multiparous) were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags; SCR Dairy, Netanya, Israel) to monitor rumination and activity from at least 21 d before expected calving until at least 80 d after calving. Of 1,121 cows enrolled in the study, 41 (3.7%) were removed from the data set due to tag malfunction or misplacement during data collection. Thus, 1,080 cows were included in the final data set for analysis. Based on rumination and activity data, an HIS (0 to 100 arbitrary units) for each cow was generated by the system software (DataFlow, Netanya, Israel) using a series of internal algorithms (proprietary to SCR Dairy). An HIS of 100 arbitrary units represents a cow with an ideal pattern of rumination and activity; an HIS value <86 arbitrary units may be indicative of a health disorder. An HIS report was generated daily to include cows with <86 arbitrary units (as determined by SCR) and stored for evaluation by the research group. During the study, farm personnel did not have access to the HIS report or any information generated by the AHMS.

Fresh Cow Monitoring Program and Case Definitions

The fresh cow health-monitoring program and definition of each particular health disorder are provided in detail in a companion manuscript (Stangaferro et al., 2016). In particular, clinical mastitis was defined as swelling or pain in the udder, or milk with abnormal appearance (milk was stripped onto the floor and observed for flakes or clots). Signs of udder inflammation may or may not have been accompanied by depressed attitude, anorexia, and fever. Mastitis monitoring was conducted during milking and during health monitoring of fresh cows. Milk culture was performed on all cows at the beginning of lactation (first milking) and on the day of mastitis diagnosis. Milk samples for pathogen detection were collected aseptically and shipped daily to the Quality Milk Production Services Laboratory at Cornell University (Ithaca, NY). Results were provided to the farm within 24 h of sample retrieval. Culture outcomes were grouped as follows: (1) *E. coli*; (2) *Klebsiella* spp.; (3) gram-positive bacteria (*Strep. agalactiae*, *Strep. dysgalactiae*, *Strep. uberis*, *Streptococcus* spp., *Staphylococcus* spp., *Trueperella* *pyogenes*); (4) *Staphylococcus aureus*; and (5) no important growth after 48 h. Two consecutives episodes of mastitis were considered separate episodes if they occurred at least 7 d apart or in a different quarter.

Blood Collection and Laboratory Analyses

Blood samples were collected from a subgroup of cows (n = 459) on d −11 ± 3 and −4 ± 3 prepartum, and 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 after calving. Plasma samples were analyzed for NEFA (d −11, −4, 0, 3, 7, 14, 28), BHB (d 0, 3, 7, 14, 28), total Ca (d 0, 3, 7, 14), and haptoglobin (d −4, 0, 3, 7, 14, 28). Details about blood collection, plasma separation, and NEFA, BHB, total Ca and haptoglobin analysis are provided in a companion manuscript (Stangaferro et al., 2016).

Statistical Analysis

System Performance. The main outcome of interest for this study was the ability of the HIS to correctly identify cows with mastitis. Clinical diagnosis by farm personnel was used as the reference test. Because HIS does not confirm the occurrence of disease or indicate the type of disease, a positive outcome was defined as an HIS value of <86 arbitrary units during at least 1 d during the 5 d preceding, the day of, or 2 d after CD. The sensitivity and 95% CI for the HIS to identify cows with mastitis was calculated using PROC FREQ in SAS (version 9.4; SAS Institute Inc., Cary, NC) and was defined as the ability of the HIS to correctly identify cows with a positive CD for mastitis. To evaluate the potential confounding effect of other health disorders (i.e., all disorders of interest, pneumonia, and lameness) on the sensitivity of HIS, we conducted 3 separate analyses. The first analysis included all cows with cases of clinical mastitis; a second analysis included cows diagnosed only with mastitis during the range of interest around CD; and a third analysis included cows diagnosed with mastitis and at least one other health disorder during the range of interest around CD. Clinical cases of mastitis were also stratified by milk culture results. For this analysis, cows were separated into the following groups: (1) *E. coli*; (2) *Klebsiella* spp.; (3) gram-positive bacteria (*Strep. agalactiae, Strep. dysgalactiae, Strep. uberis, Streptococcus* spp., *Staphylococcus* spp., *T. pyogenes*); (4) *Staph. aureus*; and (5) no important growth after 48 h. We determined differences in the sensitivity of HIS between the subgroup of cows with mastitis only and cows with mastitis and at least another disorder, and for the different pathogen subgroups, by logistic regression using the events over trials option of PROC LOGISTIC in SAS.

Interval Between the First Positive HIS Outcome and Clinical Diagnosis. To determine if HIS was capable of identifying cows with mastitis earlier than CD by farm personnel, the interval (in days) between the first positive HIS outcome during the period of interest around CD (5 d before to 2 d after) and the day of CD was evaluated. For this analysis, which included only cows flagged by the AHMS, we compared the mean number of days from the first HIS-positive outcome and the day of CD with a paired t-test conducted using the PROC TTEST in SAS.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis. Daily rumination time (min/d), daily activity (arbitrary units/d), and HIS (arbitrary units) were evaluated from 5 d before to 5 d after CD (d 0) for the first event of mastitis. Milk production (kg/d) was evaluated only from 5 d before until the day of CD, because data was not available after CD for cows treated with an antibiotic that required milk withdrawal. Before statistical analysis, we assessed normality of the data for rumination, activity, HIS, and milk production using the Shapiro-Wilk statistic and graphical methods (histogram and Q-Q plot) and using PROC UNIVARIATE in SAS. Based on this analysis, no data transformations were necessary.

Different analyses were conducted using data from groups of cows created based on specific criteria. A first analysis included a nondisease group (**ND**) and the group with clinical mastitis (for first event of mastitis). Thereafter, cows were grouped according to the following criteria: CD-positive and HIS-positive (**HI+**; HIS <86 arbitrary units at least 1 d within 5 d before, the day of, and 2 d after CD); CD-positive and HIS-negative (**HI−**; HIS ≥86 arbitrary units from 5 d before, the day of, and 2 d after CD); and CD-negative (ND; cows not diagnosed with a health disorder during the study period). For cows in the ND group, we considered the average DIM at CD for cows with mastitis "day 0." Data were analyzed by ANOVA with repeated measurements using PROC MIXED in SAS. Models for each outcome of interest included group (e.g., ND and clinical mastitis; and ND, HI+, and HI−), time, and group-by-time interaction as explanatory variables. Parity and group-by-parity interaction were also offered to the initial models. The occurrence of another health disorder (i.e., all disorders of interest, pneumonia, and lameness) during the −5 to 5 d period after CD was offered as a categorical variable (0 = no occurrence and 1 = occurrence) to the initial models to evaluate the potential effect of multiple disorders on the parameter of interest. The final model for each variable of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Akaike's information criterion. Group, time, and group-by-time interaction were forced in all models. Cow within group was included as a random effect in all models. Cow was the subject of the repeated measurements, and all models were run using an autoregressive (AR-1) covariance structure. When the main effect or interaction between explanatory variables was significant, we used the LSD post hoc mean separation test to determine differences between groups of means.

Plasma Concentration of NEFA, BHB, Ca, and, Haptoglobin. Cows were grouped in the same way as for evaluation of rumination, activity, and HIS. Data were analyzed by ANOVA with repeated measurements using PROC MIXED in SAS as described and using the same models as for the other parameters.

Data for proportions are presented as arithmetical means and 95% CI; quantitative data are presented as LSM ± SEM or 95% CI, unless otherwise stated. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Mastitis Incidence and System Performance

The incidence of mastitis and DIM at CD are presented in Table 1. Of all the cows affected with clinical mastitis during the study period (n = 123), 90.2% developed 1 event and 9.8% developed 2 events. The greatest incidence observed was for cases with a culture result for gram-positive bacteria (not including *Staph. aureus*), followed by *E. coli* and no growth after 48 h. Eleven cows with clinical mastitis (8.9% of the total clinical cases) were not included in the analysis for specific pathogens because they had the following culture results: yeast, no culture results, other, or contamination.

The sensitivity of HIS to detect cows with mastitis and the interval between an HIS-positive outcome and a CD of mastitis by farm personnel are also presented in Table 1. The overall sensitivity for clinical mastitis was 58%, with a tendency to be greater ($P = 0.08$) for cows that developed another health disorder from −5 to 2 d after CD than for cows that had mastitis only (89 vs. 55%, respectively). For cases of clinical mastitis stratified by pathogen, the sensitivity of HIS was greater ($P = 0.04$) for mastitis caused by *E. coli* (81%) than for cases caused by *Klebsiella* spp. (33%), gram-positive bacteria (49%), *Staph. aureus* (46%) or no growth after 48 h (48%). Overall, all cases of clinical mastitis were flagged earlier ($P < 0.02$) based on HIS than CD by farm personnel.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis

Daily rumination time, activity, and HIS patterns from −5 to 5 d after CD for cows with clinical mastitis (n = 110; first event of mastitis only), and cows in the ND group (n = 435) are shown in Figure 1. For all parameters, the effect of parity and the group-by-parity interaction were not significant. For rumination, we observed an interaction between group and day ($P < 0.01$). Rumination was lower for cows in the clinical mastitis group than for cows in the ND group from −1 to 3 d relative to CD. Cows with clinical mastitis reached their nadir (~397 min/d) on d −1. We detected an interaction between group and day ($P < 0.01$) for activity. Cows with clinical mastitis had lower activity than cows in the ND group during the entire period analyzed, reaching their nadir (~485 arbitrary units/d) on d 0. We observed an interaction between group and day ($P < 0.01$) for HIS in cows with mastitis. Cows in the clinical mastitis group had lower HIS than cows in the ND group from −1 to 5 d relative to CD, reaching the lowest value (~84 HIS units) on d 0. In addition, HIS was affected by the occurrence of metritis. Cows with metritis had lower HIS ($P < 0.01$) than cows without metritis during the period of interest around CD (88.3 ± 1.7 vs. 95.2 ± 0.1 units, respectively).

Figure 2 includes rumination, activity, and HIS patterns from −5 to 5 d relative to CD for cows with clinical mastitis caused by E. coli or gram-positive bacteria and included in the HI+ and HI− groups. For all parameters, the effect of parity and the group-by-parity interaction were not significant. For cows with mastitis caused by *E. coli* (Figure 2A, B, C), we observed an interaction between group and day ($P < 0.01$) for rumination. Rumination times were lower for cows in the HI+ group (n = 21) than the ND group (n = 435) from −1 to 3 d relative to CD, reaching their nadir (~291 min/d) and the greatest difference with the ND group (~183 min /d) on d 0. Cows in the HI− group (n = 5) had higher rumination time than cows in the HI+ group from −1 to 1 d relative to CD. We detected an interaction between group and day ($P = 0.01$) for activity. Cows in the HI+ group had lower activity than cows in the HI− and ND groups from −1 to 5 d relative to CD. We detected an interaction between group and day ($P < 0.01$) for HIS, such that cows in the HI+ group had lower HIS than cows in the HI− and ND groups from 0 to 2 and from 0 to 3 d after CD, respectively. The HIS for cows in the HI+ group reached their lowest value (~77 units) on the day of CD. In addition, HIS was affected by the occurrence of lameness, because cows with lameness had reduced HIS ($P < 0.01$) than cows without lameness during the period of interest around CD (94.6 ± 0.3 vs. 99.6 ± 1.8 units, respectively).

For cows diagnosed with mastitis caused by gram-positive bacteria (Figure 2D, E, F), we observed an interaction between group and day ($P < 0.01$) for rumination. Cows in the HI+ group (n = 19) had lower rumination than cows in the ND (n = 435) group from −1 to 4 d relative to CD, reaching a nadir (~330 min/d) on d 0. Cows in the HI− group (n = 23) had higher rumination than cows in the HI+ group from 0 to 4 d after CD. We detected an interaction between group and day ($P = 0.05$) for activity. Daily activity was lower in the HI+ group than in the ND group from −2 to 5 d relative to CD; cows in the HI− group exhibited no difference compared with the HI+ or ND groups. We detected an interaction between group and day ($P < 0.01$) for HIS. Cows in the HI+ group had lower HIS than cows in the HI− and ND groups from −2 to 5 d relative to CD, with the lowest values (~86 HIS units) from d 0 to 2. In addition, HIS was also affected by the occurrence of metritis, because cows with metritis had reduced HIS ($P < 0.01$) than cows without metritis during the period of interest around CD (88.6 ± 2.2 vs. 94.8 ± 0.2 units, respectively).

Daily milk production (kg/d) from 5 d before until the day of CD for cows that had clinical mastitis is presented in Figure 3. Because parity had an effect ($P < 0.01$) on milk production, results are presented separately for primiparous and multiparous cows. For primiparous cows (Figure 3A), we observed an interaction between group and day ($P < 0.01$). Cows with mastitis regardless of HIS group (HI+ and HI−) produced less milk than cows in the ND group from −3 to 0 (HI− vs. ND) and from −2 to 0 d (HI+ vs. ND) relative to CD. We observed the greatest difference between cows in the HI+ and ND groups on d −1 (12.8 kg/d), and the greatest difference between cows in the HI− and in the ND group on d 0 (9.5 kg/d). We observed no differences between primiparous cows in the HI+ and HI− groups. For multiparous cows (Figure 3B), we observed an interaction between group and day ($P < 0.01$). Multiparous cows in the HI+ and HI− groups produced less milk than cows in the ND group from −2 d until the day of CD. We observed the greatest difference between the HI+ and HI− groups compared to the ND group on d 0 (16.9 kg/d and 10.1 kg/d, respectively). Cows in the HI+ group produced less milk than cows in the HI− group on the day of CD.

Plasma Concentrations of NEFA, BHB, Ca, and Haptoglobin

Plasma concentrations of NEFA, BHB, Ca, and haptoglobin for cows that developed clinical mastitis before 30 DIM are presented in Figure 4. We detected only an effect of day ($P < 0.01$) for NEFA concentrations, because NEFA were greater on d 0, 3, and 7 than the rest of the days. We detected no effect of group ($P = 0.58$) or day ($P = 0.64$), and no interaction between group and day ($P = 0.83$) for BHB concentrations. For total Ca concentrations, we observed a group-by-day interaction ($P = 0.03$). Cows in the HI+ group had lower Ca concentrations (~1.0 mg/dL different) than cows in the HI− and ND group at 14 DIM. For haptoglobin concentrations, we observed an interaction between group and day ($P = 0.02$). Cows with clinical mastitis (both HI+ and HI− groups) had greater haptoglobin concentrations than cows in the ND group on d 14 and 28.

DISCUSSION

In the current study, we evaluated the performance an AHMS that combined rumination and activity to identify cows with mastitis. Our results support the notion that an HIS alert generated based on rumination and activity would be able to identify cows with more severe cases of mastitis. We observed moderate sensitivity when all cases of mastitis were included in the analysis but substantial differences in sensitivity when cows were stratified based on the presence or absence of another disorder from 5 d before to 2 d after clinical diagnosis of mastitis. Moreover, when cows with mastitis caused by *E. coli* were evaluated individually, the sensitivity of the AHMS was more than 20 percentage points greater than when all cases were combined. This finding was expected, because intramammary infections caused by *E. coli* are characterized by a severe inflammatory response, including sudden shock, sepsis, and often death (Burvenich et al., 2003; 2007; White et al., 2010; Schukken et al., 2011). Indeed, cows affected by *E. coli* and identified by the AHMS (HI+ group) had sudden and dramatic reductions in rumination, activity, and HIS, reaching nadirs similar than those of cows affected by metabolic and digestive disorders (Stangaferro et al., 2016). As a result of the low HIS observed during the nadir (<86 points), most cows were flagged by the AHMS. Conversely, the low sensitivity of the AHMS in detecting cows with mastitis caused by *Klebsiella* spp. was unexpected. Clinical cases caused by this pathogen are usually characterized by clinical signs similar to those observed in mastitis caused by *E. coli* (Harmon, 1994; Radostits et al., 2006). Although we did not have information about the severity of the mastitis event, we speculate that the moderate sensitivity of the AHMS in these cases was due to an over-representation of mild cases among the small number of cows with mastitis caused by this pathogen.

The observed lower sensitivity for cases of clinical mastitis caused by gram-positive pathogens and *Staph. aureus* was also expected, because these pathogens do not cause the same level of toxemia as *E. coli* (Harmon, 1994; Todhunter et al., 1995; Keefe, 1997; Schukken et al., 2011). Based on the patterns of rumination, activity, and HIS for the HI+ group, however, it seemed obvious that this subgroup contained cows with more severe cases of mastitis and systemic compromise. Cows in the HI+ group had significant reductions in rumination and activity within 1 to 2 d before CD and, for some cows, the HIS was below 86 points from the day of and up to 2 d after CD. It is uncertain whether the cases of clinical mastitis with no pathogen growth in cultured milk resembled more closely those caused by *E. coli* or the rest of the pathogens isolated during the study. However, the sensitivity of the AHMS for this subgroup of cows and the patterns of rumination, activity, and HIS (data not shown) closely matched those of cows with mastitis caused by gram-positive pathogens and *Staph. aureus*.

Another interesting aspect of cows affected by clinical mastitis was the lack of difference in milk production between cows in the HI+ and HI− groups around CD (except for d 0 in the multiparous group). Both groups produced less milk on the days leading up to and day of CD than cows with no health disorders. This was in contrast to our observations for metabolic and digestive disorders (Stangaferro et al., 2016). In such cases, milk production before CD was lowest for cows in the HI+ group; cows in the HI− group had less of a reduction or no reduction before CD compared to cows in the ND group. These contrasting results for milk production differences between the HI+ and HI− groups for cows affected by metabolic and digestive disorders or mastitis were probably a reflection of the reasons underlying reduced milk production. It is likely that in cows with clinical mastitis, the decline in milk production around CD was, for the most part, due to the direct effect of inflammation on the mammary gland (Zhao and Lacasse, 2008; Akers and Nickerson, 2011). In contrast, it is likely that cows with metabolic and digestive disorders produced less milk because of a reduction in DMI and the overall effect of disease on cow health (Bareille et al., 2003; Van Winden et al., 2003).

Similar to cows with metabolic and digestive disorders (Stangaferro et al., 2016), cows with clinical mastitis and an HIS <86 points were flagged earlier than by farm personnel (approximately half a day). In this case, the difference in favor of the AHMS was smaller than for the other disorders, and we observed no statistical differences when mastitis events were separated by pathogen. The short time frame from an HIS <86 points and CD was likely a reflection of the sudden drop in rumination and activity within no more than 1 d of CD in cows flagged based on HIS. In contrast, for disorders such as displaced abomasum or ketosis, rumination and activity were below levels observed in cows with no health disorders as early as 5 d before CD. Taken together, these results suggest that there may not be a major advantage for the AHMS in terms of the timing of mastitis diagnosis for herds with similar mastitis detection programs. The characteristic changes in milk composition, udder appearance, and consistency observed in cows with clinical cases of mastitis make other direct and simple methods of detection (e.g., milk stripping, udder visual inspection, palpation) more effective than an AHMS that is based on rumination and activity only. Nevertheless, rumination and activity or alert systems generated based on these parameters (e.g., HIS) could be used as tools for diagnosing severe cases of clinical mastitis caused by pathogens such as *E. coli*, which have profound systemic effects for the cow. Another potential application consists of using rumination and activity as markers of systemic compromise and as an aid in treatment decision-making, because changes in milk composition or udder status do not provide information about cows' overall health status.

From an on-farm implementation perspective, the HIS generated by the AHMS tested in our study could be used to detect severe cases of mastitis that affect the cow systemically. The use of HIS could be complemented by other parameters not reported in this study but generated based on rumination behavior and activity (e.g., weekly rumination and activity, deviations in the last 2 h) that could be retrieved separately for individual cows or groups of cows. However, the most likely scenario is that not all cows that develop a case of mastitis will be detected. This is because the underlying health disorder does not compromise cow health sufficiently to dramatically reduce rumination and activity in all cows. Thus, it seems reasonable to suggest that for farms with proactive health-monitoring and treatment programs, the AHMS could be used in combination with other traditional methods of mastitis detection. The AHMS may be a valuable tool for providing further insights about the overall health status of the cow.

CONCLUSIONS

Our findings demonstrated that automated rumination and activity monitoring was effective for identifying cows with clinical cases of mastitis caused by *E. coli* and cases of mastitis concurrent with another health disorder. Conversely, the ability of the AHMS to identify cows with clinical mastitis caused by pathogens other than *E. coli* was moderate. Overall, cows with clinical mastitis were identified earlier than through CD by farm personnel. The patterns of rumination, activity, and HIS from −5 to 5 d after CD for cows with clinical mastitis were characterized by marked differences compared to cows in the ND group. Among cows with clinical mastitis, those not identified by HIS had rumination and activity patterns very similar to cows in the ND group and different from cows diagnosed with the disorder but flagged by HIS. We conclude that automated health-monitoring systems that use rumination and physical activity should be used in combination with or to complement traditional methods of mastitis detection. Future research is needed to evaluate the effect of management programs that combine rumination and activity monitoring with traditional methods to diagnose mastitis on cow well-being and performance.

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Table 1. Incidence of mastitis, DIM at clinical diagnosis, sensitivity of health index score (HIS) to detect cows with mastitis, and interval between the first HIS-positive outcome and clinical diagnosis (CD) of mastitis by farm personnel.

	Cows	Incidence	DIM at event	Sensitivity		HIS positive to CD[2]		
	(n)[1]	(%)	(mean ± SD)	% (n/n)	(95% CI)	Days	(95% CI)	*P*-value
Clinical mastitis[3]	123	11.4	38 ± 24	58 (71/123)	49,67	-0.5	-1.0,-0.1	0.02
Clinical mastitis only[4]	114	10.6	40 ± 24	55 (63/114)	46,64	-0.4	-0.8,0.1	0.08
Clinical mastitis with other disorders[5]	9	0.8	20 ± 23	89 (8/9)	68,100	-1.5	-3.6,0.6	0.13
Clinical Mastitis by Pathogen[6]								
E. coli	31	25.2	40 ± 24	81 (25/31)^{a}	67,95	-0.4	-1.1,0.2	0.18
Klebsiella spp.	6	4.9	37 ± 24	33 (2/6)^{b}	1,71	-	-	-
Gram-positives[7]	39	31.7	37 ± 26	49 (19/39)^{b}	32,65	-0.5	-1.4,0.5	0.31
Staph. aureus	11	8.9	38 ± 20	46 (5/11)^{b}	17,77	-1.4	-4.1,1.3	0.23
No growth[9]	25	20.3	37 ± 23	48 (12/25)^{b}	28,69	-0.2	-1.4,1.1	0.78

$^{a-b}$ Different superscripts indicate differences ($P \leq 0.05$) between means based on mean separation with the LSD test.

[1] Number of events diagnosed.

[2] HIS-positive to CD = interval in days between the first positive HIS outcome (positive outcomes only) and CD. For cases of mastitis caused by Klebsiella spp., HIS-positive to CD was not calculated because of lack of sufficient observations.

[3] All clinical mastitis events recorded.

[4] Cows diagnosed with only clinical mastitis from −5 to 2 d relative to CD.

[5] Cows diagnosed with clinical mastitis and at least another health disorder from −5 to 2 d relative to CD.

[6] Clinical mastitis events classified by the results of milk culture [11 cows not included: no culture results (n = 6); yeast (n = 2); other (n = 2); contamination (n = 1)].

[7] Gram-positives = Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus spp., Staphylococcus spp., Trueperella pyogenes.

[8] No important growth after 48 h

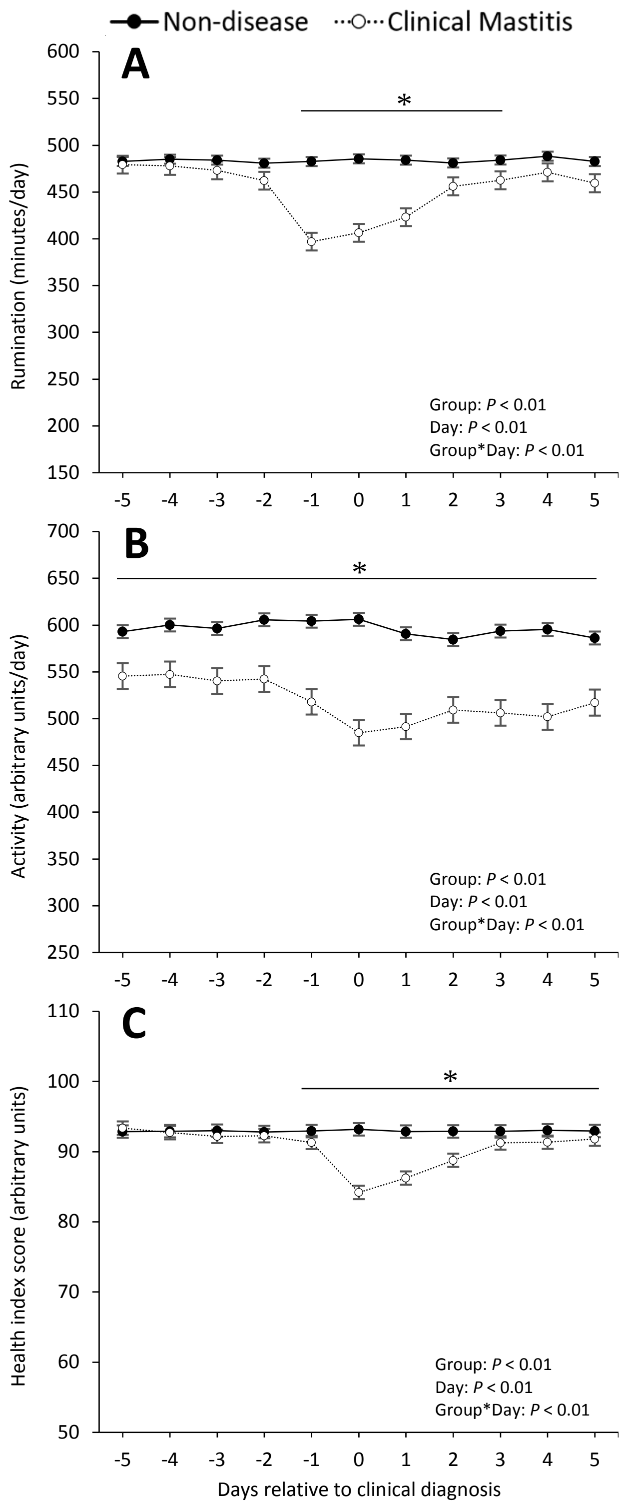

Figure 1. Rumination (A), activity (B; arbitrary units, AU), and health index score (AU; C) patterns from −5 to 5 d relative to clinical diagnosis for cows that developed clinical mastitis

(first event only; n = 110) compared with cows in the nondisease group (n = 435). For the nondisease group, the average DIM at CD for cows with mastitis was considered "day 0." Values are presented as LSM ± SEM. Within a day, differences ($P \leq 0.05$) for pairwise comparisons between the nondisease and clinical mastitis group are represented by an asterisk (*).

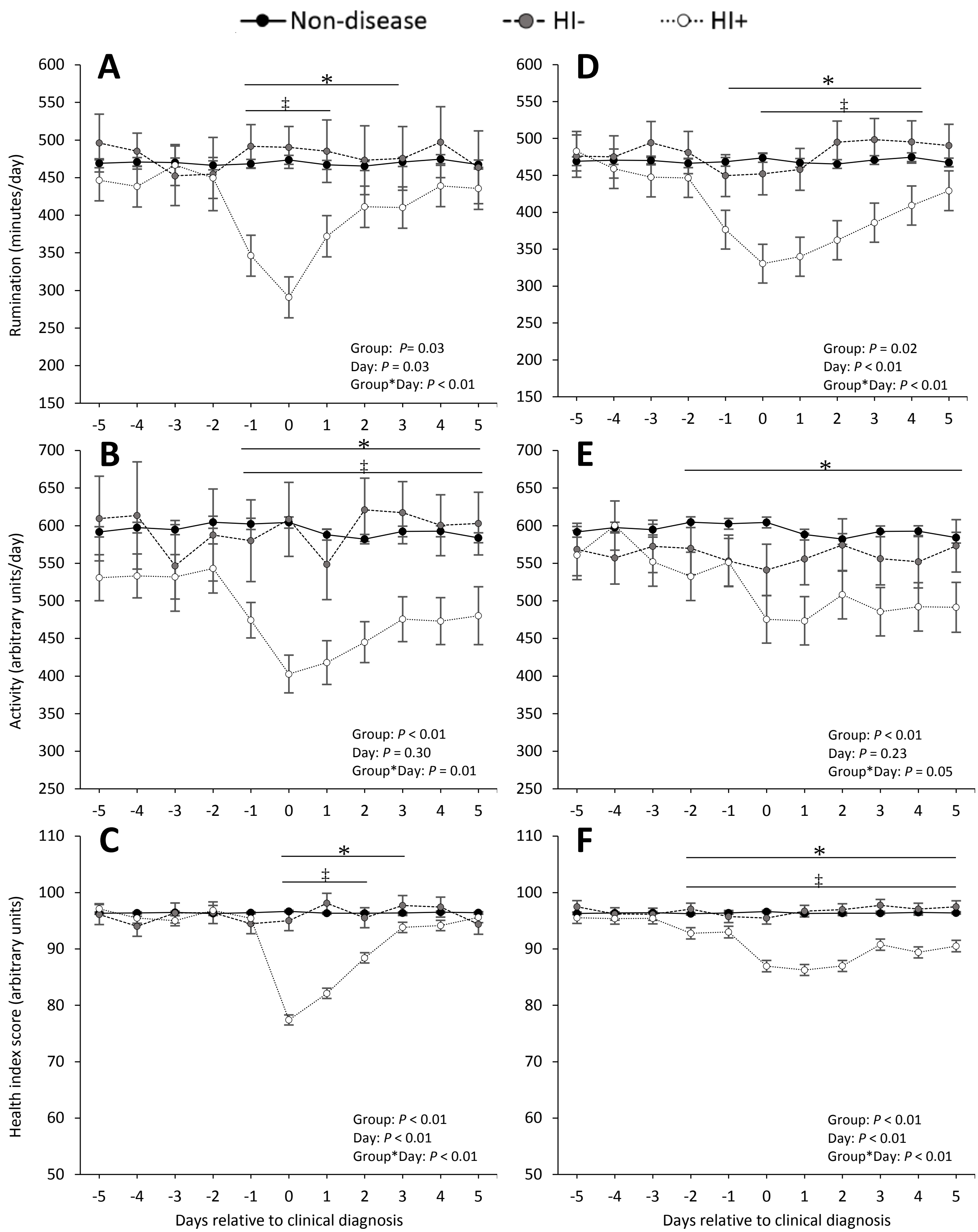

Figure 2. Rumination (A), activity (B ; arbitrary units, AU), and health index score (HIS, AU; C) patterns from −5 to 5 d relative to clinical diagnosis (CD) for cows that developed clinical

mastitis caused by *Escherichia coli* (HI+: n = 21; HI−: n = 5) compared with cows in the nondisease group (n = 435). Cows were assigned to the HI+ or HI− group if they had an HIS of <86 or ≥86 arbitrary units, respectively, during the 5 d preceding, the day of, and 2 d after CD. For cows in the nondisease group, the average DIM at CD for cows with mastitis was considered "day 0." Rumination (D), activity (E), and HIS (F) patterns from −5 to 5 d relative to clinical diagnosis for cows that developed clinical mastitis caused by gram-positive bacteria (*Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus* spp., *Staphylococcus* spp., *Trueperella pyogenes*) compared with cows in the nondisease group (n = 435). Cows with clinical mastitis caused by gram-positive bacteria were assigned to the HI+ (n = 19) and HI− (n = 18) groups following the same criteria as for cows with clinical mastitis caused by *E. coli*. Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; ‡HI+ vs. HI−.

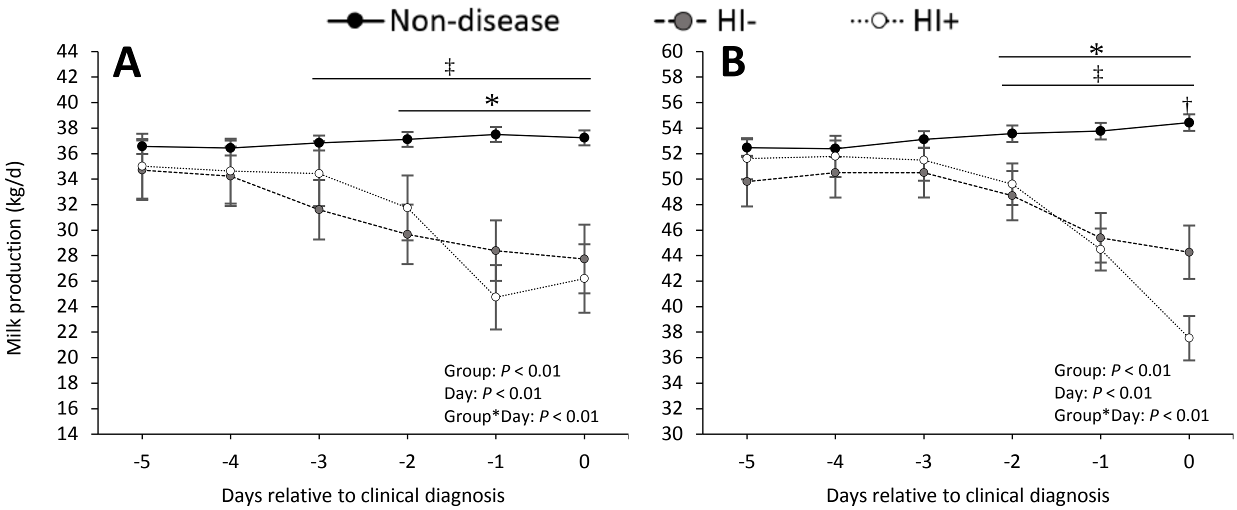

Figure 3. Milk production (kg/d) from −5 d to the day of clinical diagnosis (CD; d 0) for primiparous cows (A) that developed clinical mastitis (HI+: n = 15; HI−: n = 12) compared with cows in the nondisease group (n = 171). Cows were assigned to the HI+ or HI− group if they had a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after clinical diagnosis. Milk production (kg/d) from −5 d to the day of CD for multiparous cows (B) that developed clinical mastitis compared with cows that did not develop mastitis (nondisease; n = 264). Multiparous cows with mastitis were assigned to the HI+ (n = 49) and HI− (n = 33) groups following the same criteria as for primiparous cows with mastitis. Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; †control vs. HI−; ‡ HI+ vs. HI−.

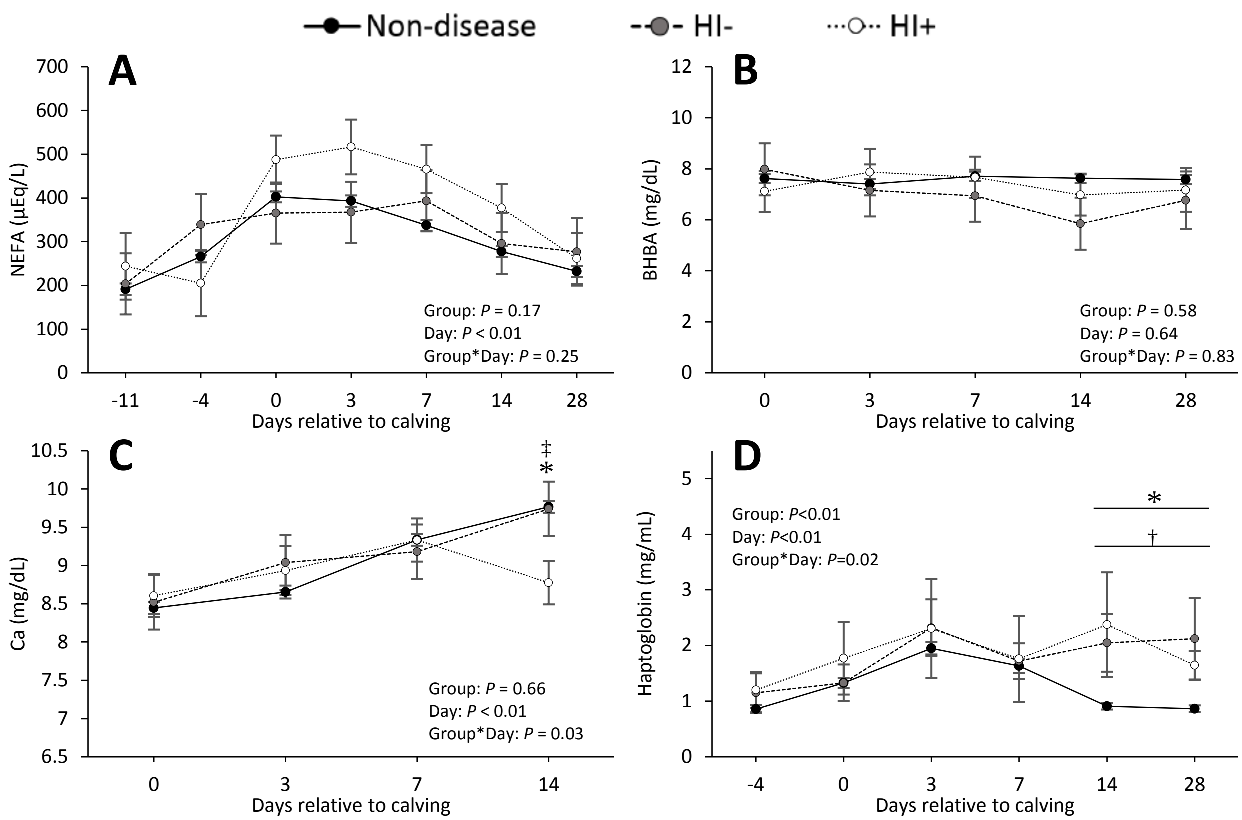

Figure 4. Plasma concentrations of nonesterified fatty acids (NEFA; A), BHB (B), total calcium (C), and haptoglobin (D) for cows that developed clinical mastitis up to 30 DIM (HI+: n = 8; HI−: n = 5) compared with cows in the nondisease group (n = 161). Cows with mastitis were assigned to the HI+ or HI− group if they had a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d preceding, the day of, and 2 d after clinical diagnosis. Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different (*P* ≤ 0.05) based on LSD are represented as follows: *control vs. HI+; †control vs. HI−; ‡ HI+ vs. HI−.

CHAPTER IV

USE OF RUMINATION AND ACTIVITY MONITORING FOR THE IDENTIFICATION OF DAIRY COWS WITH HEALTH DISORDERS: PART III. METRITIS

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ABSTRACT

The objectives of this study were to evaluate (1) the performance of an automated health-monitoring system (**AHMS**) to identify cows with metritis based on an alert system (health index score, **HIS**) that combines rumination time and physical activity; (2) the number of days between the first HIS alert and clinical diagnosis (**CD**) of metritis by farm personnel; and (3) the daily rumination time, physical activity, and HIS patterns around CD. In this manuscript, the overall performance of HIS to detect cows with all disorders of interest in this study [ketosis, displaced abomasum, indigestion (companion paper, part I), mastitis (companion paper, part II), and metritis] is also reported. Holstein cattle (n = 1,121; 451 nulliparous and 670 multiparous) were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags, SCR Dairy, Netanya, Israel) from at least −21 to 80 d in milk (**DIM**). Raw data collected in 2-h periods were summarized per 24 h as daily rumination and activity. An HIS (0 to 100 arbitrary units) was calculated daily for individual cows with an algorithm that used rumination and activity. A positive HIS outcome was defined as an HIS of <86 units during at least 1 d from −5 to 2 d after CD. Blood concentrations of nonesterified fatty acids, β-hydroxybutyrate, total calcium, and haptoglobin were determined in a subgroup of cows (n = 459) at −11 ± 3, −4 ± 3, 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 DIM. The overall sensitivity of HIS was 55% for all cases of metritis (n = 349), but it was greater for cows with metritis and another disorder (78%) than for cows with metritis only (53%). Cows diagnosed with metritis and flagged based on HIS had substantial alterations in their rumination, activity, and HIS patterns around CD, alterations of blood markers of metabolic and health status around calving, reduced milk production, and were more likely to exit the herd than cows not flagged based on the HIS and cows without disease, suggesting that cows flagged based on the HIS had a more severe episode of metritis. Including all disorders of interest for this study, the overall sensitivity was 59%, specificity was 98%, positive predictive value was 58%, negative predictive value was 98%, and accuracy was 96%. The AHMS was effective for identifying cows with severe cases of metritis, but less effective for identifying cows with mild cases of metritis. Also, the overall accuracy and timing of the AHMS alerts for cows with health disorders indicated that AHMS that combine rumination and activity could be a useful tool for identifying cows with metabolic and digestive disorders, and more severe cases of mastitis and metritis.

Keywords: rumination, activity, metritis, dairy cow

INTRODUCTION

Metritis is a prevalent disease that affects lactating dairy cows in the early postpartum period. The occurrence of this health disorder, which may affect up to 40% of cows (Markusfeld, 1987; Zwald et al., 2004; Sheldon et al., 2009), has been associated with reduced milk production, impaired reproductive performance, and increased risk of culling during lactation

(Bell and Roberts, 2007; Wittrock et al., 2011; Giuliodori et al., 2013). Therefore, commercial dairy farms implement health-monitoring programs to detect and treat cows with metritis in an attempt to reduce its consequences for cow performance. Although health-monitoring programs vary significantly among farms, the diagnosis of metritis is based primarily on evaluation of the uterus and uterine discharge. The presence of an abnormally enlarged uterus and a fetid, watery, pink-brown uterine discharge are considered evidence of metritis (Földi et al., 2006; Sheldon et al., 2006; 2009). In addition, signs of systemic illness such as anorexia, fever, depression, and dehydration provide information about the general status of the cow and the severity of the disease (Benzaquen et al., 2007; Sheldon et al., 2009; Risco and Melendez Retamal, 2011).

Beyond clinical examination and evaluation of the uterus and its contents, few diagnostic aids are available to identify cows with metritis in dairy farms. Research studies have documented moderate sensitivity (**Se**) and specificity (**Sp**) for methods such as feeding behavior (Urton et al., 2005) and blood concentrations of the acute-phase protein haptoglobin (Huzzey et al., 2009). But even though these tests may help identify cows with metritis, the greatest limitation for their widespread use in commercial dairy farms is on-farm implementation. The recent development of noninvasive sensor technologies for monitoring rumination time and physical activity could be used to assess overall cow health and aid in the detection of cows with metritis. Recent studies have documented alterations in the pattern of rumination and activity in cows diagnosed with metritis. For example, Liboreiro et al. (2015) reported lower rumination and activity after calving in cows with metritis compared with cows without metritis. Titler et al. (2013) reported that cows with metritis spent more time standing, took fewer steps, and had fewer lying bouts from 1 to 3 d before and after diagnosis than cows without a health disorder. Although results from these studies suggested that automated rumination and activity monitoring systems could be used to identify cows with metritis, additional data are needed to support their use in commercial dairy operations. In addition, little is known about the patterns of rumination and activity around the timing of clinical diagnosis of metritis. A better understanding of the changes in these parameters in cows with metritis may help improve the ability of farms to detect cows that are at risk or that already have the disorder. In addition, rumination and activity may offer unique insights into the overall health status of cows that are not provided by clinical examination and evaluation of the uterus.

We hypothesized that an automated health-monitoring system (**AHMS**) that continuously monitors rumination and activity would be able to identify cows with metritis. Also, we expected that changes in rumination and activity before evident clinical signs of disease would result in earlier identification of metritis. Thus, the objectives of this study were to evaluate (1) the performance of an automated rumination and physical activity monitoring system to identify cows with metritis; (2) the interval between the AHMS alert based on a health index score (**HIS**) and the day of clinical diagnosis (**CD**) by farm personnel; and (3) the rumination, activity, HIS, and milk-production patterns for cows with metritis. We also used culling dynamics, reproductive outcomes for first AI service, and markers of energy status [nonesterified fatty acids (**NEFA**) and BHB], mineral status (total calcium), and systemic inflammation (haptoglobin) to complement the diagnosis of metritis and the performance of the AHMS alert.

MATERIALS AND METHODS

Animals, Management, and Study Design

All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University. The study followed an observational prospective cohort design. Details about animal management and study design are provided in a companion manuscript (Stangaferro et al., 2016a). Briefly, Holstein cows (n = 1,121; 451 nulliparous and 670 multiparous) were fitted with a neck-mounted electronic rumination and activity monitoring tag (HR Tags; SCR Dairy, Netanya, Israel) to monitor rumination and activity from at least 21 d before expected calving until at least 80 d after calving. Of 1,121 cows enrolled in the study, 41 (3.7%) were removed from the data set due to tag malfunction or misplacement during data collection. Thus, 1,080 cows were included in the final data set for analysis. Based on rumination and activity data, an HIS (0 to 100 arbitrary units) for each cow was generated by the system software (DataFlow, Netanya, Israel) using a series of internal algorithms (proprietary to SCR Dairy). An HIS of 100 arbitrary units represents a cow with an ideal pattern of rumination and activity; an HIS value <86 arbitrary units may be indicative of a health disorder. An HIS report was generated daily to include cows with <86 arbitrary units (as determined by SCR) and stored for evaluation by the research group. During the study, farm personnel did not have access to the HIS report or any information generated by the AHMS.

Fresh Cow Monitoring Program and Case Definitions

Disease definitions and the health-monitoring program used in the study are provided in detail in a companion manuscript (Stangaferro et al., 2016a). In short, after calving, cows were examined daily from 1 to 10 DIM. The clinical examination included direct observation, rectal temperature, urine ketones, and rumen auscultation. For any cow not diagnosed with metritis before 8 DIM, transrectal massage of the uterus was conducted to obtain and evaluate uterine discharge. Metritis was defined as the presence of watery, pink/brown, and fetid uterine discharge with or without fever (rectal temperature ≥39.5°C).

Two consecutives episodes of metritis were considered separate episodes if they occurred at least 7 d apart.

Blood Collection and Laboratory Analyses

Briefly, blood samples were collected from a subgroup of cows (n = 459) on d −11 ± 3 and −4 ± 3 prepartum, and then at 0, 3 ± 1, 7 ± 1, 14 ± 1, and 28 ± 1 d after calving. Plasma samples were analyzed for NEFA (d −11, −4, 0, 3, 7, 14, 28), BHB (d 0, 3, 7, 14, 28), total Ca (d 0, 3, 7, 14), and haptoglobin (d −4, 0, 3, 7, 14, 28). Details about blood collection, blood sample processing, and analysis of metabolites (NEFA, BHB), calcium, and markers of systemic inflammation (haptoglobin) are provided in a companion manuscript (Stangaferro et al., 2016a).

Statistical Analysis

System Performance. The main outcome of interest for this study was the ability of the HIS to correctly identify cows with metritis. Clinical diagnosis by farm personnel was used as the reference test. Because HIS does not confirm the occurrence of disease or indicate the type of disease, a positive outcome was defined as an HIS value of <86 arbitrary units during at least 1 d during the 5 d before, the day of, or 2 d after CD. The Se and 95% CI for the HIS to identify cows with metritis was calculated using PROC FREQ in SAS (version 9.4; SAS Institute Inc., Cary, NC) and was defined as the ability of the HIS to correctly identify cows diagnosed with metritis by farm personnel. To evaluate the potential confounding effect of other health disorders (i.e., all disorders of interest, pneumonia, and lameness) on the Se of HIS, we conducted 3 separate analyses. The first analysis included all cows diagnosed with metritis; a second analysis included cows diagnosed only with metritis during the range of interest around CD; and a third analysis included cows diagnosed with metritis and at least one other health disorder during the range of interest around CD. Unfortunately, uterine discharge characteristics (appearance, color, and odor), severity of metritis, or both, based on clinical signs, were not recorded. Thus, in an attempt to identify cows with metritis cases of different severity, we also calculated the Se of HIS for subgroups of cows with metritis created based on rectal temperature at the time of CD. For this analysis, cows were included in 1 of the following rectal temperature groups: (1) ≤39.4°C, no fever; (2) 39.5 to 39.9°C, moderate fever; and (3) ≥40°C, high fever. We determined differences in Se of HIS between the subgroup of cows with metritis only and cows with metritis and at least another disorder, and for the different rectal temperature subgroups, by logistic regression using the events over trials option of PROC LOGISTIC in SAS.

Interval Between the First Positive HIS Outcome and Clinical Diagnosis. To determine if HIS was capable of identifying cows with metritis earlier than CD by farm personnel, the interval (in days) between the first positive HIS outcome during the period of interest around CD (5 d before to 2 d after) and the day of CD of metritis was evaluated. For this analysis, which included only cows flagged by the AHMS, we compared the mean number of days from the first HIS-positive outcome and the day of CD with a paired t-test using the PROC TTEST in SAS.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis. Daily rumination time (min/d), daily activity (arbitrary units/d), and HIS (arbitrary units) were evaluated from 5 d before to 5 d after CD (d 0) for the first event of metritis. Milk production (kg/d) was evaluated only from 5 d before until the day of CD, because data were not available after CD for cows treated with an antibiotic that required milk withdrawal. Before statistical analysis, we assessed normality of the data for rumination, activity, HIS, and milk production using the Shapiro-Wilk statistic and graphical methods (histogram and Q-Q plot) and using PROC UNIVARIATE in SAS. Based on this analysis, no data transformations were necessary.

For this analysis, cows were grouped according to the following criteria: CD-positive and HIS-positive (**HI+**; HIS <86 arbitrary units at least 1 d within 5 d before, the day of, and 2 d after CD); CD-positive and HIS-negative (**HI−**; HIS ≥86 arbitrary units from 5 d before, the day of, and 2 d after CD); and CD-negative (nondisease, **ND**; cows not diagnosed with a health disorder during the study period). For cows in the ND group, we considered the average DIM at CD for cows that had metritis "day 0." Data was analyzed by ANOVA with repeated measurements using PROC MIXED in SAS. Models for each outcome of interest (rumination, activity, HIS, and milk production) included group (e.g., ND, HI+, and HI−), time, parity, and the interactions between group and time, and group and parity, as explanatory variables. The occurrence of another health disorder (i.e., all disorders of interest, pneumonia, and lameness) during the −5 to 5 d period after CD was offered as a categorical variable (0 = no occurrence and 1 = occurrence) to the initial models to evaluate the potential effect of multiple disorders on the parameters of interest. Cow within group was included as a random effect in all models. The final model for each parameter of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Akaike's information criterion. Cow was the subject of the repeated measurements, and all models were run using an autoregressive (AR-1) covariance structure. Group, time, and the group-by-time interaction were forced in all models. When the main effect or interaction between explanatory variables was significant, we used the LSD post hoc mean separation test to determine differences between groups of means.

Plasma Concentration of NEFA, BHB, Calcium, and Haptoglobin. Cows were grouped in the same way as for evaluation of rumination, activity, and HIS. Data were analyzed by ANOVA with repeated measurements using PROC MIXED in SAS as described.

Culling Dynamics and Reproductive Outcomes for First AI Service. The proportion of cows coded as do not breed, sold, and dead within the first 60 DIM or up to 270 DIM were analyzed by logistic regression using PROC GLIMMIX in SAS. The proportion of cows inseminated after a detected estrus or timed AI, and pregnancies per AI after first AI service were also analyzed by logistic regression using PROC GLIMMIX in SAS. The regression models included group (HI+, HI−, and ND), parity, and their interaction as explanatory variables. The final model for each variable of interest was selected by backward elimination of explanatory variables with $P > 0.10$. The effect of group (HI+, HI−, and ND) was forced in all models. We used the LSD post hoc mean separation test was used to determine differences between groups of means. Differences in DIM at first service were analyzed by ANOVA using PROC MIXED in SAS and models that included the same explanatory variables used for the binomial outcomes.

Overall System Performance. Because HIS is designed as an alert to identify cows for further clinical examination rather than to provide a definitive diagnosis of a health disorder, false-positive outcomes could not be assigned to a particular health disorder, because the reason for the alert was unknown. Therefore, we calculated Sp, positive predictive value (PPV), negative predictive value (NPV), and accuracy as an overall test for all events recorded for the disorders of interest [metabolic and digestive (Stangaferro et al., 2016a), mastitis (Stangaferro et al., 2016b), and metritis (this paper)]. Because each day was considered a new test, the total contribution of individual cows to the number of cow-days was determined from 2 to 80 DIM. A cow could contribute with a positive or negative outcome during specific periods of the study depending on her clinical status (ND vs. with a disorder) as defined by CD. Cows that were sold or died before the end of the study did not contribute to the cow-day calculation after they left the herd.

We created a 2 × 2 frequency table based on the following criteria:

- ***True positive*** **=** positive HIS outcome (HIS <86 arbitrary units) at least 1 d within the 5 d before, the day of, and 2 d after CD. Each day within the range of interest was counted as a true positive outcome when HIS was <86 arbitrary units for at least 1 d within the range of interest.
- ***False positive*** **=** positive HIS outcome for a cow without a health disorder or positive HIS outcome outside the −5 to 2 d range relative to CD for cows with health disorders.
- ***True negative*** **=** negative HIS outcome (HIS ≥86 arbitrary units) for a cow without a health disorder or negative HIS outcome outside of the −5 to 2 d range relative to CD for cows with health disorders.
- ***False negative*** **=** negative HIS outcome during the −5 to 2 d range relative to CD for a cow with a health disorder. Each day within the range of interest was counted as a false negative outcome.

This frequency table was then used to estimate the overall Se, Sp, PPV, NPV, and accuracy of the HIS using PROC FREQ of SAS. Only the diseases of interest (displaced abomasum, ketosis, indigestion, mastitis, and metritis) could contribute as a positive disease outcome (true positive or false negative), because the window from −5 to 2 d relative to CD was removed for health disorders of no interest to the study (pneumonia and lameness).

Data for proportions are presented as arithmetical means and their 95% CI; quantitative data are presented as LSM ± SEM or 95% CI, unless otherwise stated. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Metritis Incidence and System Performance

Table 1 summarizes the incidence of metritis, DIM at CD, and Se of HIS to identify cows with metritis for all cows with a recorded case of metritis, cows diagnosed with metritis only, and cows diagnosed with metritis and another health disorder from −5 to 2 d after CD. Data are also presented for subgroups of cows with metritis and fever or no fever at the time of CD. The overall incidence of metritis was 32% (n = 349; 331 and 9 cows with 1 or 2 events of metritis recorded, respectively). The overall Se of HIS to identify cows with metritis was 55%, and it was greater ($P = 0.02$) for cows with metritis and another health disorder than for cows with metritis only. Conversely, the Se of HIS did not differ ($P = 0.52$) for subgroups of cows with or without fever. Overall, cows with metritis and a positive HIS outcome were identified earlier than CD by farm personnel.

Rumination, Activity, HIS, and Milk Production Relative to Clinical Diagnosis

Daily rumination time, activity, and HIS patterns from −5 to 5 d relative to CD for cows in the HI+, HI−, and ND groups are presented in Figure 1. For all parameters, the effect of parity and the group-by-parity interaction were not significant. For rumination, we observed an interaction between group and day ($P < 0.01$). Rumination time was lower for cows with metritis in the HI+ (n = 187) than in the HI− (n = 153) or ND groups (n = 435); however, it was similar for the HI− and ND groups over the entire period. Because metritis was diagnosed on average at ~7 DIM, the nadir for rumination for all groups (313, 376, and 368 min/d for the HI+, HI−, and ND groups) was observed on d −5 (~2 DIM average). Thereafter, rumination increased for all groups, but the increment was slower for cows in the HI+ group, and faster for cows in the HI− and ND groups. We observed the greatest difference for rumination between cows in the HI+ group and cows in the HI− (122 min/d) and ND (131 min/d) groups 1 d before CD. Rumination time for cows with metritis was also affected by the occurrence of ketosis, because cows with ketosis had reduced rumination ($P = 0.01$) than cows without ketosis during the period of interest around CD (407 ± 16.1 vs. 452 ± 2.1 min/d, respectively).

For activity, we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group had lower activity than cows in the HI− and ND groups during the entire period evaluated, reaching their nadir (434 arbitrary units/d) on d 0; cows in the HI− group had lower activity than cows in the ND group on d −5. Activity for cows with metritis was also affected ($P = 0.03$) by the occurrence of ketosis. Cows with ketosis had less activity than cows without ketosis during the period of interest around CD (467 ± 32.6 vs. 534 ± 4.1 arbitrary units/d, respectively).

For HIS, we also detected an interaction between group and day ($P < 0.01$). Cows in the HI+ group had lower HIS than cows in the HI− and ND groups, but cows in the HI− and ND group had similar HIS during the entire period analyzed. The HIS for cows in the HI+ group reached its lowest value (83 units) on d 0. The HIS was also affected by the occurrence of ketosis, because cows with metritis and ketosis had lower HIS ($P = 0.04$) than cows without ketosis during the period of interest around CD (90 ± 1.7 vs. 95 ± 0.2 arbitrary units, respectively).

Daily milk production from 5 d before until the day of CD for cows with metritis, grouped based on HIS outcome (HI+ and HI−), is presented in Figure 2. Because parity had an effect ($P < 0.01$) on milk production, results are presented separately for primiparous and multiparous cows. For primiparous cows (Figure 2A), we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group produced less milk than cows in the ND group from 4 d before until the day of CD; cows in HI+ group produced less milk than cows in the HI− group on d −2 and 0 relative to CD. The greatest difference between cows in the HI+ and cows in the HI− and ND groups was observed −2 and 0 d relative to CD (2.4 and 3.2 kg/d, respectively). For multiparous cows (Figure 2B), we observed an interaction between group and day ($P < 0.01$). Multiparous cows in the HI+ group produced less milk than cows in the HI− and ND groups from 4 d before until the day of CD, with the greatest differences −1 and 0 d relative to CD of metritis (~7 kg/d). Cows in the HI− and ND groups produced similar amounts of milk during the period analyzed.

Plasma Concentrations of NEFA, BHB, Calcium, and Haptoglobin

Plasma concentrations of NEFA, BHB, Ca, and haptoglobin relative to the day of calving for cows that developed metritis are presented in Figure 3. For NEFA, we observed an interaction between group and day ($P < 0.01$). Plasma concentrations of NEFA were greater in the HI+ than in the ND group from −11 to 7 d relative to calving. Cows in the HI− group had lower NEFA concentrations than cows in the HI+ group 0 and 3 d relative to calving, and we observed no differences with the ND group during the period analyzed. Concentrations of NEFA peaked on d 0 (488 ± 24 μEq/L) for cows in the HI+ group and differed by 85 and 126 μEq/L compared with cows in the ND and HI− group, respectively. For BHB, we observed an effect of day ($P = 0.04$) and a tendency for an interaction between group and day ($P = 0.08$). In addition, BHB concentrations were also affected ($P < 0.01$) by the occurrence of displaced abomasum. Cows with a displaced abomasum had greater BHB concentrations than cows without (13.4 ± 1.5 vs. 7.6 ± 0.1 mg/dL, respectively). For concentrations of total calcium in plasma, we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group had total calcium concentrations that were lower than the ND group from 3 to 14 d after calving, and lower than the HI− group from 7 to 14 d after calving. Cows in the HI+ group had their lowest calcium concentration (8.4 mg/dL) on d 3 after calving, and the greatest difference with cows in the HI− (0.5 mg/dL) and ND group (0.6 mg/dL) 14 d after calving. Cows in the HI− and ND group had similar calcium concentrations during the entire period analyzed. For concentrations of haptoglobin, we observed an interaction between group and day ($P < 0.01$). Cows in the HI+ group presented greater haptoglobin concentrations than cows in the ND group from 0 to 14 d after calving reaching peak concentrations (4.0 mg/mL) and the greatest difference with the ND group (2.1 mg/mL) 3 d after calving. Cows in the HI− group presented greater haptoglobin concentrations than cows in the ND group from 0 to 7 d after calving (greatest difference 1.3 mg/mL on d 3), but lower than cows in the HI+ group from 3 to 14 d after calving.

Culling Dynamics and Reproductive Outcomes for First AI Service

The proportion of cows dead up to 60 DIM ($P = 0.74$) or up to 270 DIM ($P = 0.33$) was similar for cows in the HI+, HI−, and ND groups (Table 2). In contrast, the proportion of cows coded as do not breed or sold up to 60 DIM (end of the voluntary waiting period), as well as up to 270 DIM, were greater ($P < 0.05$) for the HI+ than the HI− and ND groups. No differences in the proportion of cows inseminated based on a detected estrus or timed AI ($P = 0.76$), DIM at first AI ($P = 0.73$), or pregnancies per AI ($P = 0.80$) among groups were detected.

Overall system performance

From 2 to 80 DIM, the overall Se of HIS to detect all the health disorders of interest for this study (displaced abomasum, ketosis, indigestion, mastitis, and metritis) was 59.0% (95% CI: 57.5, 60.5%; 2,416/4,096 cow-days) and Sp was 97.6% (95% CI: 97.5, 97.7%; 70,695/72,423 cow-days). Based on the prevalence observed in the study, the PPV was 58.3% (95% CI: 56.8, 59.8%; 2,416/4,144 cow-days), the NPV was 97.7% (95% CI 97.6, 97.8%; 70,695/72,375 cow-days), and accuracy was 95.6% (95% CI: 95.4, 95.7%; 73,111/76,519 cow-days).

DISCUSSION

In the current study, we evaluated the performance of an AHMS that combined rumination and activity to identify cows with metritis. The overall Se of the AHMS was moderate and slightly lower for cows diagnosed with metritis only during the −5 to 2 d range relative to CD. Conversely, the Se of HIS was substantially greater (25 percentage points) for cows with metritis and another health disorder during the window of interest around CD. The incidence of metritis in our study was within the upper level of the range reported for lactating dairy cows (Bell and Roberts, 2007; Sheldon et al., 2009; Toni et al., 2015), likely because the farm standard operating procedures for diagnosis and treatment of metritis determined that cows with very mild watery, foul smelling, pink/brown discharge should be treated. Therefore, we speculated that the major reason for the Se of HIS observed in our study was a wide range in severity of the disorder, which, in turn, caused a wide range of alterations to the rumination and activity patterns around CD. Thus, we evaluated the performance of the AHMS for subgroups of cows in an attempt to identify different levels of severity. In this regard, the greater Se of HIS for cows that experienced metritis along with another disorder supports the notion that the AHMS was capable of detecting cows in a poorer health state. Moreover, when HIS group (HI+ and HI−) was used as a proxy for severity and systemic compromise, cows in the HI+ group had more dramatic reductions in rumination and activity, and a greater reduction in milk production around the time of metritis CD than cows in the HI− and ND groups. For example, milk production before CD was lower in primiparous and multiparous cows in the HI+ group than in the ND group, but cows in the HI− group did not have decreased milk production. Moreover, the differences observed for NEFA, Ca, and haptoglobin around calving indicated that cows in the HI+ group were in a more negative plane of energy balance, were more Ca deficient, and presented more severe systemic inflammation than cows in the HI− group. The lack of difference in BHB concentrations was unexpected because of the differences for the other blood markers and the results of previous studies, which have reported an association between BHB concentrations and the risk of metritis (Duffield et al., 2009; Galvão et al., 2010; Ospina et al., 2010). Nevertheless, in agreement with the current results, other studies have not reported differences in BHB concentrations between cows with or without metritis (Chapinal et al., 2011; Martinez et al., 2012). For cows in the HI− group, intermediate haptoglobin concentrations suggested that they underwent systemic inflammation, but that the degree of inflammation was likely lower than for cows in the HI+ group. Furthermore, cows in the HI+ group were about twice as likely to exit the herd or be coded as do not breed (up to 60 DIM and up to 270 DIM) than cows in the HI− and ND groups. The differences in culling dynamics also help explain the lack of differences in first service reproductive performance, because many cows expected to have poor fertility and production left the herd before their first AI service. Taken together, these observations suggest that not only were cases of metritis for cows in the HI+ group more severe, but also that these cows did not cope as well with the disease, leading to their earlier removal from the herd.

Rectal temperature was also explored as a potential indicator of severity of metritis, because fever may be an indication of infection and inflammation (Mackowiak et al., 1997; Leon, 2002; Steiner et al., 2006). Nevertheless, the Se of the AHMS did not increase substantially for cows with fever compared with that of cows without fever, suggesting that in this case fever was not a good marker of severity. This was not surprising, because a previous study (Benzaquen et al., 2007) reported that up to 58% of lactating dairy cows diagnosed with metritis did not develop fever (defined as rectal temperature of ≥39.4°C).

Collectively, our findings seem to support the notion that there are varying levels of severity and systemic compromise for metritis, and that the ability of the HIS generated by the AHMS to identify cows with metritis depends upon the degree to which overall cow health and behavior are compromised. Based on our current limited knowledge of the physiological effect of metritis on rumination and physical activity, we speculate that the patterns of these parameters change more drastically when the uterine infection affects cows systemically rather than when it affects the uterus only. Additional research studies that include objective definitions of severity and systemic compromise for cases of metritis are needed to corroborate our observations and continue improving algorithms used to identify cows with metritis.

For cows with metritis detected based on HIS, the AHMS identified them earlier than farm personnel. This is in agreement with our data for cows diagnosed with metabolic and digestive disorders (Stangaferro et al., 2016a) and cows diagnosed with mastitis, which were also identified earlier based on HIS than by farm personnel (Stangaferro et al., 2016b). The earlier identification of cows based on HIS was expected, because cows in the HI+ group presented substantial reductions in rumination and activity on the days leading up to CD. The value of identifying cows with metritis approximately 1 d earlier based on HIS than with traditional health-monitoring programs is currently unknown. Earlier treatment could be beneficial by halting the progression of the disorder and improving treatment response. Earlier treatment could also help mitigate the negative effect of the disease on milk production and reduce the likelihood of culling. Additional studies are necessary to determine the value of earlier treatment on cow well-being and performance during lactation.

The pattern of rumination and activity for cows with metritis reflected the timing of metritis diagnosis during lactation. Dairy cattle have a rumination nadir on the day of calving (Soriani et al., 2012; Calamari et al., 2014; Liboreiro et al., 2015), followed by a sharp increase until a plateau is reached at ~7 DIM. Activity patterns are also profoundly altered around calving, but a sharp increase rather than a decrease is usually observed. The peak observed on the day of calving is followed by a steady decline up to ~6 DIM, after which activity remains steady (Liboreiro et al., 2015; M. L. Stangaferro and J. O. Giordano, unpublished results). These expected patterns of rumination and activity in dairy cows explain the general trends observed for all cows regardless of health status. For cows in the ND group, d −5 corresponded to an average of 2 DIM because d 0 for this group was equal to the mean DIM for diagnosis of metritis (6.8 DIM). For cows diagnosed with metritis, d −5 included some cows that were as early as 1 DIM because the earliest time of metritis diagnosis was 3 DIM. Regardless of the influence of DIM on the pattern of rumination and activity, cows affected by metritis showed differences compared to cows without a health disorder. Liboreiro et al. (2015) have also reported reduced rumination time and activity within the first week after calving in lactating dairy cows diagnosed with metritis.

From an on-farm implementation perspective, the results of the current experiment suggest that the HIS generated by the AHMS tested in our study could be used to detect severe cases of metritis that affect the cow systemically or cases of metritis accompanied by another health disorder. However, the most likely scenario is that not all cows that develop metritis will be detected. This is because the underlying health disorder may not compromise cow health sufficiently to affect rumination and activity. Thus, it seems reasonable to suggest that for farms with proactive health-monitoring and treatment programs, the AHMS could be used in combination with other traditional methods of metritis detection. For example, the AHMS could be used to identify cows with severe cases of metritis up to 7 to 10 DIM when most cases are expected (Sheldon et al., 2009; current study). Thereafter, cows not diagnosed with metritis could be examined to rule out the presence of the disorder. For cows with metritis detected during the mandatory examination (mild cases without a significant systemic compromise), a minor delay from the onset of the disorder to diagnosis may not comprise cow well-being, the outcome of the treatment, or the future productive and reproductive performance of the cow. Research experiments should be conducted to test the feasibility of on-farm implementation of these types of strategies. Unlike farms with proactive health-monitoring programs, farms that do not actively examine cows to detect metritis may benefit from using rumination and activity to detect cows with the most severe cases of the disorder. In this case, it is also unlikely that cows with mild or less severe cases would be identified by farm personnel because of the lack of a systematic health-monitoring program.

The HIS generated by the AHMS presented high accuracy when all the disorders of interest (displaced abomasum, ketosis, indigestion, metritis and mastitis) were included in the analysis. This is likely a reflection of the high Sp and NPV (both ≥97%) and the considerably greater number of cow-days during which cows did not have a health disorder (n = 72,423) rather than when they did have a health disorder (n = 4,096). These observations also suggest that a HIS value of ≥86 arbitrary units is a reasonable indicator that cows are not affected by a health disorder. Generating the fewest false-positive alerts is an important attribute of an AHMS to avoid the unnecessary inclusion of cows without a health disorder in reports created to select cows for clinical examination. Conversely, the Se and PPV for all disorders combined were close to 60%, considerably lower and undesirable for on-farm implementation. The main reason, however, for the relatively low Se was the wide range of Se for the disorders monitored in the study and the considerably greater number of cows with disorders for which the HIS presented moderate Se. For all metabolic and digestive disorders combined, the overall Se was 93%, and the number of health events represented only 20% of the total number recorded (Stangaferro et al., 2016a). In contrast, the overall Se for cases of metritis and mastitis was ~55% (Stangaferro et al., 2016b) but they represented 80% of all health events. Two other factors also contributed to the moderate PPV. First, the restricted window around CD (−5 to 2 d after CD) to consider a positive outcome from the AHMS as true positive led to the misclassification of some outcomes as false positives. Indeed, 10.7% of the cows presented an HIS-positive outcome 6 to 8 d before CD, and 13.8% continued to present an HIS-positive outcome 3 to 5 d after CD. On the other hand, a very low daily incidence of health disorders favors a lower PPV, because the presence of a few false-positive outcomes per day outweighs the number of true-positive outcomes (PPV =

number of true positive outcomes divided by the total number of true-positive + false-positive outcomes).

To determine the Sp of the HIS in a valid way, we eliminated data from 3.7% of the cows enrolled. In these cows the rumination and activity monitoring tag was either misplaced or malfunctioned during the study, resulting in a rumination and activity pattern characterized by consistently low values (usually <100 min/d of rumination or <100 arbitrary units/d for activity) during prolonged periods (at least 14 d). Because this is a known limitation of the AHMS used in our study, specific reports including cows with a malfunctioning or misplaced tag can be created to replace the tag or place it in the correct position.

We are uncertain whether the AHMS used in our study would be equally effective in identifying cows with health disorders in other commercial dairy herds with similar management. Indeed, using CD by farm personnel as reference for the occurrence and timing of diagnosis of clinical health disorders could make our results unique to the farm where the study was conducted. Therefore, additional studies are needed to confirm our findings. However, data from the present study provides compelling evidence that automated rumination and activity monitoring systems could be incorporated either alone or in combination with other traditional health-monitoring strategies to identify cows with metabolic and digestive disorders and severe cases of clinical mastitis and metritis. For dairy herds with very intensive health-monitoring programs, such as the herd that participated in our study, some cows with health disorders may not be identified by the AHMS. Nevertheless, our current data suggest that cows not flagged based on HIS may have had a less severe episode of the disorder and experienced a less severe reduction in performance (i.e., milk production). Because rumination and activity patterns for individual cows can also be evaluated separately, under some circumstances it may be possible to identify cows for clinical examination based on rumination and activity alone, rather than by HIS. Thus, in herds with intensive health-monitoring programs, the AHMS could be used as an alternative or a complement to human monitoring. Such a strategy would also reduce the resources and time needed for health monitoring while minimizing disruption of cow normal behavior and time budgets. Conversely, for dairy farms with passive health-monitoring programs (i.e., no monitoring or minimal monitoring) the AHMS may help improve overall cow health and performance by identifying cows with health disorders that would not be recognized otherwise. Regardless of the level of intensity of the current health-monitoring program, all farms may benefit by early disease detection (for cows detected by the AHMS) by improving the treatment response and reducing the negative consequences of health disorders on overall cow health, productive performance, and reproductive performance. Likely, the greatest benefit would be realized in herds with passive health-monitoring programs. In such herds, detection of cows with health disorders may be important not only to optimize productivity but also to improve overall cow health and well-being.

CONCLUSIONS

Our findings demonstrated that automated rumination and activity monitoring was effective for identifying cows with severe cases of metritis or cows with metritis and another health disorder. Conversely, the ability of the AHMS to identify cows with mild cases of metritis was moderate. Overall, cows with metritis were identified earlier than through CD by farm personnel. The patterns of rumination, activity, and HIS from −5 to 5 d after CD for cows with metritis identified based on HIS were characterized by marked differences compared to cows in the ND group as early as 5 d before CD. Conversely, cows with metritis not identified based on

HIS had rumination and activity patterns very similar to cows in the ND group and different from cows diagnosed with the disorder but flagged based on HIS. We conclude that automated health-monitoring systems that use rumination and physical activity should be used in combination with or to complement traditional methods of metritis detection. Future research is needed to evaluate the effect of management programs that combine rumination and activity monitoring with traditional methods to diagnose metritis on cow well-being and performance.

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Table 1. Incidence of metritis, DIM at clinical diagnosis, sensitivity of health index score (HIS) to detect cows with metritis, and interval between the first HIS-positive outcome and clinical diagnosis (CD) of metritis by farm personnel.

	Events (n)[1]	Incidence (%)	DIM at event (mean ± SD)	Sensitivity % (n/n)	(95% CI)	HIS positive to CD[2] Days	(95% CI)	*P*-value
Metritis[3]	349	32	6.8 ± 2.6	55 (191/349)	49,60	-1.2	-1.6,-0.7	<0.01
Metritis only[4]	322	30	6.8 ± 2.6	53 (170/322)[a]	47,58	-1.2	-1.6,-0.7	<0.01
Metritis with other disorders[5]	27	2	7.0 ± 2.4	78 (21/27)[b]	62,91	-1.3	-2.4,-0.2	0.03
Metritis by rectal temperature								
≤ 39.4 °C	165	52	7.2 ± 1.8	56 (92/165)	48,64	-1.4	-1.9,-1.0	<0.01
39.5 – 39.9 °C	79	25	6.2 ± 2.0	49 (39/79)	38,61	-1.3	-2.9,0.4	0.13
≥ 40.0 °C	74	23	5.2 ± 1.8	58 (43/74)	46,70	-0.2	-0.9,0.4	0.46

[a-b]Different superscripts indicate differences ($P \leq 0.05$) between means based on mean separation with the LSD test.
[1]Number of events diagnosed (331 and 9 cows with 1 or 2 events of metritis, respectively).
[2]HIS-positive to CD = interval in days between the first positive HIS outcome (positive outcomes only) and clinical diagnosis.
[3]All metritis events recorded.
[4]Cows diagnosed with only metritis from −5 to 2 d relative to CD.
[5]Cows diagnosed with metritis and at least another health disorder from −5 to 2 d relative to CD.

Table 2. Culling dynamics and reproductive outcomes for first AI service for cows diagnosed with metritis included in the ND, HI−, and HI+ groups[1]

	ND[1]	HI-[2]	HI+[3]	*P*-value
Dead ≤ 60 DIM, % (n/n)	0.9 (4/451)	0 (0/153)	1.6 (3/187)	0.74
Dead up to 270 DIM, % (n/n)	2.9 (13/451)	0.7 (1/153)	2.1 (4/187)	0.33
DNB/Sold[4] ≤ 60 DIM, % (n/n)	2.5 (11/451)[a]	3.3 (5/153)[a]	7.0 (13/187)[b]	0.03
DNB/Sold up to 270 DIM, % (n/n)	18.6 (84/451)[a]	14.4 (22/153)[a]	31.0 (58/187)[b]	<0.01
Cows AI to estrus, % (n/n)	91.7 (367/400)	91.4 (128/140)	89.8 (141/157)	0.76
Cows AI by timed AI, % (n/n)	8.3 (33/400)	8.6 (12/140)	10.2 (16/157)	0.76
DIM at 1st AI, days (n)	79 (400)	79 (140)	80 (157)	0.73
P/AI[5] at 1st AI, % (n/n)	46.0 (184/400)	42.9 (60/140)	45.9 (72/157)	0.80

[a-b]Different superscripts within a row indicate differences ($P \leq 0.05$) between means based on mean separation with the LSD test.

[1]ND (n = 451) = cows not diagnosed with a health disorder during the study period; HI− (n = 153) = health index score ≥86 arbitrary units during the 5 d before, the day of, or 2 d after clinical diagnosis; HI+ (n = 187) = health index score <86 arbitrary units during at least 1 d during the 5 d before, the day of, or 2 d after clinical diagnosis.

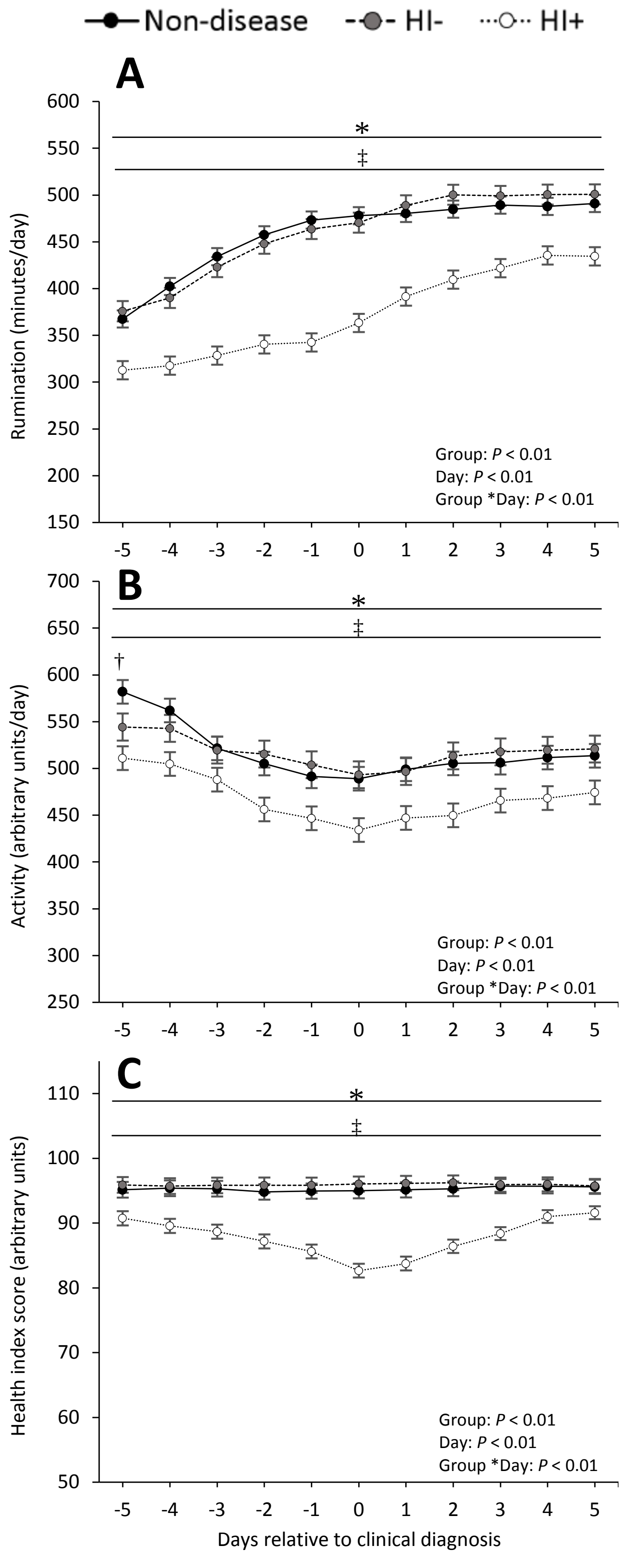

Figure 1. Rumination (A), activity (arbitrary units, AU; B), and health index score (AU; C) patterns from −5 to 5 d relative to clinical diagnosis (CD) for cows that developed metritis (HI+:

n = 187; HI−: n = 153; first event only) compared with cows in the nondisease group (n = 435). Cows were assigned to the HI+ or HI− group if they presented a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after CD. For the nondisease group, the average DIM at CD for cows with metritis was considered "day 0." Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; ‡HI+ vs. HI−.

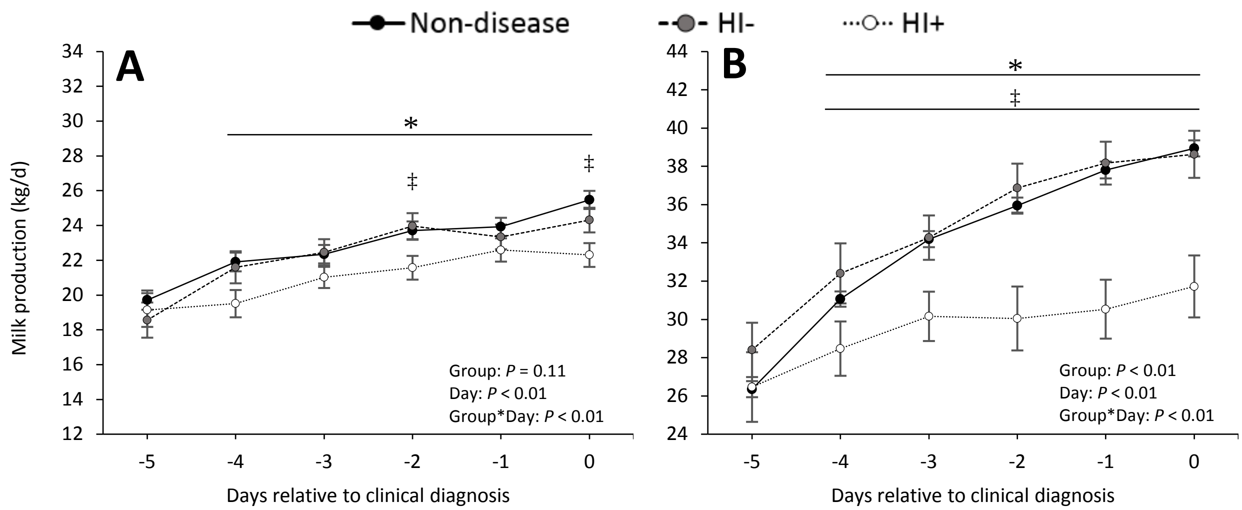

Figure 2. Milk production (kg/d) from −5 d to the day of clinical diagnosis (CD; d 0) for primiparous cows (A) that developed metritis (HI+: n = 110; HI−: n = 96) compared with nondisease cows (n = 171). Cows were assigned to the HI+ or HI− group if they had a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after CD. Milk production (kg/d) from −5 d to the day of CD for multiparous cows (B) that developed metritis compared with nondisease cows (n = 264). Multiparous cows with metritis were assigned to the HI+ (n = 77) and HI− (n = 57) groups following the same criteria as for primiparous cows with metritis. Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; ‡HI+ vs. HI−.

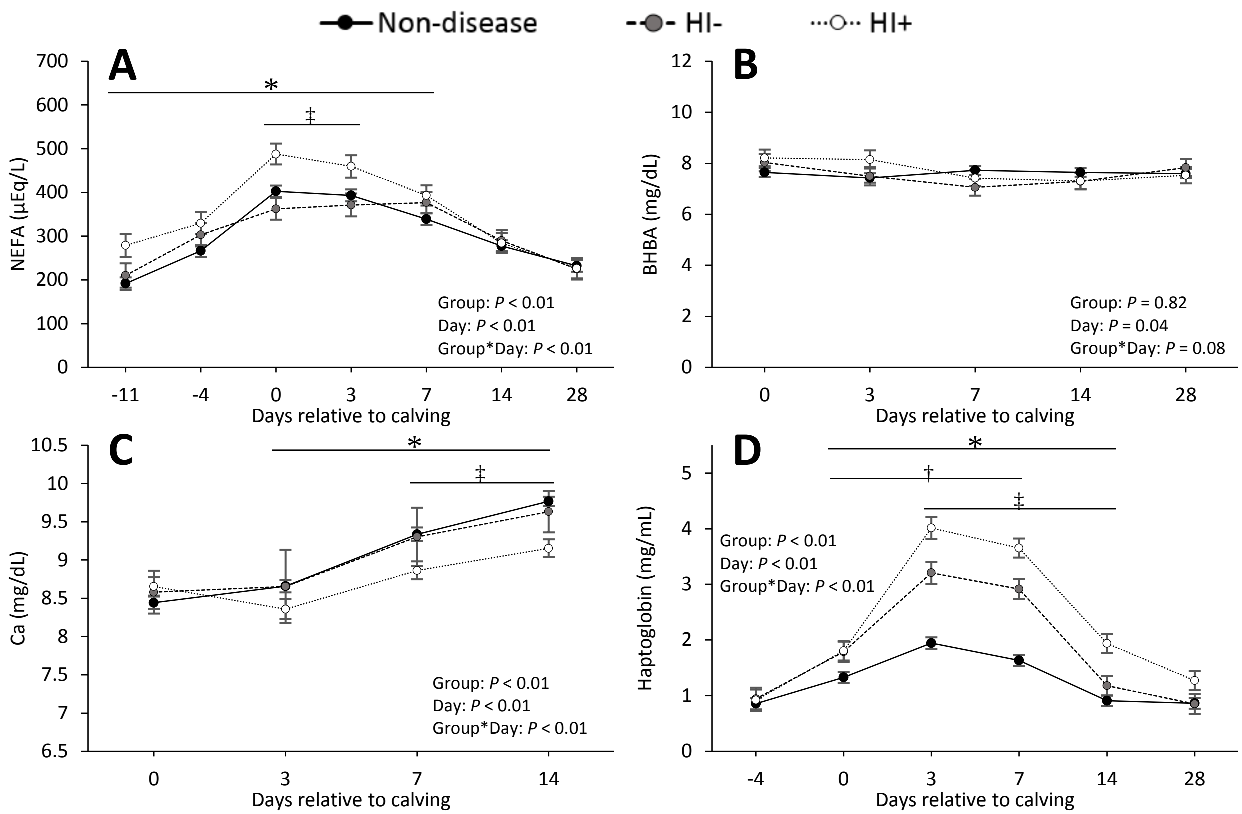

Figure 3. Plasma concentrations of nonesterified fatty acids (NEFA; A), BHB (B), total Ca (C), and haptoglobin (D) for cows that developed metritis compared with cows in the nondisease group (n = 161). Cows with metritis were assigned to the HI+ (n = 51) or HI− (n = 48) group if they had a health index score of <86 or ≥86 arbitrary units, respectively, during the 5 d before, the day of, and 2 d after clinical diagnosis. Values are presented as LSM ± SEM. Within a day, pairwise comparisons that were statistically different ($P \leq 0.05$) based on LSD are represented as follows: *control vs. HI+; †control vs. HI−; ‡HI+ vs. HI−.

SECTION II

IMPACT OF POSTPARTUM VOLUNTARY WAITING PERIOD DURATION ON DAIRY COW PHYSIOLOGY AND HERD PERFORMANCE

CHAPTER V

VOLUNTARY WAITING PERIOD IN DAIRY CATTLE: PHYSIOLOGICAL CHANGES AND EFFECTS ON REPRODUCTIVE PERFORMANCE AND PROFITABILITY

1. General introduction

Determining the duration of the voluntary waiting period (**VWP**) is an important management decision in dairy farms because of the important implications of VWP duration for the productive and reproductive performance of cows (Arbel et al., 2001; Chebel and Santos, 2010; Gobikrushanth et al., 2014). Although several definitions have been proposed, a simple definition of the VWP is the time from calving until cows become eligible for insemination (Inchaisri et al., 2011). However, the duration of the VWP may be quite variable across herds, and it may also vary according to parity group or milk production level within a herd (DeJarnette et al., 2007; Norman et al., 2009).

Although the VWP duration can be quite flexible, a minimum of 40 to 45 d is needed to allow recovery of the reproductive tract to its non-pregnant condition (Risco and Melendez, 2011; Senger, 2012). Inseminating cows as early as possible after calving may not only result in reduced fertility (Britt, 1975; Risco and Melendez, 2011) but also may not be the most profitable strategy (De Vries, 2006), especially for high yielding cows (Arbel et al., 2001). Therefore, extending the VWP duration by a reasonable amount of time beyond the period necessary to recover reproductive tract health may be a simple and inexpensive management change with potential to improve the reproductive performance and profitability of dairy herds.

Timing of pregnancy during lactation is paramount to the profitability of dairy herds because it affects the calving interval, milk production efficiency, and herd replacement dynamics of dairy herds (De Vries, 2006; Inchaisri et al., 2011; Giordano et al., 2012). In this regard, the DIM at which cows become pregnant during lactation is determined by two factors: the VWP duration and the rate at which cows become pregnant after the end of the VWP, also known as pregnancy rate. Traditionally, dairy farms in the US began inseminating cows as early as ~40 to 50 DIM (Caraviello et al., 2006; DeJarnette et al., 2007; Miller et al., 2007), mainly because sub-optimal estrous detection and fertility to AI required that cows received multiple services to conceive. In recent years, however, improvements in herd management and the implementation of effective reproductive management strategies (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008) led to increases of the service rate and pregnancies per artificial insemination (**P/AI**) in well-managed dairy herds. Collectively, these changes resulted in dramatic reductions of the variation and number of days to pregnancy after the end of the VWP for some herds. Thus, farms with excellent reproductive performance of their lactating cow herds have the unique opportunity to better control the timing of pregnancy by modifying the VWP duration thereby; favor the establishment of pregnancy at a stage of lactation that maximizes profitability. Unfortunately, scarce scientific data are available on the effect of VWP duration on dairy herd performance; therefore, decisions about VWP duration for a dairy herd are usually made based on assumptions of expected performance or anecdotal evidence.

2. Physiological changes during the VWP in dairy cattle

After calving, cows undergo a sequence of physiological events that prepare them to receive a new pregnancy: the puerperium. During this process, the reproductive tract returns to its non-pregnant condition, involving two major events: uterine involution (repair) and resumption of ovarian cyclicity (Jainudeen and Hafez, 2000; Senger, 2012). In addition, cows experience major metabolic adaptations to support the demands of lactation (Bauman and Currie, 1980; Drackley, 1999; Ingvartsen, 2006). For example, cows mobilize a significant amount of body tissue reserves (Erdman and Andrew, 1989; Komaragiri and Erdman, 1997; Komaragiri et al., 1998). Although the time needed to complete these processes is quite variable among animals, a minimum of 40 to 45 d seems to be necessary for dairy cows (Risco and Melendez, 2011; Senger, 2012), mainly because of the time required to complete uterine involution.

2.1. Uterine involution

The process of uterine involution after calving includes three major events: myometrial contractions and expulsion of lochia, regeneration of the endometrium, and elimination of bacterial contamination from the reproductive tract (Kiracofe, 1980; Sheldon et al., 2008; Senger, 2012). Strong and frequent myometrial contractions after parturition are essential to facilitate the discharge of fluids and tissue debris from the endometrium (lochia), reduce the size of the uterus, and compress the uterine vasculature (Jainudeen and Hafez, 2000; Senger, 2012). Vasoconstriction of the maternal caruncle stalk occurs immediately after delivery and is critical to induce necrosis of the caruncular tissue. Necrotic uterine caruncles are sloughed and shredded into the uterine lumen between day 5 and 12 postpartum and later discharged with the rest of tissues and fluid in the form of lochia (Sheldon et al., 2008). Expulsion of lochia out of the uterus is usually observed between 2 and 12 days postpartum and should completely disappear by 14 to 23 days postpartum (Lewis, 1997; LeBlanc, 2008). During these early stages of puerperium, uterine size and weight is reduced dramatically with a reduction from ~13 kg after calving to ~1 kg after the first 3 weeks (Sheldon et al., 2008).

Regeneration of the endometrium usually starts after the caruncular tissue has been removed and sloughed into the uterine lumen. Initially, epithelial regeneration takes place in the inter-caruncular area because this part of the endometrium did not undergo necrosis and sloughing. Inter-caruncular epithelium growths faster than in the caruncular areas and, by centripetal cell growth, caruncular epithelium is fully regenerated by about 25 d after parturition (Sheldon et al., 2008; Senger, 2012). Although gross uterine involution defined by rectal palpation may be variable, ranging from 25 to 47 days after calving (Gier and Marion, 1968; Marion et al., 1968; Harrison et al., 1986), complete microscopic regeneration of all layers of the uterine wall usually takes longer, ranging from 6 to 8 weeks postpartum (LeBlanc, 2008; Sheldon et al., 2008).

Immediately after calving, the uterine lumen of almost all cows is contaminated with a wide range of bacteria (Sheldon et al., 2002; Sheldon et al., 2004; Singh et al., 2008). However, most cows successfully overcome this contamination without developing clinical disease. Whether or not cows develop clinical uterine disease ultimately depends on the balance between the immune response mounted against pathogens and the pathogenicity of bacteria (Sheldon et al., 2006; Sheldon et al., 2009; LeBlanc et al., 2011). After calving, the main defense mechanism acting in the uterus to fight bacterial contamination is mediated by innate immunity. Large amounts of polymorphonuclear cells (**PMN**), in particular neutrophils, migrate from the peripheral circulation to the endometrium and uterine lumen (Dhaliwal et al., 2001; Sheldon et al., 2008; Singh et al., 2008). In order to eliminate bacterial contamination and allow endometrial regeneration, the immune response must be quick, strong, and effective but still constrained. Either an excessive or a weak and prolonged inflammatory response can contribute to the pathogenesis of uterine disorders (LeBlanc, 2012, 2014).

Recent research demonstrated a link between metabolic status and impaired inflammatory response in the uterus of dairy cows. For example, cows with more severe negative energy balance (**NEB**) after calving, especially those suffering uterine disorders, had a more pronounced impaired neutrophil killing ability (Hammon et al., 2006). Elevated non-esterified fatty acids (**NEFA**) concentrations similar to that observed around calving can exert a direct inhibitory effect on PMN function *in vitro* (Scalia et al., 2006; Ster et al., 2012). In addition, when adipose tissue is mobilized, not only fatty acids and glycerol are released into the circulation, but also pro-inflammatory cytokines such as tumor necrosis factor α (**TNF-α**) and interleukin- 6 (**IL-6**). These cytokines can stimulate the release of acute phase proteins affecting neutrophil chemotaxis, diapedesis, and migration (LeBlanc, 2014) and can promote insulin resistance, further exacerbating the release of NEFA (Ingvartsen and Moyes, 2013; Sordillo and Raphael, 2013).

Circulating NEFA not only affect PMN function systemically, but they may also contribute directly to endometrial inflammation by binding to the Toll-like receptor 4 (**TLR4**; main receptor for LPS signaling) in the uterus (LeBlanc, 2014). Similarly, excessive formation of reactive oxygen species (**ROS**) due to high metabolism to support milk production (oxidative stress) may also increase inflammation by activating pro-inflammatory (NF-κB and TNFα) pathways (Sordillo et al., 2009; LeBlanc, 2014). Glucose levels and glycogen content in neutrophils have also been related to uterine disease. Galvão et al. (2010a) showed that cows with uterine health disorders had lower neutrophil glycogen content at calving compared with healthy cows. This is important because glycogen is necessary for phagocytosis and the occurrence of the oxidative burst to kill bacteria. Collectively, a wealth of evidence suggests that severe NEB and oxidative stress after calving in dairy cows may contribute to impaired inflammatory response making these two conditions in the early postpartum period risk factors for the development of uterine disease.

The establishment of a uterine infection depends not only on the presence of bacteria in the uterus and a poor immune response, but also on the adherence of the pathogenic microorganisms to the endometrium, colonization or penetration of the epithelium, and/or the release of bacterial toxins (Sheldon et al., 2006). In recent years, mounting evidence supports the idea that certain strains of bacteria have specific virulence factors that favor the occurrence of uterine disorders. For example, recent studies suggested that FimH, a virulence factor present in some strains of *E. coli*, plays a role in adhesion (Krogfelt et al., 1990) and colonization of the uterine epithelium (Mooi and De Graaf, 1985), hence it is an important risk factor for uterine infections in dairy cows (Bicalho et al., 2010; Sheldon et al., 2010; Bicalho et al., 2012). Pyolysin and leukotoxin A found in *T. pyogenes* and *F. necrophorum*, respectively, were also linked to uterine infections (Santos et al., 2010; Bicalho et al., 2012). In summary, impaired immune response and the presence of certain strains of bacteria with specific virulence factors has been proposed as major risk factors for the development of uterine disorders after calving.

2.2. Resumption of ovarian cyclicity

After calving, estradiol concentrations (high in late pregnancy) are drastically reduced to basal levels allowing a transient release of follicle stimulating hormone (**FSH**) from the pituitary gland (Murphy et al., 1990; Butler, 2001; Crowe, 2008). Circulating concentrations of FSH peak at about 4 to 5 days postpartum and stimulate the initiation of a new follicular wave (Beam and Butler, 1997; Crowe et al., 1998). Thereafter, selection of the first dominant follicle occurs around 10 to 12 days postpartum (Savio et al., 1990; Beam and Butler, 1997). These events take place in all cows independent of energy balance, occurrence of periparturient diseases, and environmental conditions (Beam and Butler, 1997; Beam and Butler, 1998; Sheldon et al., 2002; Sheldon et al., 2008).

Even though most dairy cows have a dominant follicle present on their ovaries by the first couple of weeks postpartum, ovulation of the dominant follicle depends upon the re-establishment of pulsatile LH secretion and the ability of the follicle to produce sufficient estradiol to trigger ovulation (Butler, 2001; Crowe, 2008). Consequently, based on the pattern of luteinizing hormone (LH) secretion after calving, the first dominant follicle has three possible fates: ovulation and formation of a corpus luteum (CL), non-ovulation followed by atresia and the initiation of new follicular wave (turnover), or non-ovulation followed by the formation of a follicular cyst (Savio et al., 1990; Beam and Butler, 1997; Butler, 2001). In a recent study, Cheong et al. (2016) demonstrated that cows having an ovulatory first dominant follicle postpartum had higher plasma LH concentrations because of higher LH pulse frequency but not greater pulse amplitude than cows with non-ovulatory follicles. Moreover, the follicular fluid of ovulatory cows had greater estradiol and androstenedione concentrations but similar estradiol/androstenedione ratio, suggesting a deficiency in androstenedione synthesis (because of lower LH pulses) rather than in the capacity of follicles to convert androstenedione to estradiol in non-ovulatory cows (Cheong et al., 2016).

It is well documented that early postpartum ovulation and cyclicity leads to improved reproductive performance in dairy cows (Smith and Wallace, 1998; Westwood et al., 2002; Galvão et al., 2010b). In this regard, postpartum severe NEB and uterine infection may negatively affect the resumption of ovarian cyclicity. For example, severe NEB after calving has been associated with anovulation of the first dominant follicle by decreasing pulsatile LH secretion and increasing the sensitivity to the negative feedback of estradiol (Butler et al., 1981; Canfield and Butler, 1990; Beam and Butler, 1997). Likewise, reduced estradiol production and delayed first postpartum ovulation were described in cows with low glucose, insulin and insulin like growth factor-1 (IGF-1) concentrations, and high levels of NEFA and BHBA (Butler, 2003; Webb et al., 2004; Bossaert et al., 2008).

Uterine infection may also be associated with delayed ovulation postpartum and impaired corpus luteum function. The dominant follicle of the first postpartum follicular wave in cows with uterine infection grew at a slower rate, produced less estradiol and, if ovulation occurred, the CL produced less progesterone (Dohmen et al., 2000; Sheldon et al., 2002). One possible explanation for this link might be the absorption of LPS from the uterus which can act systemically to suppress gonadotropin-releasing hormone (**GnRH**) and LH secretion (Battaglia et al., 2000; Karsch et al., 2002), and locally to reduce estradiol production by the follicle (Herath et al., 2007; Sheldon et al., 2009). On the other hand, uterine infection may also affect resumption of regular ovarian cyclicity by altering luteolysis. Damage to the endometrium by bacterial infections can induce a shift from prostaglandin PGF2α (luteolytic) to prostaglandin E (luteotropic) production which can ultimately result in an extension of the luteal phase and delayed return to estrus and ovulation (Opsomer et al., 2000; Herath et al., 2007; Sheldon et al., 2009).

2.3. Energy balance

It is well recognized that modern dairy cows experience NEB after calving as a result of an abrupt increase in energy requirements to support the onset lactation and a concurrent depression in dry matter intake (**DMI)** (Grummer et al., 2004; Ingvartsen, 2006; Esposito et al., 2014). To overcome this situation, cows undergo major metabolic adaptations (homeorhetic adaptations) involving a variety of tissues (fat, liver, skeletal muscle, and mammary gland among others) to provide enough glucose to the mammary gland for milk synthesis (Bauman and Currie, 1980; Bell, 1995). Glucose output is drastically increased by the liver while other peripheral tissues (like skeletal muscle) reduce glucose uptake and rely on fatty acids as the main source of energy (Bauman and Elliot, 1983; Petterson et al., 1993; Reynolds et al., 2003). Therefore, adipose tissue mobilization is essential during the transition period to maintain major physiological functions while privileging the use of glucose by the mammary gland.

Although NEB is not routinely assessed in dairy cows because of the difficulty to measure individual cow dry matter intake, indirect measures of adipose tissue reserves such as BCS and markers of fatty mobilization in circulation such as NEFA and BHBA have been widely used to supplant energy balance determinations. Using these parameters, a strong link between energy balance and reproductive performance has been established. For instance, the effect of changes in BCS on fertility has been studied over the last 30 years and produced highly reproducible results. Butler and Smith (1989) found a major reduction in P/AI at first service in cows losing more than one unit of BCS (scale 1 to 5) compared to cows losing less BCS. Likewise, Santos et al. (2009) showed that cows losing more than one unit of BCS after calving had reduced P/AI at first service than cows losing less than one unit or not losing BCS. In a recent study, Carvalho et al. (2014) also observed the lowest P/AI for cows that lost BCS, intermediate for cows that maintained BCS, and greatest for cows that gained BCS. Collectively, these results highlight the importance of reducing the severity of NEB after calving (measured as changes in BCS) to maximize reproductive performance.

Circulating concentrations of NEFA during the transition period may also be indicative of reproductive performance during lactation. Garverick et al. (2013) reported that the probability of pregnancy at first service through timed artificial insemination (**TAI**) was reduced as serum concentration of NEFA on day 3 postpartum increased. An epidemiological study conducted in the Northeast United States showed that NEFA concentrations above certain levels during the transition period ($\geq$ 0.27 mEq/L prepartum or $\geq$ 0.72 mEq/L postpartum) reduced the risk of pregnancy within 70 d after the end of the VWP (Ospina et al., 2010a). In addition, herds with more than 15% of the cows above the pre-established thresholds (herd-alarm level) had reduced pregnancy rate (Ospina et al., 2010b). Although different thresholds for NEFA and herd alarm levels were used, another study found a reduction in P/AI at first service for herds with a greater proportion of cows than the herd alarm level supporting previous results (Chapinal et al., 2012).

Several mechanisms linking the detrimental effect of severe NEB on reproductive performance have been proposed. Severe postpartum NEB has been associated with failure to ovulate the first dominant follicle and longer intervals to first ovulation (Butler et al., 1981; Canfield and Butler, 1990; Beam and Butler, 1997; Rukkwamsuk et al., 1999). As a result, severe BCS loss after calving can increase the proportion of cows not cyclic at the end of the VWP (Gümen et al., 2003; Lopez et al., 2005; Santos et al., 2009). Negative energy balance can impair immune responses after calving through reduced neutrophil function, excessive release of ROS, and activation of pro-inflammatory cytokines (i.e., IL-6 and TNF-α) which can lead to the development of uterine disorders, and thereby affect fertility (Hammon et al., 2006; Sordillo et al., 2009; LeBlanc, 2014). Finally, excessive NEB may be deleterious for oocyte development and embryo quality. Changes in circulating levels of metabolites (e.g., NEFA, BHBA, glucose, insulin, IGF-1) due to severe NEB in early lactation may affect oocytes directly or indirectly through changes in the follicular fluid and surrounding follicular cells (e.g., altering cumulus expansion and nuclear maturation and inducing apoptosis in granulosa cells), all of which may have carry-over effects on oocyte and embryo quality.

3. Effect of the VWP duration on reproductive performance of dairy cows

3.1. Potential physiological advantages of extending VWP duration on reproductive performance of lactating dairy cows

Extending the duration of the VWP may improve reproductive outcomes, especially at first service, through multiple mechanisms. A longer VWP duration may provide cows more time to recover their uterine heath, reducing the proportion of cows affected by uterine disease (clinical or cytological) around the time of insemination (LeBlanc et al., 2002; Gilbert et al., 2005; Sheldon et al., 2009; Gautam et al., 2010). This is important because a substantial proportion of cows can be affected by clinical (i.e., presence of purulent vaginal discharge in the cranial portion of the vagina) or subclinical (i.e., cytological; characterized by the infiltration with large number of PMNs in the endometrium) endometrial inflammation during the postpartum period (Gilbert et al., 2005; McDougall et al., 2007; Barlund et al., 2008; Cheong et al., 2011; Wagener et al., 2017). In this regard, Gautam et al. (2010) reported that through spontaneous recovery the prevelance of clinical endometritis decreased from 45% at 15 to 20 d to 15% at 29 to 60 d after calving. Improved uterine health might be the consequence of better immune status later in lactation, extra time available to resolve the inflammatory process established immediately after calving, or both (LeBlanc et al., 2011; LeBlanc, 2014). Thus, an extended VWP may reduce the proportion of cows with poor uterine health around first service reducing the detrimental effect of uterine disease on P/AI and time to pregnancy during lactation (Barlund et al., 2008; Dubuc et al., 2011; Denis-Robichaud and Dubuc, 2015).

Additional time from calving to the end of the VWP may also provide cows more time to return to hormone secretion patterns that promote ovulation and resumption of normal ovarian cyclicity (Butler, 2003; Kawashima et al., 2012; Cheong et al., 2016). It is well documented that early postpartum ovulation and cyclicity lead to improved reproductive performance (Smith and Wallace, 1998; Westwood et al., 2002; Galvão et al., 2010b) and more estrous cycles before first service were linked to reduced days to first service and greater P/AI (Thatcher and Wilcox, 1973; Butler and Smith, 1989; Darwash et al., 1997). Conversely, anovular cows have lower reproductive efficiency regardless of the programs used to conduct first service (i.e., at detected estrus or TAI) (Gümen et al., 2003; Santos et al., 2009). For example, cows that remained anovular for more than 50 DIM were less likely to become pregnant during lactation and were more likely to leave the herd than cows that resumed cyclicity before 50 DIM (Butler, 2003). Thus, extra days of VWP may increase the number of estrous cycles and the proportion of cyclic cows before first service hence, improve overall reproductive performance of the herd.

Finally, delaying first service to avoid the period of NEB in early lactation and provide time to recover body tissue reserves mobilized after calving may also improve reproductive performance. In fact, a BCS of ≥ 2.75 units (scale of 1 to 5) at the time of insemination has been strongly associated with higher probability of pregnancy at first service (Moreira et al., 2000; Souza et al., 2007; Souza et al., 2008; Santos et al., 2009; Herlihy et al., 2012; Carvalho et al., 2014). This effect may be explained by the well-recognized impact of postpartum NEB on return to cyclicity (Gümen et al., 2003; Lopez et al., 2005; Santos et al., 2009), uterine inflammation (Hammon et al., 2006; Galvão et al., 2010b; LeBlanc, 2014), and oocyte quality (Leroy et al., 2008a; Leroy et al., 2008b).

Thus, extending the duration of the VWP may be a simple and inexpensive strategy that dairy farmers can use to optimize fertility after calving, affecting first service outcomes and overall reproductive performance during lactation.

3.2. Effect of manipulating VWP duration on reproductive performance of lactating dairy cows

Despite the potential benefits of extending the duration of the VWP on reproductive performance, the impact of this management strategy on overall herd performance has not been fully elucidated. Although many studies manipulated the duration of the VWP to some extent, simultaneous changes to the management strategy used to submit cows for first service (for instance, combination of estrus detection and TAI versus 100% TAI) confounded the evaluation of different VWP durations (Chebel and Santos, 2010; Machado et al., 2017). In addition, some studies limited enrollment to cows to certain parity groups or milk production level (Van Amburgh et al., 1997; Arbel et al., 2001; Gobikrushanth et al., 2014). Therefore, there are few studies which truly investigated the effect of extra days of VWP on reproductive outcomes for cows of all parities and production level.

Using a randomized control design, Van Amburgh et al. (1997) evaluated the effect of extending the VWP duration from 60 to 150 DIM on reproductive and productive performance. In this experiment with a limited number of second lactation cows (54 cows per group) inseminated at detected estrus (**EDAI**) only, no differences in P/AI (short VWP = 71%; long

VWP = 62%), heat detection efficiency (short VWP = 49%; long VWP = 62%), and services per conception (shot VWP = 1.4; long VWP = 1.6) were observed. Extra days from calving until first service in cows with extended VWP duration did not improve reproductive performance. Hence, cows with longer VWP had greater days open (short VWP = 122 d; long VWP = 212 d), and consequently, longer lactations (short VWP = 346 d; long VWP = 492 d) and longer calving interval (short VWP = 401 d; long VWP = 547 d).

Similarly, Arbel et al. (2001) conducted a randomized control experiment to evaluate the effect of extending the VWP by 60 d (from 90 to 150 in primiparous, and from 60 to 120 in multiparous cows) on reproductive performance of dairy cows under Israeli conditions. For this experiment conducted in 19 commercial dairy herds, only cows with above average milk production (primiparous cow that reached at least 30 kg of milk in one of the first three milk records, or multiparous cows that had a value-corrected milk above the herd average in its previous lactation) and cows that calved during the cool months of the year were included. In addition, potential differences in management strategies to submit cows for first service, which may have varied between farms, were not specifically reported. Similarly to the study by Van Amburgh et al. (1997), P/AI at first service was similar for the control and extended VWP group in both primiparous (40.3% vs. 43.5%, respectively) and multiparous cows (36.6% vs. 38.7%, respectively). Consequently, days open and calving interval were approximately 60 days longer (i.e., the approximate difference in VWP duration) for cows with extended VWP. Collectively, these two studies suggested that extending the duration of the VWP did not improve P/AI and overall reproductive performance, therefore cows with longer VWP became pregnant later in lactation and had longer calving interval.

In an experiment conducted in Germany using only cows with above (> 30 and > 40 kg/d at week 5 of lactation for primiparous and multiparous cows, respectively) or below (≤ 25 and ≤ 32 kg/d at week 5 of lactation for primiparous and multiparous cows, respectively) average herd milk production, the effect of extending the duration of the VWP from 77 to 98 DIM (high producing cows) or 56 to 77 DIM (low producing cows) was evaluated (Tenhagen et al., 2003). In this experiment, all cows were submitted to TAI for first service after synchronization of ovulation with the Ovsynch protocol (Pursley et al., 1995). An increment of ~13 (28.2% vs. 41.4%) and ~20 (14.4% vs. 34.5%) percentage points in P/AI to first service was observed for high and low producing cows with the extended VWP, respectively. Conversely, no differences were observed in overall P/AI for all AI services during lactation and the proportion of cows pregnant at 200 DIM. Despite increased P/AI at first service and similar overall P/AI during the rest of lactation, high producing cows with longer VWP duration had ~10 more days open (113 d vs. 123 d) than high producing cows with shorter VWP. For that reason, the 13-percentage point increment in P/AI at first service in high producing cows was not sufficient to compensate for the extra 21 days of VWP, which delayed time to pregnancy during lactation.

More recently, Gobikrushanth et al. (2014) reported the results of a retrospective cohort study using data from a commercial farm in Florida that extended the duration of the VWP during summer months only. In this study, synchronization of estrus was performed using the Presynch-Ovsynch protocol (Moreira et al., 2001), and cows were inseminated if estrus was detected after the second (short VWP) or after the first or second (long VWP) PGF2α treatment of Presynch. Unfortunately, the reproductive programs used to submit cows for first service resulted in overlapped DIM at first service for a substantial proportion of cows, which limited the ability of truly comparing different VWP durations. In addition, results from this study might have been confounded by season of AI because cows with short VWP (57 to 63 d) received first service during summer and fall, whereas cows with long VWP (64 to 121) received first service during fall only. Under these conditions, cows with the extended VWP had improved first service P/AI, with a 3 and 8-percentage point difference in favor of the extended VWP group for primiparous (36% vs. 39%) and multiparous cows (29% vs. 37%), respectively. However, similar to the study by Tenhagen et al. (2003) the increased P/AI at first service did not compensate for the extra days of VWP. Therefore, cows with longer VWP became pregnant later in lactation (median days to pregnancy were 113 and 130 for short and long VWP group, respectively) and consequently both primiparous (404 vs. 427 d for short and long VWP, respectively) and multiparous cows (413 vs. 430 d for short and long VWP, respectively) had longer calving interval.

Collectively, these previous reports did not provide conclusive evidence that extending the duration of the VWP is beneficial or detrimental for the reproductive performance of dairy herds. Ambiguous results, multiple exclusion criteria, and use of different reproductive management strategies to submit cows for first service preclude drawing definitive conclusions. Therefore, more research including all cows in the herd and seasons of insemination, as well as cows submitted to first service using the same reproductive management strategy, is needed to accurately test the effect of extra days of VWP on reproductive performance.

3.3. Interactions between method of submission for first service and VWP duration on reproductive performance.

In recent years, reproductive management strategies including synchronization of estrus and ovulation protocols have been widely implemented in the dairy industry (Caraviello et al., 2006; Ferguson and Skidmore, 2013; Wiltbank and Pursley, 2014). Indeed, nowadays most reproductive programs employed to submit lactating dairy cows for first service consist of either a combination of AI at detected estrus and TAI, or submission of all cows for TAI (Caraviello et al., 2006; Ferguson and Skidmore, 2013; Scott, 2016).

The choice of method of submission for first service depends upon many farm- and protocol-specific factors among which the pattern of insemination after the end of the VWP and P/AI to first service are some of the most relevant. Combined strategies have the advantage of reducing the number of cows submitted to TAI thereby, the number of hormonal treatments and associated labor. Using protocols like Presynch-Ovsynch, up to 50 to 70% of cows may be inseminated at detected estrus after the PGF2α treatments of the protocol (Chebel et al., 2006; Fricke et al., 2014; Giordano et al., 2016; Machado et al., 2017). On the other hand, using an all TAI program to submit cows for first service can be beneficial by reducing the number of and the variation for days to first service (Pursley et al., 1997). In addition, an all TAI program may increase P/AI because some protocols improve synchrony of ovulation and the endocrine environment before insemination (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008). In this regard, several experiments compared P/AI after submission to first service with a combined or an all TAI strategy in cows synchronized with the Presynch-Ovsynch protocol (Chebel and Santos, 2010; Gumen et al., 2012; Fricke et al., 2014; Borchardt et al., 2016; Machado et al., 2017). Some of these studies (Gumen et al., 2012; Fricke et al., 2014; Borchardt et al., 2016; Machado et al., 2017) have shown that a combined strategy (EDAI + TAI) resulted in reduced P/AI at first service when compared with all TAI. For example, Gumen et al. (2012) and Fricke et al. (2014) reported a 20 (EDAI + TAI = 36%; TAI = 56%) and 8-percentage point (EDAI and TAI = 32%; TAI = 40%) increase in overall P/AI for cows submitted to all TAI rather than a combination of EDAI and TAI. Similarly, Machado et al. (2017) reported greater P/AI at first service in cows submitted to an all TAI program, but this difference was smaller than in previous studies (5.5-percentage point; EDAI and TAI = 35.7%; TAI = 41.2%) and only for multiparous cows. Furthermore, a recent meta-analysis (Borchardt et al., 2016) of 20 articles including 9,813 cows presynchronized with the Presynch-Ovsynch protocol for first AI, reported overall P/AI of 30.9% and 41.7% for the combination of EDAI and TAI and all TAI, respectively. Of note, in many of these studies results may have been confounded, at least in part, by the effect of VWP duration because cows in the combined program received AI either at detected estrus immediately after the end of the VWP (usually coincident with one of the PGF treatments of Presynch) or TAI several days later (at least 20 d) at the same time that cows in the all TAI program.

First service management programs for lactating dairy cows have implications for reproductive performance other than first service P/AI. Reductions in first service P/AI for cows inseminated at detected estrus may be compensated by a shorter VWP and/or earlier re-insemination of cows failing to conceive to first service. Therefore, some studies evaluated reproductive performance during an entire lactation rather than at first service only. For example, Chebel and Santos (2010) randomly assigned cows synchronized with a modified Presynch-Ovsynch protocol to receive first service after a combination of EDAI and TAI with a VWP of 49 ± 3 DIM or all TAI with a VWP of 72 ± 3 DIM. No differences were observed between groups for P/AI at first service (described above), hazard of pregnancy during lactation (HR = 1.12; 95% CI 0.94-1.35), mean and median days to pregnancy (154 and 125 d for EDAI + TAI; and 153 and 134 d for all TAI, respectively), and number of inseminations (EDAI + TAI = 3.6; all TAI = 3.8). On the other hand, Machado et al. (2017) found greater P/AI at first service for multiparous cows but not for primiparous cows synchronized with the Presynch-Ovsynch protocol that received TAI compared to cows inseminated through a combination of EDAI and TAI. As a result, time to pregnancy during lactation for multiparous cows in that experiment was similar between experimental treatments (134 d for both groups) suggesting that lower P/AI at first service was compensated by additional opportunities to receive inseminations in cows submitted to the program combining EDAI and TAI. Conversely, for primiparous cows the additional opportunities for insemination and similar P/AI at first service than cows that received TAI in the combined EDAI and TAI program resulted in greater hazard of pregnancy during lactation (HR = 1.40; 95% CI 1.24-1.58) and, consequently, reduced median days to pregnancy (113 vs. 134 EDAI and TAI vs. all TAI, respectively). Of note, in both studies (Chebel and Santos, 2010; Machado et al., 2017) the VWP duration was different between groups (EDAI and TAI vs. all TAI) and about 40% of the cows assigned to the combined approach with short VWP received TAI at the same DIM as cows in the all TAI group. These experiments provided evidence that programs which combine submission of cows for first service at detected estrus and TAI can lead to similar overall reproductive performance during lactation when compared to all TAI programs. Nevertheless, it is possible that P/AI to first service for the all TAI program in these experiments was not maximized because the Presynch-Ovsynch protocol was used to synchronize ovulation. Some recent evidence suggest that GnRH-based protocols such as Double-Ovsynch (Souza et al., 2008) increase P/AI when compared with PGF-based protocols such as Presynch-Ovsynch, in particular for primiparous cows (Souza et al., 2008; Herlihy et al., 2012; Borchardt et al., 2017). Therefore, it remains to be determined whether using a GnRH-based protocol like Double-Ovsynch, which is expected to maximize first service P/AI, results in better reproductive performance than a combined approach (i.e., at estrus and TAI) to submit cows for first service. It also remains to be determined if interactions between the method of submission to first service, using different types of synchronization protocols (i.e., PGF- vs. GnRH-based), and VWP duration have an impact on the reproductive, productive, and economic performance of lactating dairy cows.

4. Profitability of dairy cows managed with different VWP duration

4.1. Timing of pregnancy during lactation and dairy herd profitability

Decisions about VWP duration for the lactating cow herd have major implications not only for first service P/AI, but also for time to pregnancy during lactation (referred to in some studies as days open), lactation length, and herd exit dynamics, all of which affect profitability. Therefore, a better understanding of how timing of pregnancy affects cow and herd profitability is key to unravel the interactions between VWP duration and timing of pregnancy during lactation. In this regard, although the effect of time to pregnancy and calving interval on the productive performance and profitability of dairy herds has been extensively studied, ambiguous results have been reported.

Traditionally, it has been generally accepted that a calving interval of approximately 12 months, achieved by reducing days open, maximizes milk production and profitability (Olds et al., 1979; Oltenacu et al., 1981; Holmann et al., 1984; Dijkhuizen et al., 1985; Schmidt, 1989). For example, Olds et al. (1979) detected that milk per day of a calving interval decreases as days open increase, and reported that each additional day open from 40 to 140 days resulted in reduced income over feed cost ($0.71 for primiparous and $1.18 for multiparous cows). Similarly, Oltenacu et al. (1981) observed greater annualized milk yield when cows had fewer days open, with an increment in net return per cow of $60 when days open decreased from 136 to 119. Additionally, some studies found that fewer days open (about 40 to 60 days) were optimal to maximize milk yield per unit of time (milk yield per year or per day of calving interval) rather than per lactation, as well as profitability (Holmann et al., 1984; Dijkhuizen et al., 1985).

On the other hand, several contemporary studies to those discussed above have shown a productive benefit of delaying pregnancy during lactation. For example, Weller et al. (1985) observed that maximum cumulative milk production for two consecutive annualized lactations was achieved at 117 and 98 days open for primiparous and multiparous cows, respectively, and conception before 60 days of lactation had a detrimental effect on cumulative milk yield. Similarly, Weller and Folman (1990) found that optimum days open were longer for primiparous than multiparous cows, ranging from 91 to 110 DIM, and that expected losses for early insemination (around 40 DIM) could reach up to 780 kg of fat-corrected milk per cow per lactation. Similarly, Bar-Anan and Soller (1979) reported that high yielding cows respond positively to some extension of the calving interval, proposing that high milk yielding herds should start inseminating primiparous cows not earlier than 70 DIM.

A more recent study using modeling techniques investigated the economic value of pregnancy during lactation in dairy cows, according to the stage of lactation, lactation number, milk yield, milk price, replacement heifer cost, probability of pregnancy, probability of involuntary culling, and breeding decisions (De Vries, 2006). Results from this study showed that cows with more persistent lactation curves had a greater value for a new pregnancy (i.e. greater profitability) when they became pregnant later in lactation (60 DIM used as reference). Moreover, the economic value of a new pregnancy was negative at 60 DIM in first lactation cows when milk production was more persistent, cows had greater probability of pregnancy, and were high producing. Therefore, this author concluded that first AI should be delayed past 60 d (used in this study) to maximize profitability in some specific group of animals (especially first lactation cows and cows with high milk production persistency).

Overall, these studies showed ambiguous results about the timing of pregnancy during lactation that maximizes production performance and cow profitability. While some studies reported better economic revenues associated with shorter days open and, consequently, shorter calving intervals, others suggested that early insemination during lactation could be detrimental to farm profitability. In addition, the latter studies suggested that primiparous and high yielding cows (groups with greater milk production persistency) might benefit the most from a delay in days at first insemination. As many of the studies that suggested an economic benefit of very short calving intervals were conducted more than 20 to 30 years ago, it is possible that their results do not necessarily represent current dairy production conditions. Indeed, during the last 20 to 30 years many factors important for the effect of timing of pregnancy during lactation on profitability have changed dramatically. Increases in milk production and persistency of lactation, increased culling pressure, increased availability of replacements, and largely different economic conditions are some examples. Thus, it is possible that under current conditions, delayed pregnancy and extended calving intervals may be beneficial for all or some subgroups of cows as suggested by some of the most recent studies (De Vries, 2006).

4.2. Effect of manipulating VWP duration on dairy herd profitability

Studies conducted to evaluate the effect of VWP duration on dairy cow profitability are scarce, and those conducted have shown contradictory results. Van Amburgh et al. (1997) observed greater income over feed cost, lower replacement cost, and greater profitability per productive life as well as per year and day of productive life for second lactation cows with long rather than short calving interval (18 vs. 13.2 mo, respectively). In this experiment with a limited number of cows, the differences in calving interval were attained by extending the VWP from 60 to 150 DIM. However, this study assumed that herd life expressed as number of lactations (2.9 lactations per productive life) would not change with the extended calving interval (i.e., fewer replacements needed to maintain herd size per unit of time in the group with extended calving interval) and that cows with longer calving interval would spend fewer days of their life in the post-partum period, thus reducing the risk of health disorders. Another caveat of this experiment was modeling rather than collecting data for the majority of the lifecycle of cows.

In another randomized controlled experiment conducted by Arbel et al. (2001) under Israeli conditions, the effect of extending the duration of the VWP by 60 d (from 90 to 150 in primiparous, and from 60 to 120 in multiparous cows) on cow profitability was evaluated. Both primiparous and multiparous cows with extended lactations due to a longer VWP had greater total net returns and net returns per day of calving interval during the experimental lactation. The advantage was, however, greater in primiparous cows because of their greater persistency of lactation than for multiparous cows. Furthermore, differences in favor of longer VWP duration were maintained during the first 150 d of the following lactation which resulted in greater total net returns and net returns per day of the study period (experimental lactation plus 150 d of the following lactation). In this study, only cows that did not calve during summer months and cows with above average milk production were included, limiting the relevance of findings to certain groups of cows within a herd.

On the other hand, two recent studies showed similar profitability for cows submitted to a short or a long VWP duration. Chebel and Santos, (2010) found no difference in income over feed cost, replacement cost, and overall cow profitability of cows managed with a short (49 ± 3 DIM) or long (72 ± 3 DIM) VWP duration. However, this study only evaluated economic outcomes during the experimental lactation, without considering a time component (lactation length was longer in cows with long VWP duration) and the effect of parity groups. Moreover, the method of submission to first service was different between groups (EDAI and TAI vs. all TAI), resulting in more than 40% of the cows in the short VWP groups (EDAI and TAI) inseminated at the same DIM than cows in the long VWP group (all TAI). Therefore, the lack of differences found in this experiment could be a consequence of the small difference in the average DIM at first service between groups (65 vs. 74 d for short and long VWP, respectively). Likewise, Gobikrushanth et al. (2014) observed similar profitability during the experimental lactation, subsequent lactation, and total cash flow simulated for up to 6 years of enrollment in a study in which cows had a regular (57 to 63 DIM) or extended (64 to 121 DIM) VWP during summer. This observational retrospective cohort study had some design limitations that confound interpretation of results [i.e., delayed inseminations during summer only, wide range of DIM at first service for cows in the long VWP group (i.e., 64 to 121), effect of season on first service, and overlapped DIM at first service for a substantial proportion of cows].

Finally, using Monte-Carlo simulation models, Inchaisri et al. (2011) reported that under Dutch conditions, 63% of the animals had an optimal VWP shorter than 8 weeks and the largest proportion of cows with a similar optimal VWP (i.e., 6 weeks) was 37% of the cows. Conversely, only about 10% of the cows beneficiated from a VWP of 10 weeks or longer (up to 15 weeks). In particular, longer VWPs were optimal for cows in their first lactation, cows with high milk persistency, cows with delayed time to first postpartum ovulation, and cows that suffered health disorders after calving.

In summary, results from different studies over the last 20 years have been inconclusive about the effect of VWP duration on dairy cow and herd profitability. Study design limitations, multiple exclusion criteria, potential confounders (e.g., season, parity group, and milk yield strata) may explain the ambiguity of results. Moreover, the use of different methods to calculate profitability as well as different consideration of time effects (i.e., analysis based on a fixed number of lactation(s) versus a fixed period of time) could have also played an important role in generating conflicting results. Therefore, more research is needed to elucidate the effect of extending the duration of the VWP on cow and herd profitability.

5. Summary

This literature review intended to summarize the major physiological changes that dairy cows experience during their VWP. Moreover, it provides an overview of currently available research on the effect of extending the duration of the VWP on reproductive performance and profitability of individual dairy cows and herds.

Timing of pregnancy during lactation affects the profitability of dairy herds by defining the calving interval, milk production efficiency, and herd replacement dynamics. Given that the VWP has a major impact on time to pregnancy during lactation, several studies over the last 40 years were aimed at identifying a VWP duration that maximizes cow reproductive performance and profitability. Nonetheless, results from these experiments have been inconsistent, limited to only certain groups of cows or seasons of the year, obtained by computer simulation, or through studies with observational rather than randomized controlled designs. Therefore, we conducted two randomized controlled experiments including cows from all parities and all seasons of the year to answer questions about this topic. The experiment presented in Chapter VI of this section had the primary objectives of evaluating the reproductive performance, herd exit dynamics, and lactation performance of dairy cows managed with a VWP of 60 or 88 days. Thereafter, Chapter VII presents a deterministic and a stochastic analysis of profitability for dairy cows managed with a VWP of 60 or 88 days. Finally, Chapter VIII presents the results of an experiment aimed at investigating the effect of submitting lactating dairy cows for first service with a management strategy that relied on a combination of EDAI and TAI versus an all TAI program with a GnRH-based synchronization of ovulation protocol and different VWP duration on time to pregnancy and herd exit dynamics during lactation.

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CHAPTER VI

EXTENDING THE DURATION OF THE VOLUNTARY WAITING PERIOD FROM 60 TO 88 DAYS IN COWS THAT RECEIVED TIMED ARTIFICIAL INSEMINATION AFTER THE DOUBLE-OVSYNCH PROTOCOL AFFECTED THE REPRODUCTIVE PERFORMANCE, HERD EXIT DYNAMICS, AND LACTATION PERFORMANCE OF DAIRY COWS

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ABSTRACT

This experiment evaluated the reproductive performance, herd exit dynamics, and lactation performance of dairy cows managed with a voluntary waiting period (**VWP**) of 60 or 88 d. Secondary objectives were evaluating VWP effect on cyclicity status, uterine health, systemic inflammation, and body condition score (**BCS**) before first service. Lactating Holstein cows from 3 commercial farms in New York State cows were blocked by parity group and total milk yield in their previous lactation and then randomly assigned to VWP of 60 (**VWP60**; n = 1,352) or 88 (**VWP88**; n = 1,359) days in milk (**DIM**). All cows received the Double-Ovsynch protocol (GnRH-7 d-PGF2α-3 d-GnRH-7 d-GnRH-7 d-PGF2α-56 h-GnRH-16 to 20 h-timed artificial insemination; **TAI**) for synchronization of ovulation and TAI. For second and greater artificial insemination (**AI**), cows received AI after detection of estrus or the Ovsynch protocol (GnRH-7 d-PGF2α-56 h-GnRH-16 to 20 h-TAI) initiated 32 ± 3 d after AI for cows not re-inseminated at detected estrus. Cyclicity status (progesterone concentration), uterine health (vaginal discharge and uterine cytology), BCS, and systemic inflammation (haptoglobin concentration) were evaluated at baseline (33 ± 3 DIM for both treatments), beginning of the Double-Ovsynch protocol, and 10 d before TAI. Effects of treatments were assessed with multivariable statistical methods relevant for each outcome variable. Extending duration of VWP from 60 to 88 DIM increased pregnancies per AI (**P/AI**) to first service (VWP60 = 41%; VWP88 = 47%). Nonetheless, the greatest benefit of extending VWP on first-service P/AI was for primiparous cows (VWP60 = 46%; VWP88 = 55%), as P/AI did not differ within the multiparous cow group (VWP60 = 36%; VWP88 = 40%). Physiological status more conducive to pregnancy—characterized by improved uterine health, greater BCS, reduced systemic inflammation, and to a lesser extent more time to resume ovarian cyclicity—explained the increment in P/AI to first service. Our data also indicated that despite having greater P/AI to first service, cows with the longer VWP had delayed time to pregnancy during lactation (hazard ratio = 0.72; 95% confidence interval 0.69–0.98) and greater risk of leaving the herd, particularly for multiparous cows (hazard ratio = 1.34; 95% confidence interval 1.23–1.47). This shift in pregnancy timing led to an overall extension of the lactation length (+13 d), which resulted in greater total milk yield per lactation (+491 kg) but not greater milk yield per day of lactation. In conclusion, data from this experiment highlight the importance of considering the complex interactions between reproductive performance, herd exit dynamics, and lactation performance as well as the effects of parity at the time of defining the duration of the VWP for lactating dairy cows.

Keywords: voluntary waiting period, reproductive performance, herd exit dynamics, dairy cow

INTRODUCTION

Timing of pregnancy during lactation affects the profitability of dairy herds by defining calving interval, milk production efficiency, and herd replacement dynamics (De Vries, 2006; Inchaisri et al., 2011; Giordano et al., 2012). Although insemination and conception risk after the end of the voluntary waiting period (**VWP**) are the 2 major determinants of time to pregnancy during lactation, the duration of the VWP can also influence timing of pregnancy because it determines when cows become eligible for insemination. Traditionally, VWP duration in US dairy farms has been ~60 d (Miller et al., 2007). Recent changes in reproductive performance and management of dairy herds, however, may allow the duration of the VWP to be manipulated to optimize reproductive performance and profitability. For example, aggressive management programs that substantially reduce the variation of time to pregnancy may enable dairy farms to select the optimal timing to achieve pregnancy in a substantial proportion of cows.

Extending the duration of the VWP may improve reproductive performance of cows through multiple mechanisms. For example, it may provide more time to recover uterine health (Gilbert et al., 2005; Gautam et al., 2009; Sheldon et al., 2009) through improved immune status, more time to resolve the inflammatory process established immediately after calving, or both (LeBlanc et al., 2011; LeBlanc, 2014). Likewise, a longer VWP may provide cows more time to return to reproductive hormone secretion patterns and a metabolic status that promotes resumption of ovarian cyclicity (Butler, 2003; Kawashima et al., 2012; Cheong et al., 2016). Earlier ovulation postpartum and more estrous cycles before first service were linked to reduced days to first service and greater pregnancies per AI (**P/AI**; Thatcher and Wilcox, 1973; Butler and Smith, 1989; Darwash et al., 1997). Finally, delaying first service beyond the negative energy balance nadir in early lactation as much as possible may improve reproductive performance by avoiding insemination of cows during severe negative energy balance and provide additional time to recover body tissue reserves. Optimum BCS at the time of insemination is strongly associated with high probability of pregnancy (Souza et al., 2007, 2008; Carvalho et al., 2014).

Despite the potential benefits of extended VWP on reproductive performance, the effect of this management strategy on overall herd performance has not been fully elucidated. Few randomized controlled experiments have evaluated the implications of VWP duration on first-service P/AI and whole-lactation reproductive performance of dairy cows. In an experiment with a limited number of second-lactation cows, Van Amburgh et al. (1997) found no differences in reproductive performance when comparing VWP of 60 versus 150 d. Similarly, Arbel et al. (2001) observed no effect of extending VWP by 60 d on reproductive performance of cows with above-average milk yield under Israeli conditions. In contrast, in an experiment conducted with above- or below-average milk-producing cows, an increment of 13 and 20 percentage points in P/AI was observed when VWP was extended from 77 to 98 or from 56 to 77 DIM for high- and low-producing cows, respectively (Tenhagen et al., 2003). More recently, Gobikrushanth et al. (2014) reported the results of a retrospective cohort study at a commercial farm in Florida that extended VWP duration during summer months only. Cows with the extended VWP had greater first-service P/AI, more days open, and longer calving intervals. Nonetheless, season of AI might have confounded results as cows with short VWP (57 to 63 d) received first service during summer and fall, whereas cows with long VWP (64 to 121) received first service during fall only. Moreover, the reproductive program used resulted in overlapped DIM at first service for a substantial proportion of cows. Collectively, the ambiguous results and multiple exclusion criteria of previous reports did not allow decisive conclusion that extending the duration of the VWP is beneficial for the performance of dairy herds.

Thus, our primary objective was to evaluate the reproductive performance, herd exit dynamics, and lactation performance of dairy cows managed with a VWP of 60 or 88 DIM. Secondary objectives were evaluating the effect of longer VWP on uterine health, cyclicity status, BCS, and systemic inflammation before first service. We hypothesized that extending VWP duration from 60 to 88 DIM would increase P/AI to first service and improve overall reproductive performance. We further hypothesized that cows with extended VWP would have improved reproductive performance because of improved uterine health, reduced rate of anovulation, improved BCS, and reduced systemic inflammation before first service.

MATERIALS AND METHODS

All procedures performed with cows were approved by the Animal Care and Use Committee of Cornell University.

Farms and Cow Management

Lactating Holstein cows from 3 commercial farms (A, B, and C) in New York State (2 in Cayuga County and 1 in Lewis County) were enrolled in this experiment from March 2014 to March 2015. During the study period the average numbers of milking cows were 1,034, 1,248, and 793 at farms A, B, and C, respectively. Average milk yield per cow per day was 39, 40, and 40 kg for farms A, B, and C, respectively. Data for cow numbers and milk yield were retrieved from the dairy management software (DairyComp305, Valley Ag Software, Tulare, CA) using the ECON\ID command. All farms housed cows in free-stall barns with 4 or 6 rows of stalls, concrete flooring, self-locking headgates in the feedline, and fans and sprinklers in the feedline. On farms A and B, freestall surfaces were covered with either mattresses covered with sawdust or deep sand bedding, whereas on farm C freestall surfaces were covered with deep sand bedding. Farms B and C milked cows thrice daily at approximately 8-h intervals, and farm A milked cows twice daily at approximately 12-h intervals. Cows were supplemented with recombinant bovine somatotropin (**rbST**; Sometribove zinc, Posilac, Elanco Animal Health, Indianapolis, IN) at all farms. On farm A, cows were supplemented with rbST every 10 or 11 d until dry-off beginning at 120 DIM; on farm B, cows were supplemented with rbST from 110 DIM until dry-off following a 10 and 11 d schedule; and on farm C, cows were supplemented every 10 or 11 d from 65 DIM until dry-off.

Experimental Treatments

The experimental design was a complete randomized block design with parity (primiparous vs. multiparous) as the blocking factor. Milk yield in the previous lactation for multiparous cows was also used as a stratification factor at enrollment. At each farm a list of all cows eligible for enrollment in the experiment (i.e., all ambulatory cows at 7 ± 3 DIM) was generated weekly and transferred to Excel (Microsoft Corporation, Redmond, WA). Cows were blocked by parity group (primiparous vs. multiparous) and within the multiparous group cows were stratified by total milk yield recorded for the previous lactation. Thereafter, cows were randomly assigned to a VWP of 60 [**VWP60**; n = 1,352 (farm A = 575; farm B = 458; farm C = 319)] or 88 [**VWP88**; n = 1,359 (farm A = 578; farm B = 462; farm C = 319)] DIM (Figure 1). All cows received the Double-Ovsynch (**DO**) protocol (GnRH-7 d-PGF2α-3 d-GnRH-7 d-GnRH-7 d-PGF2α-56 h-GnRH-16 to 20 h-TAI; Souza et al., 2008) for synchronization of ovulation. Cows received timed AI (**TAI**) at 60 ± 3 or 88 ± 3 DIM in the VWP60 and VWP88 treatments, respectively, as cows were enrolled in the Double-Ovsynch protocol on a weekly basis (i.e., Fridays on all farms: 33 ± 3 and 61 ± 3 DIM for cows in the VWP60 and VWP88 treatments, respectively). For second and greater AI services, cows were submitted for insemination after detection of estrus through visual observation (farms A and C) or a combination of visual observation and physical activity monitoring (farm B) using neck-mounted activity tags (DeLaval Activity Meter System, DeLaval International AB, Tumba, Sweden). In the 3 farms, cows not re-inseminated at detected estrus received TAI after resynchronization of ovulation with the Ovsynch protocol (GnRH-7 d-PGF2α-56 h-GnRH-16 to 20 h-TAI) initiated 32 ± 3 d after AI (**D32-Resynch**). On farm C, cows not having a corpus luteum ≥15 mm in diameter at the time of the nonpregnancy diagnosis and PGF2α injection of D32-Resynch received a CIDR-Synch protocol (GnRH+CIDR-7 d-CIDR removal+PGF2α-56 h-GnRH-16 to 20 h-TAI) as described in Giordano et al. (2016).

On the 3 farms, GnRH treatments consisted of 100 µg of gonadorelin diacetate tetrahydrate given i.m. and PGF2α treatments consisted of 500 µg of cloprostenol sodium given i.m. On farms A and B, the GnRH product used was Fertagyl (Merck Animal Health, Madison, NJ), whereas on farm C, GonaBREED (Parnell Technologies Pty Ltd., Alexandria, Australia) was used. On farms A and B, the PGF2α product used was Estrumate (Merck Animal Health, Summit, NJ), whereas on farm C, EstroPLAN (Parnell Technologies Pty Ltd.) was used.

Out of 2,711 cows enrolled in the experiment at 7 ± 3 DIM, 186 cows (VWP60, n = 87; VWP88, n = 99) were excluded because they left the herd or were classified as “do not breed” by farm personnel before 30 DIM. Cows were also excluded if TAI occurred outside the DIM range specified for their respective experimental group due to a farm management decision. After removal of these cows, the final number of cows per treatment was 1,265 for VWP60 and 1,260 for VWP88.

Blood Sample Collection and Laboratory Assays

Blood samples (~8 to 9 mL) were collected by puncture of the caudal vein or artery using evacuated tubes containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ) from a subgroup of cows on farms A and B (n = 684). After collection, samples were immediately placed in crushed ice until transported to the laboratory for further processing. At the laboratory, blood samples were centrifuged at 2,000 × g for 20 min at 4°C in a refrigerated centrifuge. After centrifugation, 3 aliquots of each sample were placed in Eppendorf storage vials and stored at –20°C until assayed.

A schematic representation of the blood-sample collection schedule is presented in Figure 1. The first 2 samples from cows in both treatments were collected at 26 ± 3 and 33 ± 3 DIM. For cows in the VWP60 treatment, 33 ± 3 DIM was the time of the first GnRH treatment of the Pre-Ovsynch portion of the DO protocol (hereafter referred to as the beginning of the DO protocol). For cows in this treatment, another blood sample was collected at 50 ± 3 DIM, coincident with the first GnRH treatment of the Breeding-Ovsynch portion of the DO protocol or 10 d before TAI (hereafter referred to as 10 d before TAI). For cows in the VWP88 treatment, the third and fourth samples were collected at 54 ± 3 and 61 ± 3 DIM to document the status of cows immediately before the beginning of the DO protocol. Another sample was collected 10 d before first-service TAI at 78 ± 3 DIM, coincident with the first GnRH injection of the Breeding-Ovsynch portion of the DO protocol. Samples collected to represent baseline and the beginning of the DO protocol for both VWP treatments were collected 7 d apart to calculate the proportion of cyclic cows [i.e., circulating concentrations of progesterone (P4) ≥1 ng/mL] at each of these time points. This approach was used to reduce misclassification of cows as not cyclic due to stage of the estrous cycle at time of sampling.

Blood samples were assayed for P4 at all time points for all cows sampled and for haptoglobin in a subgroup of cows at 33 ± 3 and 50 ± 3 DIM for the VWP60 treatment (n = 120), and at 33 ± 3, 61 ± 3, and 78 ± 3 DIM for the VWP88 treatment (n = 120).

Plasma P4 concentrations were analyzed in duplicate using a commercial solid-phase, no-extraction RIA (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA). To assess precision of the assay, control samples with high (4.8 ng/mL) and low (0.4 ng/mL) P4 concentrations were included at the beginning and end of each assay (n = 20 assays). Average detection limit of the assay was 0.05 ng/mL. For the high-concentration sample, intraassay coefficient of variation was 6.0% and interassay coefficient of variation was 10.0%. For the low-concentration sample, intraassay coefficient of variation was 14.2% and interassay coefficient of variation was 20.7%.

Plasma haptoglobin was analyzed in duplicate following an enzymatic analysis that quantified haptoglobin-hemoglobin complex by estimated differences in peroxidase activity as described in Bicalho et al. (2014). Concentrations were calculated against a standard curve from 0 to 2.5 mg/mL (Molecular Innovations, Novi, MI). Measurements were conducted using a SpectraMax 190 microplate reader. Intra- and interassay coefficients of variation were 9.3 and 21.3%, respectively.

Evaluation of Reproductive Tract Health

Vaginal discharge and uterine cytology were evaluated in a subgroup of cows (n = 263) at the baseline, beginning of DO, and 10 d before TAI for both treatments (Figure 1). Vaginal discharge was examined using a Metricheck device (Simcro, Hamilton, New Zealand), and scored on a 0 to 5 scale (0 = no discharge, 1 = clear mucus, 2 = clear mucus with flecks of pus, 3 = mucopurulent but <50% pus, 4 = mucopurulent with >50% pus, and 5 = foul-smelling discharge) as described in McDougall et al. (2007). Purulent vaginal discharge (**PVD**) was defined as a Metricheck score ≥2. This cut-off was selected based on the minimum value associated with a reduction in P/AI for first service in the current experiment regardless of experimental treatment (data presented in Results section).

Uterine cytology samples were collected through the cytobrush technique to determine the percentage of PMN in the uterine lumen. Samples were collected using a stainless steel gun attached to a sterile brush (Medscand Cytobrush Plus GT, CooperSurgical Inc., Trumbull, CT) following the technique described by Madoz et al. (2013). The smears were air-dried and stained with Dip Quick kit (Jorgensen Laboratories Inc., Loveland, CO). Each slide was evaluated at 400× magnification by a single observer. Percentage PMN in each slide was calculated from 2 counts of 100 cells each in 2 different locations of the slide. If the difference between the 2 counts was greater than 10 percentage points, a third count was conducted and the average of the 3 counts was used. Cutoff values to classify cows with or without cytological endometritis (CYTO) were determined using receiver operating characteristic curves generated with MedCalc (version 17.2; MedCalc Software BVBA, Ostend, Belgium). The cutoff selected represented the minimum percentage of PMN that indicated with greatest combined sensitivity and specificity a positive pregnancy outcome at first service (data presented in Results section).

Body Condition Score

Body condition score was recorded every time blood was collected. A scale of 1 (emaciated) to 5 (fat) with increments of 0.25 was used (Edmonson et al., 1989). Data for BCS were dichotomized using a threshold BCS of 2.75 units (high ≥2.75, low <2.75).

Pregnancy Diagnosis

Pregnancy testing was conducted at 39 ± 3 d after AI at the 3 farms. Transrectal palpation of the uterine contents was used on farm A and transrectal ultrasonography on farms B and C. On farm A, rectal palpation was conducted by a trained farm technician whereas transrectal ultrasonography was conducted by veterinarians using an Ibex Pro (Ibex, Loveland, CO) machine on farm B and an Easi-Scan (BCF Technology Ltd., Livingston, UK) machine on farm C. Reconfirmation of pregnancy status in pregnant cows was conducted by transrectal palpation (farm A) or transrectal ultrasonography (farms B and C) at 67 ± 3, 95 ± 3, and 109 ± 3 d after AI on farms A, B, and C, respectively. Therefore, pregnancy loss for each farm was estimated from the day of the initial pregnancy diagnosis (39 ± 3 d after AI) until reconfirmation of pregnancy.

Estimation of Daily Milk Yield

Daily milk yield for the entire lactation for each cow was estimated with the MilkBot model (Ehrlich, 2011) using monthly test records retrieved from the dairy management software. Briefly, the model predicts daily milk yield as a function of DIM and 4 MilkBot parameters: scale, ramp, offset, and decay. Tertiles of milk yield, based on the accumulated milk yield up to 30 DIM (**MK30**), were calculated to classify cows in high, medium, and low milk yield groups.

To account for the effect of farm and parity on milk yield, cutoff values for milk yield tertiles were obtained for individual farms and parity groups. Cutoff points for MK30 are presented in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13046). To test if MK30 was representative of milk yield of cows at the approximate DIM for first service, the correlations between MK30 versus milk yield up to 60 (**MK60**) and 90 (**MK90**) DIM were estimated. Correlations were MK30 versus MK60, R2 = 0.997 (P < 0.01) and MK30 versus MK90, R2 = 0.973 (P < 0.01). Therefore, we used MK30 instead of MK60 or MK90 to group cows in milk yield groups because the first test date data were available for every cow.

Statistical Analysis

A sample size calculation was performed using the "sample size calculation" option of WinPepi version 11.54 (Abramson, 2011). Based on an expected minimum difference in the proportion of cows pregnant at first TAI of 6 percentage points, assuming 47% P/AI for the VWP88 (approximation based on Souza et al., 2008; Herlihy et al., 2012; Giordano et al., 2013) and a 10% correction factor for cows not expected to have a first service outcome, a total of 1,175 cows per treatment were needed for a 2-tailed test with probability of type I error rate of 5% and probability of type II error rate of 20%.

Dichotomous outcomes [P/AI, pregnancy loss, proportion of cows AI after estrus detection, proportion of cows that were sold, died, or left the herd (sold plus dead), and proportion of cows with P4 ≥ 1 ng/mL, PVD, CYTO, and BCS ≥2.75] were analyzed by logistic regression using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Continuous outcomes were analyzed by ANOVA with (monthly milk test) or without (inter-service interval, haptoglobin concentrations, milk yield, lactation length, DIM, and days of gestation at dry-off) repeated measurements using the MIXED procedure of SAS. Time to event outcomes (time to pregnancy and time to herd exit) were analyzed by Cox's proportional hazards regression using the PHREG procedure of SAS. Kaplan-Meier survival curves generated using the Survival Analysis option of MedCalc were used for illustration of time to pregnancy and herd exit. For time-to-pregnancy analysis, a cow was considered pregnant only if pregnancy was maintained up to 150 d of gestation. Analysis of count data (total number of AI, number of PMN) was performed by Poisson regression using the GENMOD procedure of SAS.

The effect of treatment (VWP60 or VWP88), parity (primiparous vs. multiparous), and treatment by parity interaction were offered as explanatory variables to all models, whereas farm was included as a random effect. In addition, for outcomes related to first service, the effect of season of insemination (cold vs. warm), MK30 (low, medium, and high), treatment by MK30, and treatment by season interactions were offered to the models. Season of insemination was defined as warm (from June 21 to September 20) or cold (September 21 to June 20) based on the date of first TAI.

For physiological parameters (cyclicity, reproductive tract health, systemic inflammation, and BCS) and time to event data, the predictors were the same as previously described, except that season of first service was removed from the models.

The effect of VWP duration on milk yield measured in monthly milk tests was evaluated with a model that included treatment, monthly milk test number, and the treatment by test number interaction.

Assumptions of normality of residuals, linear relationship, and homoscedasticity for linear regression models were tested by evaluating the normal probability plot (normal Q-Q plot) and plotting residuals versus predicted values. All parameters evaluated by linear regression models met the aforementioned assumptions. For logistic regression models, goodness of fit was evaluated using the Hosmer-Lemeshow test. In all cases, model fit was deemed acceptable based on $P > 0.10$ for the Hosmer-Lemeshow test. For Poisson regression, goodness of fit of all models was evaluated with the Pearson chi-square test. Because overdispersion was present in the 2 Poisson regression models used, standard errors were scaled using the Pearson dispersion factor (Pearson $\chi 2$/degrees of freedom). The proportional hazard assumption for time to event data analysis was evaluated by graphical examination of the log[−log(survival probability)] function obtained from the PROC LIFETEST of SAS. According to this analysis, the assumption of proportional hazards was met for all models. The final model for each binary outcome of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Aikaike information criterion. Further, the potential effect of confounders with $P > 0.10$ (i.e., milk yield and season of insemination) was evaluated by calculation of the change in the estimate value for the outcome of interest with models containing or not containing the confounder. If inclusion of a confounder changed the estimate for the outcome of interest by more than 30%, it was retained in the model (Dohoo et al., 2014). Treatment and parity were forced in all models. When appropriate, the least significant difference post hoc mean separation test was used to determine differences between least squares means (**LSM)**.

All explanatory variables and their interactions were considered significant if $P \leq 0.05$, whereas $0.05 < P \leq 0.10$ was considered a tendency. Means for binomial outcomes are reported as LSM and the 95% confidence interval obtained with the LSMEANS option of the PROC GLIMMIX of SAS, whereas for quantitative variables, LSM ± standard error of the mean obtained with the LSMEANS option of PROC MIXED of SAS are reported. To simplify interpretation, count data evaluated through Poisson regression were reported as arithmetic means generated with PROC MEANS of SAS.

In all tables, results for the effect of VWP treatments are presented for both parity groups combined and for individual parity groups. Superscript letter differences within parity groups are used to report statistically significant differences between VWP treatment groups. This approach to reporting is used because of the known biological and performance differences between parity groups and the observed effects of parity in the data analyses.

RESULTS

Pregnancies per AI and Pregnancy Loss

At 39 d after first-service TAI, P/AI was greater ($P < 0.01$) for cows in the VWP88 treatment than for those in the VWP60 treatment (Table 1) and greater ($P < 0.01$) for primiparous than multiparous cows (50.4 vs. 38.0%, respectively). Cows with low (45.7%) and medium (46.7%) MK30 had greater ($P = 0.02$) P/AI than cows with high (39.9%) milk yield. The effects of season of AI, treatment by parity, treatment by MK30, and treatment by season interaction were not significant ($P > 0.10$).

Although a treatment by parity interaction for P/AI was not detected, the effect of treatment on P/AI was evaluated for primiparous and multiparous cows separately. For primiparous cows, P/AI at 39 d after TAI was greater ($P < 0.01$) for cows in the VWP88 than the VWP60 treatment, whereas for multiparous cows P/AI was similar ($P = 0.16$) for cows in both treatment treatments (VWP60 = 36.2%, VWP88 = 39.9; Table 1).

Pregnancy loss for cows pregnant to first-service TAI did not differ ($P = 0.36$) for cows in the VWP60 and VWP88 treatments (Table 1) but was greater ($P = 0.04$) for multiparous

(7.2%) than primiparous (4.1%) cows. The interaction between treatment and parity (*P* = 0.68) and the effect of MK30 (*P* = 0.57) were not significant.

The proportion of cows inseminated after a detected estrus for second and greater AI services (Table 2) was similar (*P* = 0.11) for the VWP60 and VWP88 treatments, but was greater (*P* < 0.01) for multiparous (49.9%) than for primiparous cows (42.2%). As expected, the inter-service interval was shorter for cows inseminated after estrus detection (24.3 d) than for TAI (42.8 d), but no differences were observed for the overall inter-service interval between VWP60 and VWP88 (*P* = 0.62; Table 2) and between primiparous and multiparous cows (*P* = 0.11).

Pregnancies per AI for all second and greater AI services combined was similar (*P* = 0.14) for the VWP60 and VWP88 treatments and for the warm (37.9%) and cold (34.7%) season (*P* = 0.13), but were greater (*P* < 0.01) for primiparous (40.3%) than for multiparous (30.9%) cows. Inseminations after a detected estrus (37.9%) resulted in greater P/AI (*P* < 0.01) than TAI services (33.0%). Within the primiparous group, P/AI for second and greater AI was greater (*P* = 0.04) for the VWP60 than the VWP88 treatment (Table 2), and for cows inseminated after a detected estrus (47.0%) than for those receiving TAI (34.1%; *P* < 0.01). No effect of treatment (*P* = 0.68) or type of insemination (*P* = 0.87) was observed for P/AI at second and greater AI for the multiparous group.

Total number of AI services up to 350 DIM was greater (*P* = 0.04) for the VWP60 treatment than for the VWP88 (Table 2) treatment and for multiparous than primiparous cows (2.7 vs. 2.2; *P* < 0.01).

Physiological Parameters Before First Service

The proportion of cows with P4 >1 ng/mL (resumed ovarian cyclicity) at baseline was similar ($P = 0.26$) for cows in the VWP60 and VWP88 treatments but was greater ($P < 0.01$) for primiparous than multiparous cows (Table 3). No interaction between treatment and parity was detected ($P = 0.30$). In addition, a lower ($P = 0.02$) proportion of cyclic cows was observed in the high (61.1%) than in the medium (73.1%) and low (68.0%) MK30 groups. At the beginning of DO, more ($P < 0.01$) cyclic cows were observed in the VWP88 than the VWP60 treatment and in the primiparous than the multiparous group ($P < 0.01$). Conversely, 10 d before TAI the proportion of cyclic cows was similar ($P = 0.14$) for both treatments and was not affected by parity ($P = 0.33$), treatment by parity interaction ($P = 0.93$), or MK30 level ($P = 0.11$).

Cows were considered to have PVD if at any time point the Metricheck score was ≥2. This cutoff was selected because cows with a Metricheck score ≥2 10 d before TAI had reduced ($P < 0.01$) P/AI after first service as compared with cows with a Metricheck score of 0 and 1 (26.3 vs. 52.6%, respectively; Supplemental Table S2; https://doi.org/10.3168/jds.2017-13046). As expected, at baseline the proportion of cows with PVD was similar ($P = 0.51$) for both treatments; it was also similar for primiparous and multiparous cows ($P = 0.99$). No interaction was observed between treatment and parity ($P = 0.48$), and no effect of MK30 group was observed ($P = 0.75$). Conversely, the proportion of cows with PVD was greater for the VWP60 treatment than for the VWP88 treatment at the beginning of DO ($P = 0.03$) and 10 d before TAI ($P = 0.01$; Table 3).

The percentage of PMN in uterine cytology samples followed a pattern similar to that of PVD. At baseline, no effect was observed of treatment ($P = 0.61$), parity ($P = 0.26$), treatment by parity interaction ($P = 0.70$), or MK30 ($P = 0.20$; Table 3). Conversely, cows in the VWP60 treatment had a greater percentage of PMN than did cows in the VWP88 treatment at the beginning of DO ($P < 0.01$) and 10 d before TAI ($P = 0.02$). No effects ($P > 0.10$) of parity, treatment by parity interaction, and MK30 were detected at these 2 time points.

At baseline, the proportion of cows with BCS ≥2.75 was similar ($P = 0.19$) for cows in both treatments, but was affected by parity group ($P < 0.01$) because more primiparous than multiparous cows had BCS ≥2.75 (Table 3). No treatment by parity interaction ($P = 0.12$) was detected at this time point. At the beginning of the DO protocol, the proportion of cows with BCS ≥2.75 was also similar for both VWP treatments ($P = 0.16$) and was greater for primiparous than multiparous cows ($P < 0.01$), but no treatment by parity interaction was observed ($P = 0.11$). At 10 d before TAI, the proportion of cows with BCS ≥2.75 was greater ($P < 0.01$) for the VWP88 treatment and for primiparous than multiparous cows ($P < 0.01$). Milk yield up to 30 DIM did not affect ($P > 0.10$) the proportion of cows with BCS ≥2.75 except 10 d before TAI when a greater proportion ($P = 0.02$) of cows had BCS ≥2.75 in the low (96.4%) than the medium (92.8%) and high (91.1%) MK30 groups.

Haptoglobin concentrations in plasma were not affected ($P > 0.10$) by VWP duration, parity group, or the interaction between treatment and parity at baseline or at the beginning of DO (Table 3). At 10 d before TAI, cows in the VWP60 treatment had greater ($P = 0.05$) haptoglobin concentration than cows in the VWP88 treatment, but no effects of parity ($P = 0.14$) or treatment by parity interaction ($P = 0.61$) were observed at this point. A tendency ($P = 0.06$) was observed for MK30 to affect haptoglobin concentration at baseline. No effect of MK30 on haptoglobin concentrations was observed at the beginning of DO ($P = 0.79$) and 10 d before TAI

(P = 0.45). The effect of cyclicity status, uterine health, and BCS on pregnancies per AI after first service is presented in Supplemental Table S2 (https://doi.org/10.3168/jds.2017-13046).

Time to Pregnancy and Nonpregnant Cows at 350 DIM

The hazard of pregnancy from calving until 350 DIM was greater (P < 0.01) for the VWP60 than the VWP88 treatment [hazard ratio (**HR**) 1.34, 95% CI 1.23 to 1.47; Figure 2A] and greater (P < 0.01) for primiparous than multiparous cows (HR 1.48, 95% CI 1.36 to 1.62). Median and mean days to pregnancy were 102 and 132 d for the VWP60 treatment and 128 and 154 d for the VWP88 treatment. The hazard of pregnancy was also affected by milk yield up to 30 DIM (P < 0.01), as it was lower for cows with high (HR 0.84, 95% CI 0.76 to 0.94) than medium milk yield. The HR for time to pregnancy did not differ for cows with low and medium milk yield (HR 0.94, 95% CI 0.84 to 1.05) or for cows with high and low milk yield (HR 0.90, 95% CI 0.81 to 1.01). At 350 DIM, the proportion of nonpregnant cows did not differ (P = 0.28) for the VWP60 (7.3%) and VWP88 (8.6%) treatments but was greater (P < 0.01) for multiparous (12.1%) than primiparous cows (2.5%).

When evaluated within parity group, the hazard of pregnancy was greater for cows in the VWP60 than in the VWP88 treatment both in the primiparous (P < 0.01, HR 1.38, 95% CI 1.21 to 1.58; Figure 2B) and multiparous group (P < 0.01; HR 1.31, 95% CI 1.19 to 1.50; Figure 2C). For primiparous cows, median and mean days to pregnancy were 85 and 115 d for the VWP60 treatment and 91 and 135 d for the VWP88 treatment. In addition, the proportion of nonpregnant cows at 350 DIM did not differ (P = 0.51) between VWP60 (2.3%) and VWP88 (2.9%) within the primiparous group. For multiparous cows, median and mean days to pregnancy, respectively, were 104 and 142 d for the VWP60 treatment and 132 and 163 d for the VWP88 treatment. The proportion of nonpregnant cows at 350 DIM was similar (*P* = 0.20) between treatments (VWP60 = 11.0%, and VWP88 = 13.0%).

On the other hand, the hazard of pregnancy from the end of the VWP until 350 d after the first TAI did not differ (*P* = 0.30) for cows in the VWP60 and VWP88 treatment (HR 0.96, 95% CI 0.88 to 1.14; Figure 3A) but was greater (*P* < 0.01) for primiparous than multiparous cows (HR 1.40, 95% CI 1.28 to 1.53). Median and mean days to pregnancy were 42 and 72 d for the VWP60 treatment and 41 and 66 d for the VWP88 treatment. The hazard of pregnancy was also similar for cows in the VWP60 and VWP88 treatment within both the primiparous (*P* = 0.23; HR 0.92, 95% CI 0.81 to 1.05; Figure 3B) and multiparous group (*P* = 0.73; HR 0.98, 95% CI 0.87 to 1.10; Figure 3C). For primiparous cows, median and mean days to pregnancy were 25 and 55 d for the VWP60 treatment and 0 and 47 d for the VWP88 treatment. For multiparous cows, median and mean days to pregnancy were 42 and 82 d for the VWP60 treatment and 42 and 74 d for the VWP88 treatment.

Herd Exit Dynamics

The hazard of culling up to 350 DIM was affected by the interaction between treatment and parity group (*P* = 0.05) and milk yield up to 30 DIM (*P* < 0.01). The hazard of culling was similar (*P* = 0.56) for primiparous cows in both treatment treatments (HR 1.12, 95% CI 0.77 to 1.61; Figure 4A) but was greater (*P* = 0.03) for multiparous cows in the VWP88 than the VWP60 treatment (HR 1.21, 95% CI 1.02 to 1.44; Figure 4B). Mean days to culling were 324 and 332 for primiparous cows in the VWP60 and VWP88 treatment, respectively. For multiparous cows, mean days to culling were 302 and 295 for the VWP60 and VWP88 treatments, respectively. The hazard of culling was greater (*P* < 0.01) for cows in the low milk yield group than for cows in the medium (HR 1.72, 95% CI 1.43 to 2.07) and high (HR 2.04, 95% CI 1.69 to 2.47) milk yield groups, but was similar for cows in the medium and high milk yield groups (HR 1.18, 95% CI 0.96 to 1.46; Figure 4C).

The total proportion of cows sold during the experimental lactation (Table 4) was similar ($P = 0.78$) for primiparous cows in both treatments but was greater ($P = 0.04$) for multiparous cows in the VWP88 than the VWP60 treatment. No differences were observed between treatments in the proportion of cows that died during the experimental lactation in the primiparous ($P = 0.45$) or multiparous ($P = 0.87$) group (Table 4). Consequently, the proportion of total cows that left the herd (sold plus died) was similar ($P = 0.58$) between VWP60 and VWP88 treatments for primiparous cows, but it was greater ($P = 0.03$) for multiparous cows in the VWP88 than the VWP60 treatment.

Lactation Performance

The effect of VWP treatment on milk yield during the first 10 monthly milk tests was evaluated for all cows enrolled in the experiment (Figure 5A and 5C) and for cows pregnant at first service only (Figure 5B and 5D). For all primiparous cows enrolled in the experiment (Figure 5A), an interaction was observed between VWP treatment and test number ($P = 0.04$), whereby cows in the VWP88 treatment produced more milk than cows in the VWP60 treatment on tests 8 to 10. No effect of VWP duration on milk yield at dry-off ($P = 0.24$) and on days of gestation at dry-off ($P = 0.17$) was observed for primiparous cows (Table 5). Nevertheless, DIM at dry-off were different ($P < 0.01$) between treatments. For primiparous cows pregnant at first service only (Figure 5B), there was also a treatment by test number interaction ($P < 0.01$) because cows in the VWP88 treatment produced more milk than cows in the VWP60 treatment from test 6 to 9. Milk yield at dry-off was higher ($P = 0.03$) for the VWP88 than for the VWP60 treatment, and DIM at dry-off was also higher ($P < 0.01$) for the VWP88 than for the VWP60 treatment (Table 5). Days of gestation at dry-off for primiparous cows pregnant at first TAI was similar ($P = 0.48$) between treatments.

For all multiparous cows enrolled in the experiment (Figure 5C), a treatment by test number interaction ($P = 0.03$) was observed whereby cows in the VWP88 treatment produced more milk than did cows in the VWP60 treatment on test 9. The DIM at dry-off were greater ($P < 0.01$) for the VWP88 treatment than the VWP60 treatment, but milk yield ($P = 0.17$) and days of gestation ($P = 0.12$) were similar between treatments at this time point (Table 5). For multiparous cows pregnant at first service only (Figure 5D), a treatment by test interaction ($P < 0.01$) was also observed whereby cows in the VWP88 treatment produced more milk than cows in the VWP60 treatment on tests 7 to 9. No differences were observed in milk yield ($P = 0.26$) and days of gestation ($P = 0.11$) at dry-off between VWP treatments, but DIM at dry-off was greater ($P < 0.01$) for the VWP88 than the VWP60 treatment (Table 5).

For all cows enrolled in the experiment (Table 5), total milk yield during the experimental lactation was greater ($P = 0.01$) for the VWP88 than the VWP60 treatment. Primiparous cows in the VWP88 treatment produced more milk ($P < 0.01$) than those in the VWP60 treatment (~817 kg difference), but no significant differences ($P = 0.50$) were observed within the multiparous group (~164 kg difference). Accordingly, length of the lactation was also greater ($P < 0.01$) for primiparous cows in the VWP88 than the VWP60 treatment, but no differences ($P = 0.47$) were observed within the multiparous group. Nonetheless, lactation length was longer ($P < 0.01$) for multiparous cows in the VWP88 treatment when only cows that did not left the herd were included in the analysis (data not shown). No differences (*P* = 0.94) were observed for daily milk yield (kg/d) between cows in the VWP60 and VWP88 treatments.

For cows pregnant at the first service only (Table 5), milk yield during the experimental lactation was greater (*P* < 0.01) for cows in the VWP88 treatment than in the VWP60 treatment (∼1,117 kg difference). This difference in favor of the VWP88 treatment was present in both primiparous (*P* < 0.01; ∼1,321 kg difference) and multiparous (*P* < 0.01; ∼913 kg difference) cows. Lactation length was also different (*P* < 0.01) between VWP60 and VWP88 by ∼27 d, and reflected the 28-d difference in DIM at first service. Daily milk yield (kg/d) was greater (*P* < 0.01) for primiparous cows in the VWP88 than for those in the VWP60 treatment (∼1.3 kg/d difference), but no difference (*P* = 0.38) was observed for multiparous cows.

DISCUSSION

Reproductive Performance and Markers of Physiological Status Before First Service

In support of our main hypothesis, extending the duration of the VWP from 60 to 88 DIM after synchronization of ovulation with the Double-Ovsynch protocol increased P/AI after first service in lactating dairy cows. Nevertheless, most of the observed difference could be attributed to the greater P/AI of primiparous cows in the VWP88 treatment. Our overall results are in agreement with previous studies, which documented improved P/AI after extending the duration of the VWP (Tenhagen et al., 2003; Gobikrushanth et al., 2014). Nevertheless, direct comparisons between studies are difficult because of differences in experimental design and interactions between treatments and other confounders. For example, a 21 d longer VWP increased P/AI 20 and 13 percentage points for cows with low and high milk yield submitted to TAI after the Ovsynch protocol, respectively (Tenhagen et al., 2003). In a retrospective cohort study, a 23 d extension (range = 1 to 64 d) of the VWP for cows that calved during summer (AI in fall) increased overall P/AI by 6 percentage points when compared with cows with shorter VWP (AI in summer and fall; Gobikrushanth et al., 2014). In contrast, Arbel et al. (2001) reported similar P/AI for cows with above-average milk yield inseminated at detected estrus after a 60 d extension of the VWP (from 90 and 60 DIM in primiparous and multiparous cows, respectively), and Van Amburgh et al. (1997) reported no benefit of extending the duration of the VWP from 60 to 150 d on P/AI for cows in their second lactation inseminated at detected estrus. Thus, in spite of substantial variation across studies, the collective results of the current experiment and others (Tenhagen et al., 2003; Gobikrushanth et al., 2014) conducted under conditions more similar to ours (i.e., using TAI and less difference in VWP duration) suggest that extending VWP duration increases P/AI to first service. The magnitude of the increment in P/AI, however, may be affected by parity, method of insemination, season, milk yield level, and the magnitude and timing of the extension of the VWP.

The observed difference in first-service P/AI between parity groups was expected because it has been extensively documented (Souza et al., 2008; Herlihy et al., 2012; Giordano et al., 2013). In part, it can be explained by the greater incidence of anovulation and greater proportion of cows with low BCS at different time points in the multiparous group (no difference for other parameters). Nonetheless, the reason for the different response to treatments by parity is unclear at the moment because both groups presented a fairly similar physiological response to the extension of the VWP. Differences between parities in metabolic status, health, or both not captured by the parameters monitored in this experiment may explain such a discrepancy. For example, whereas primiparous cows received first service when milk yield per day was still increasing in both VWP treatments, milk yield was increasing at a greater rate in the VWP60 than the VWP88 treatment. Conversely, milk yield per day was already declining for multiparous cows from both VWP treatments. It is also possible that the 28 d extension of the VWP was insufficient for multiparous cows to attain a physiological status substantially better than that of cows in the VWP60 group. To some extent this was supported by data for PVD because the difference in proportion of cows with PVD 10 d before TAI was of only ~7 percentage points for multiparous, whereas the difference was of ~22 percentage points for primiparous. Likewise, the difference in haptoglobin levels 10 d before TAI was ~0.10 mg/mL for multiparous cows, whereas it was ~0.18 mg/mL for primiparous cows. This reasoning, however, did not apply to data for cyclicity status, uterine cytology, and BCS.

As timing of pregnancy in lactating dairy cow herds is determined by the combined effect of all AI services rather than first service only, evaluating the pattern of pregnancy creation during the entire lactation is essential to truly determine the effect of VWP duration on reproductive performance. In this regard, cows in the VWP60 treatment became pregnant at a faster rate after calving than cows in the VWP88 treatment. The reduced P/AI to first service for cows in VWP60 was fully compensated by the creation of more pregnancies at earlier DIM due to more and earlier opportunities for re-insemination. The faster rate of pregnancy creation did not result, however, in a reduced proportion of nonpregnant cows at 350 DIM. In agreement, 2 other studies reported the same pattern of pregnancy creation and similar proportion of nonpregnant cows in late lactation (Tenhagen et al., 2003; Gobikrushanth et al., 2014). Collectively, these data suggest that the greatest consequence of longer VWP is shifting timing of pregnancy toward later lactation rather than generating a greater proportion of pregnant cows.

Previous data are inconclusive about the effect of milk yield on fertility and suggest that the interaction between milk yield and fertility is complex (Faust et al., 1988; López-Gatius et al., 2006; Bello et al., 2013). In our experiment, not all parameters of reproductive performance were affected equally by milk yield level. Whereas first-service P/AI was reduced for cows in the high milk yield group, the hazard of pregnancy for high-producing cows was lower than for cows in the medium but not the low milk yield group. At least in part, the discrepancy for the effect of milk yield on P/AI and time to pregnancy can be explained by the outcomes measured and the influence of others factors on these outcomes.

Our results for the multiple markers of physiological status support the hypothesis that a longer VWP would lead to an improved uterine environment, reduced anovulation, improved BCS, and reduced systemic inflammation before first service. Our data are also in agreement with many previous reports that documented reduced P/AI at first service in cows with PVD, CYTO, BCS <2.75, and elevated circulating haptoglobin levels (Barlund et al., 2008; Souza et al., 2008; Dubuc et al., 2011; Huzzey et al., 2015). The effect on uterine health of extending VWP duration was evident, as fewer cows were considered to have PVD and CYTO at the beginning of DO and 10 d before TAI. As PVD and CYTO alone or combined reduce P/AI of dairy cows (Gilbert et al., 2005; Barlund et al., 2008; Dubuc et al., 2010), the reduction in proportion of cows affected likely contributed to the greater P/AI for the VWP88 treatment. The longer VWP also resulted in more cows with a BCS ≥2.75, which has been associated with greater first-service P/AI (Souza et al., 2007, 2008; Carvalho et al., 2014). Assuming that most cows lost body reserves after calving, our data suggest that the longer interval from calving to first service for cows in the VWP88 treatment allowed recovery of more body reserves. Collectively, these observations for physiological markers and overall metabolic status help explain, at least in part, the greater P/AI for cows with longer VWP and suggest that providing cows more time to recover before first service is a feasible strategy to promote a physiological status more conducive to pregnancy.

Finally, the greater proportion of cyclic cows at the beginning of the DO protocol reflected the effect of additional time for resumption of cyclicity, whereas the similar proportion of cyclic cows 10 d before TAI reflected the efficacy of the DO protocol to resolve anovulation. This was expected because previous studies have demonstrated that GnRH-based presynchronization protocols are effective for reducing the proportion of anovular cows before TAI (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013). Thus, in our experiment any potential benefit of extending the VWP on reducing the incidence of anovulation must have been neutralized by the induction of ovulation in early lactation for cows in the VWP60 treatment.

The effect of extending the VWP from 60 to 88 DIM on first-service P/AI and subsequent reproductive performance in the current experiment may be specific to the use of all TAI after synchronization of ovulation with a GnRH-based fertility protocol. Compared with reproductive management programs designed to submit all cows for AI at detected estrus or through a combination of insemination at detected estrus with TAI, all TAI with a GnRH-based synchronization protocol may have conditioned the effect of extending the VWP. For example, using all TAI for first service results in a narrow range of DIM to first service regardless of the ability of cows to display estrous behavior, reducing variation of not only DIM to first service but also for second and greater AI services. By resolving anovulation, proper synchronization of ovulation, and optimization of the endocrine environment before insemination in most cows (Souza et al., 2008; Herlihy et al., 2012; Giordano et al., 2013); GnRH-based protocols may also offset the detriment of shorter VWP on P/AI to a greater extent than programs not including synchronization of ovulation or synchronization of ovulation with PGF2α-based protocols. Thus, the method of submission to first service and the type of synchronization of ovulation protocol, if any is used, are important considerations at the time of defining the duration of the VWP.

Herd Exit Dynamics and Lactation Performance

Cow parity and pregnancy status are major determinants of the herd exit dynamics in dairy farms. Pregnant cows and cows in lower parity groups have lesser risk of removal from the herd (De Vries et al., 2010; Pinedo et al., 2010). The smaller proportion of primiparous than multiparous cows leaving the herd and similar herd exit dynamics across VWP treatments for primiparous cows reflected the protective effect for culling of early pregnancy and younger age. Conversely, for multiparous cows the greater proportion of cows exiting the herd from the VWP88 treatment as lactation progressed reflected the compounded effect of delayed pregnancy and greater culling pressure in older cows. Evidently milk yield level also played a role and added another layer of complexity because nonpregnant cows with medium and high milk yield had lower culling pressure than cows with low milk yield.

A direct consequence of the different herd exit dynamics across parities was a smaller difference between treatments in lactation length and milk yield per lactation for multiparous cows when all cows (i.e., culled and not culled) in the experiment were included in the calculations. Whereas the difference in lactation length and milk yield per lactation for multiparous cows was 4 d and 164 kg, respectively, the differences for primiparous cows were 22 d and 817 kg, respectively. Cows that left the herd earlier in lactation contributed shorter lactations and less milk yield per lactation than cows that left the herd at later DIM or stayed in the herd. Indeed, when only cows that were not culled were evaluated, the differences between treatments for multiparous cows (21 d and 790 kg) better reflected the effect of delayed pregnancy on lactation length and milk yield (i.e., longer lactation and more milk per lactation). For primiparous cows the differences remained unchanged (21 d and 807 kg) because fewer cows left the herd. Therefore, the collective results for herd exit, timing of pregnancy, and effect of milk yield level on herd exit dynamics suggest that multiple, complex interactions are present that should be accounted for when defining the duration of the VWP for a dairy herd.

The shift in overall timing of pregnancy toward later DIM in the VWP88 treatment resulted in extension of average lactation length. This was clearly due to delayed pregnancy, as cows were dried off at the same days in gestation. Longer lactations coupled with high milk yield persistency at later DIM resulted in greater accumulated milk yield for cows in the VWP88 treatment, primarily due to greater milk yield in primiparous cows. This is relevant because milk yield per lactation is one the major factors affecting cow profitability (Van Amburgh et al., 1997; Arbel et al., 2001; Österman and Bertilsson, 2003). Nonetheless, it is important to note that not only total milk yield but also efficiency of milk yield during lactation influences cow profitability (Pecsok et al., 1994; Ferguson and Galligan, 1999; Britt et al., 2003). Thus, lactation performance data from this experiment should be interpreted with caution because the data are insufficient to determine the true economic effect of different durations of the VWP.

The effect of extending the VWP and shifting timing of pregnancy toward later DIM affected the pattern of milk yield in mid to late lactation. This effect was more evident in primiparous cows and cows pregnant to first service. As for the other lactation parameters, the observed pattern of milk yield for each treatment was also affected by the herd exit dynamics. Cows leaving the herd at earlier DIM, which were more likely to be low-producing cows, reduced the difference between VWP treatments when all cows were included in the calculation. In contrast, for cows pregnant at first service the greater milk yield in late lactation for cows in the VWP88 treatment may be explained by the effect of gestation on milk yield. It is well known that after the fifth month of pregnancy, milk yield declines more steadily (Olori et al., 1997; Van Amburgh et al., 1997; Roche, 2003). This was evident in our data, as cows pregnant at first service in the VWP60 treatment had lower milk yield than cows in the VWP88 starting at the sixth (primiparous) and seventh (multiparous) monthly test, which was the approximate time at which cows were starting or completing the fifth month of gestation. At the same monthly tests, cows in the VWP88 treatment were approximately 1 mo earlier in gestation.

CONCLUSION

In conclusion, extending the duration of the VWP from 60 to 88 DIM in lactating dairy cows that received TAI after synchronization of ovulation with the Double-Ovsynch protocol increased P/AI to first service. Nonetheless, the greatest gain in P/AI was observed in primiparous cows. Cows with the extended VWP had a physiological status more conducive to pregnancy characterized by improved uterine health, greater BCS, reduced systemic inflammation, and (to a lesser extent) reduced anovulation before insemination. In spite of increasing P/AI to first service, extending the VWP delayed time to pregnancy during lactation and increased the risk of leaving the herd, in particular during late lactation. This overall extension of lactation length resulted in greater total milk yield per lactation but not greater milk yield per day of lactation. Thus, data from the current experiment highlight the importance of considering the complex interactions between reproductive performance, herd exit dynamics, and lactation performance when defining VWP duration for lactating dairy cows.

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Table 1. Effect of extending duration of the voluntary waiting period (VWP) from 60 to 88 DIM on pregnancies per AI (P/AI) and pregnancy loss after first-service timed AI (TAI) in lactating dairy cows[1]

	Primiparous		Multiparous		All Parities		*P*-value[2]		
	VWP60[3]	VWP88[4]	VWP60	VWP88	VWP60	VWP88	Trt	Parity	Trt x Parity
	% (CI)		% (CI)		% (CI)				
P/AI[4] 39 d after TAI	46.0[a] (41.4-50.6)	55.3[b] (50.6-59.9)	36.2 (32.6-40.1)	39.9 (36.0-43.8)	41.1 (37.4-44.8)	47.1 (43.2-51.0)	<0.01	<0.01	0.19
P/AI at pregnancy reconfirmation	43.6[a] (38.8-48.5)	52.5[b] (47.5-57.4)	32.9 (29.0-37.0)	36.2 (32.1-40.4)	38.2 (34.3-42.2)	43.6 (39.6-47.8)	0.01	<0.01	0.22
Pregnancy loss	3.3 (1.4-7.5)	4.7 (2.3-9.4)	6.7 (4.2-10.5)	7.7 (5.1-11.6)	4.9 (3.2-7.4)	6.1 (4.2-8.7)	0.36	0.04	0.68

[a,b]Different superscripts within a row indicate significant differences ($P \leq 0.05$) within the same parity.
[1]All values are presented as LSM and the 95% CI.
[2]The effect of additional explanatory variables is described in the text. Trt = treatment.
[3]VWP60 = first-service TAI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[4]VWP88 = first-service TAI at 88 ± 3 DIM after the Double-Ovsynch protocol.

Table 2. Effect of extending the duration of the voluntary waiting period (VWP) from 60 to 88 DIM on percentage of cows inseminated at detected estrus, interval between AI services, pregnancies per AI (P/AI) after second and greater AI services, and total number of inseminations up to 350 DIM in lactating dairy cows[1]

	Primiparous		Multiparous		All Parities		*P*-value[2]		
	VWP60[3]	VWP88[4]	VWP60	VWP88	VWP60	VWP88	Trt	Parity	Trt x Parity
EDAI[5] [%, (CI)]	40.5 (17.2-69.0)	45.8 (20.5-73.5)	48.5 (18.0-80.1)	50.4 (19.2-81.3)	44.5 (17.0-75.8)	47.5 (18.8-78.0)	0.11	<0.01	0.43
Inter-service interval[6] (days)	33.7 ± 0.6	33.8 ± 0.6	33.3 ± 1.0	33.4 ± 1.0	33.5 ± 0.8	33.6 ± 0.8	0.62	0.11	0.67
P/AI [%, (CI)]	43.6[a] (38.2-49.2)	37.2[b] (31.9-42.8)	31.5 (26.9-36.4)	30.6 (26.0-35.7)	36.7 (31.9-41.8)	34.2 (29.4-39.3)	0.14	<0.01	0.23
Total number of services[7]	2.3 ± 0.05	2.2 ± 0.05	2.8 ± 0.07	2.6 ± 0.08	2.6 ± 0.07	2.4 ± 0.07	0.04	<0.01	0.72

[a,b]Different superscripts within a row indicate significant differences ($P \leq 0.05$) within the same parity.
[1]Values for binomial outcomes are presented as LSM and the 95% CI, values for inter-service interval are presented as LSM ± SEM, and values for total number of services are presented as arithmetic means ± SE.
[2]The effect of additional explanatory variables are described in the text. Trt = treatment.
[3]VWP60 = first-service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch protocol.
[4]VWP88 = first-service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch protocol.
[5]EDAI = cows inseminated after estrus detection.
[6]Inter-service interval = interval between 2 consecutive AI services.
[7]Total number of services = total number of AI services received during the lactation (up to 350 DIM).

Table 3. Effect of extending the duration of the voluntary waiting period (VWP) from 60 to 88 DIM on cyclicity status, uterine health, BCS, and haptoglobin circulating concentrations in lactating dairy cows[1]

	Primiparous		Multiparous		All Parities		*P*-value[2]		
	VWP60[3]	VWP88[4]	VWP60	VWP88	VWP60	VWP88	Trt	Parity	Trt x Parity
P4[5] >1 ng/mL (%, n/n)									
Baseline	72.9 (59.4-83.2)	79.8 (67.3-88.4)	56.9 (43.2-69.7)	57.4 (43.4-70.3)	65.4 (52.5-76.3)	69.8 (57.1-80.0)	0.26	<0.01	0.30
Beginning DO[6]	72.9 (58.8-83.5)	91.6 (82.9-96.1)	57.1 (42.7-70.4)	79.1 (67.1-87.5)	65.4 (52.5-76.3)	86.5 (77.4-92.3)	<0.01	<0.01	0.41
Ten d before TAI[7]	89.2 (75.9-97.1)	92.6 (81.9-98.3)	87.2 (72.4-96.2)	91.1 (78.7-97.5)	90.2 (75.2-96.5)	93.3 (81.6-97.7)	0.14	0.33	0.93
PVD[8] (%, n/n)									
Baseline	52.2 (37.9-66.1)	43.4 (30.8-57.0)	47.7 (37.5-58.2)	48.0 (36.9-59.3)	50.0 (41.1-58.8)	45.7 (37.1-54.6)	0.51	0.99	0.48
Beginning DO	52.8 (36.7-68.4)	32.9 (20.2-48.6)	48.3 (35.7-61.2)	39.8 (27.5-53.6)	50.6 (38.9-62.2)	36.3 (26.0-48.0)	0.03	0.82	0.36
Ten d before TAI	40.0 (26.8-54.8)	17.7 (9.4-30.6)	34.9 (25.4-45.8)	27.9 (18.6-39.8)	37.4 (29.1-46.6)	22.4 (15.5-31.1)	0.01	0.53	0.17
PMN[9] (%)									
Baseline	11.0 ± 2.9	11.7 ± 2.4	13.3 ± 2.3	15.4 ± 2.6	12.5 ± 1.8	13.9 ± 1.8	0.61	0.26	0.70
Beginning DO	11.0 ± 2.9	6.0 ± 2.1	13.3 ± 2.3	6.0 ± 1.7	12.5 ± 1.8	6.0 ± 1.3	<0.01	0.72	0.91
Ten d before TAI	5.7 ± 1.4	3.3 ± 1.3	7.4 ± 1.7	3.5 ± 1.1	6.8 ± 1.2	3.4 ± 0.8	0.02	0.55	0.76
BCS[6] ≥ 2.75 (%, n/n)									
Baseline	93.1 (87.1-96.5)	97.6 (92.6-99.3)	78.7 (70.9-84.8)	75.4 (66.9-82.4)	87.6 (81.9-91.7)	91.8 (85.4-95.6)	0.19	<0.01	0.12
Beginning DO	93.4 (86.1-97.0)	97.7 (92.4-99.3)	79.0 (67.5-87.2)	78.0 (65.9-86.6)	87.9 (79.7-93.1)	92.5 (84.9-96.4)	0.16	<0.01	0.11
Ten d before TAI	95.0 (-[10])	100 (-[10])	79.0 (72.4-84.3)	87.9 (82.0-92.0)	90.8 (85.3-94.4)	95.9 (92.5-97.8)	<0.01	<0.01	-
HAPTO[11] (mg/mL)									
Baseline	0.51 ± 0.09	0.48 ± 0.08	0.47 ± 0.06	0.51 ± 0.05	0.48 ± 0.05	0.50 ± 0.04	0.50	0.95	0.42
Beginning DO	0.51 ± 0.09	0.50 ± 0.07	0.47 ± 0.06	0.59 ± 0.07	0.48 ± 0.05	0.55 ± 0.05	0.15	0.93	0.34
Ten d before TAI	0.64 ± 0.09	0.46 ± 0.06	0.52 ± 0.06	0.42 ± 0.05	0.56 ± 0.05	0.43 ± 0.04	0.05	0.14	0.61

[1]Values for binomial outcomes are presented as LSM and the 95% CI, whereas values for quantitative outcomes are presented as LSM ± SEM.
[2]Trt = treatment.
[3]VWP60 = first-service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[4]VWP88 = first-service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[5]P4 = cows with progesterone concentration >1 ng/mL.
[6]DO = Double-Ovsynch.
[7]TAI = timed artificial insemination.
[8]PVD = cows with purulent vaginal discharge (Metricheck score ≥2; Simcro, Hamilton, New Zealand). Scored on a 0 to 5 scale (0 = no discharge, 1 = clear mucus, 2 = clear mucus with flecks of pus, 3 = mucopurulent but <50% pus, 4 = mucopurulent with >50% pus, and 5 = foul-smelling discharge) as described in McDougall et al. (2007).
[9]PMN = percentage polymorphonuclear cells in uterine cytology sample.
[10]No confidence interval because for one of the groups all observations had the same outcome.
[11]HAPTO = haptoglobin concentration.

Table 4. Effect of extending the duration of the voluntary waiting period (VWP) from 60 to 88 DIM on herd exit up to 350 d after calving in lactating dairy cows[1]

	Primiparous		Multiparous		All Parities		P-value[2]		
	VWP60[3]	VWP88[4]	VWP60	VWP88	VWP60	VWP88	Trt	Parity	Trt x Parity
	% (n/n)		% (n/n)		% (n/n)				
Sold	7.6 (2.3-22.8)	7.2 (2.1-21.8)	25.5[a] (20.3-31.7)	30.1[b] (24.3-36.7)	19.4 (14.3-25.7)	22.1 (16.5-29.0)	0.45	<0.01	0.24
Died	1.8 (0.9-3.7)	1.2 (0.5-2.9)	4.1 (2.6-6.4)	4.6 (2.9-7.1)	2.6 (1.6-4.1)	2.6 (1.6-4.2)	0.63	<0.01	0.39
Left herd	8.6 (2.3-27.5)	7.8 (2.1-25.3)	29.9[a] (25.3-34.9)	35.1[b] (30.2-40.4)	22.8 (17.8-28.7)	25.7 (20.3-32.0)	0.54	<0.01	0.13

[a,b]Different superscripts within a row indicate significant differences ($P \leq 0.05$) within the same parity.
[1]All values are presented as LSM and the 95% CI.
[2]Trt = treatment.
[3]VWP60 = first-service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch program.
[4]VWP88 = first-service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch program.

Table 5. Effect of extending the duration of the voluntary waiting period (VWP) from 60 to 88 DIM on lactation parameters and performance in lactating dairy cows[1]

	Primiparous		Multiparous		All Parities		P-value[2]		
	VWP60[3]	VWP88[4]	VWP60	VWP88	VWP60	VWP88	Trt	Parity	Trt x Parity
Milk dry-off, kg/d									
All cows[5]	32.4 ± 0.3	32.9 ± 0.3	32.5 ± 0.3	31.9 ± 0.3	32.4 ± 0.2	32.4 ± 0.2	0.82	0.22	0.14
Preg 1st AI[6]	31.8 ± 0.5[a]	33.1 ± 0.4[b]	33.9 ± 0.4	33.1 ± 0.4	32.9 ± 0.3	33.1 ± 0.3	0.59	0.02	0.02
DIM at dry-off, d									
All cows	325.4 ± 2.8[a]	344.7 ± 2.8[b]	326.4 ± 2.5[a]	346.9 ± 2.6[b]	325.9 ± 1.9	345.8 ± 1.9	<0.01	0.56	0.83
Preg 1st AI	283.9 ± 0.8[a]	312.3 ± 0.8[b]	284.4 ± 0.8[a]	309.7 ± 0.8[b]	284.1 ± 0.6	311.0 ± 0.5	<0.01	0.20	0.11
DG[7] at dry-off, d									
All cows	223.7 ± 0.9	222.6 ± 0.9	221.6 ± 0.8	220.5 ± 0.8	222.7 ± 0.6	221.1 ± 0.6	0.11	0.13	0.54
Preg 1st AI	223.8 ± 0.8	224.4 ± 0.8	224.4 ± 0.8	223.9 ± 0.8	224.1 ± 0.6	223.1 ± 0.5	0.20	0.22	0.12
Milk, kg/lact									
All cows	10,831 ± 689[a]	11,648 ± 689[b]	12,249 ± 677	12,413 ± 677	11,540 ± 671	12,031 ± 671	0.01	<0.01	0.07
Preg 1st AI	9,464 ± 293[a]	10,785 ± 291[b]	12,001 ± 290[a]	12,914 ± 292[b]	10,732 ± 279	11,849 ± 279	<0.01	<0.01	0.12
Lact. length, d									
All cows	319.4 ± 14.0[a]	341.3 ± 14.0[b]	300.7 ± 13.7	305.0 ± 13.7	310.0 ± 13.6	323.1 ± 13.5	<0.01	<0.01	0.03
Preg 1st AI	283.9 ± 0.8[a]	312.3 ± 0.8[b]	284.4 ± 0.8[a]	309.7 ± 0.8[b]	284.1 ± 0.6	311.0 ± 0.5	<0.01	0.20	0.11
Milk, kg/d									
All cows	33.2 ± 0.7	33.6 ± 0.7	40.5 ± 0.6	40.1 ± 0.6	36.9 ± 0.6	36.9 ± 0.6	0.94	<0.01	0.14
Preg 1st AI	33.2 ± 0.7[a]	34.5 ± 0.7[b]	42.1 ± 0.7	41.6 ± 0.7	37.6 ± 0.7	38.0 ± 0.7	0.31	<0.01	0.02

[a,b]Different superscripts within a row indicate significant differences ($P \leq 0.05$) within the same parity.
[1]All values are presented as LSM ± SEM.
[2]Trt = treatment.
[3]VWP60 = first-service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch protocol.
[4]VWP88 = first-service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the D32-Resynch protocol.
[5]All cows = all cows enrolled in the study (n = VWP60: 1,265; VWP88: 1,260).
[6]Preg first AI = only cows pregnant at first service that did not abort or exit the herd during the lactation (421 in VWP60 and 425 in VWP88).
[7]DG = days of gestation at dry-off.

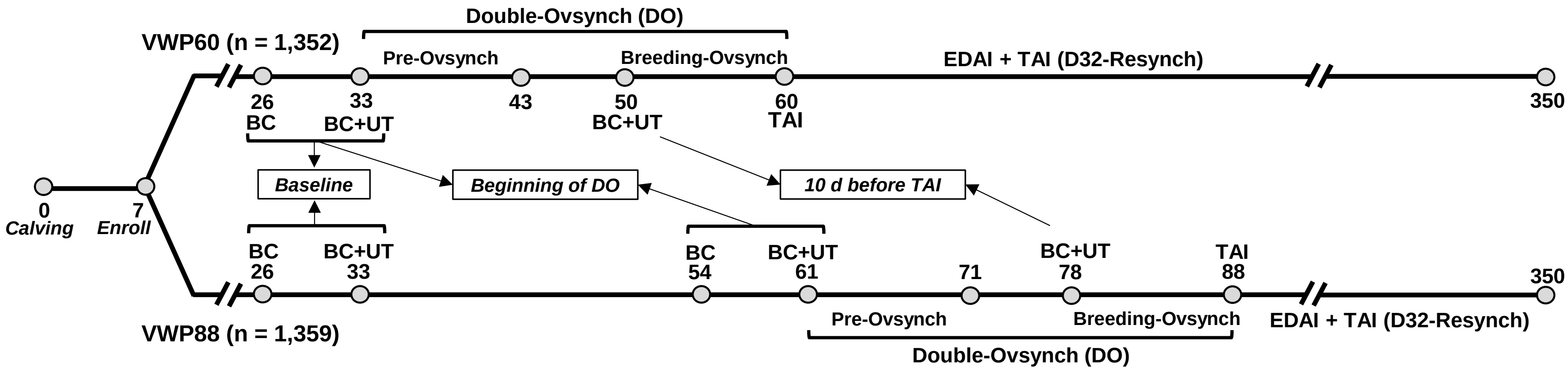

Figure 1. Graphical depiction of experimental procedures. Cows were randomly assigned to a voluntary waiting period (VWP) of 60 (VWP60; n = 1,352) or 88 (VWP88; n = 1,359) DIM. All cows received the Double-Ovsynch (DO) protocol for synchronization of ovulation and timed AI (TAI) at 60 ± 3 or 88 ± 3 DIM. Cyclicity (progesterone concentration in circulation), uterine health (UT; vaginal discharge and uterine cytology), BCS, and systemic inflammation (haptoglobin concentration in circulation) were determined at baseline (33 ± 3 DIM for both treatments), the beginning of the DO protocol (33 ± 3 DIM for VWP60 and 61 ± 3 DIM for VWP88), and 10 d before TAI (50 ± 3 DIM for VWP60 and 78 ± 3 DIM for VWP88). For determination of cyclicity status at baseline and beginning of DO, another sample was collected 7 d before the time point of interest to reduce misclassification of cows as not cyclic due to stage of the estrous cycle at time of sampling. Cows received second and greater AI at detected estrus or after resynchronization of ovulation with the D32-Resynch protocol. BC = blood collection; EDAI = estrus detected AI.

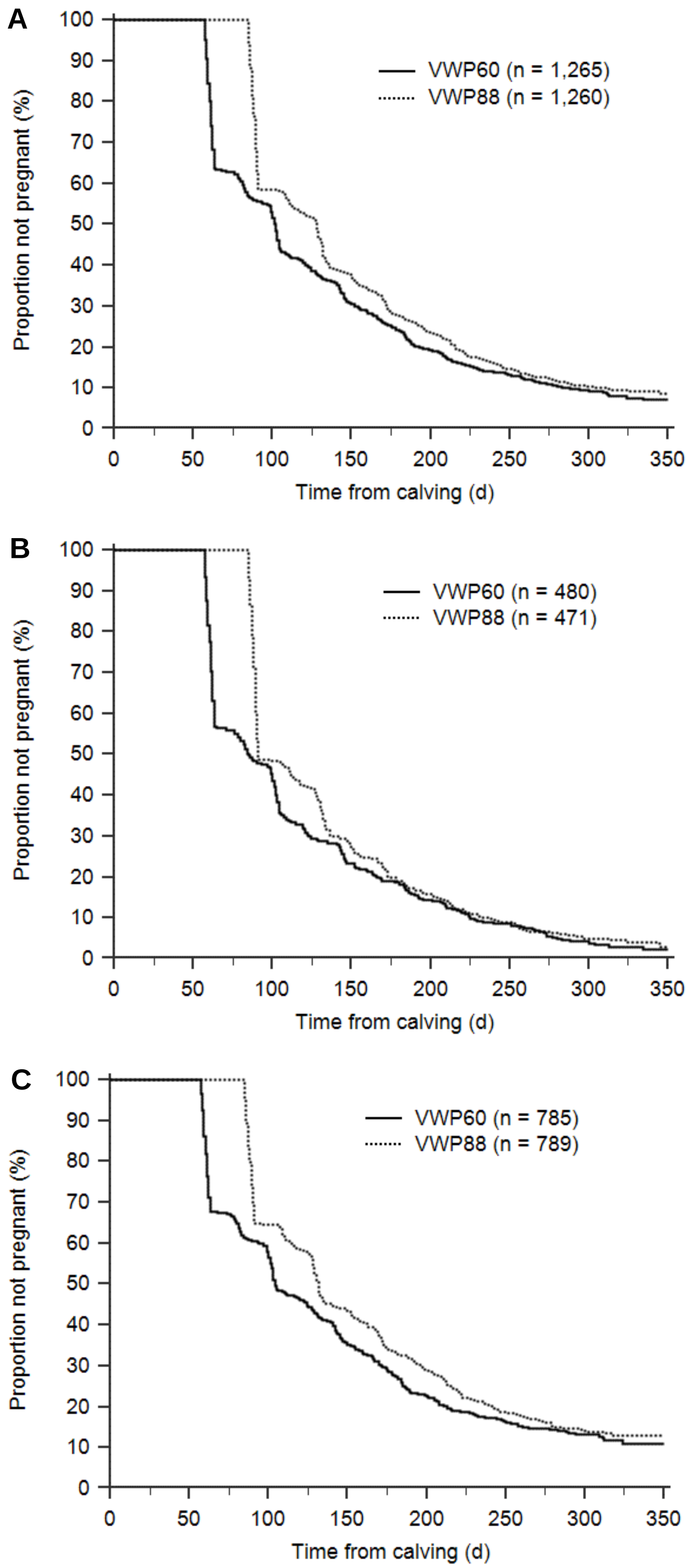
A
Proportion not pregnant (%)
Time from calving (d)
VWP60 (n = 1,265)
VWP88 (n = 1,260)
B
Proportion not pregnant (%)
Time from calving (d)
VWP60 (n = 480)
VWP88 (n = 471)
C
Proportion not pregnant (%)
Time from calving (d)
VWP60 (n = 785)
VWP88 (n = 789)

Figure 2. (A) Kaplan-Meier survival curves for time to pregnancy after calving. The hazard of pregnancy was greater (*P* < 0.001) for cows in the voluntary waiting period of 60 DIM (VWP60) than the voluntary waiting period of 88 DIM (VWP88) treatment [hazard ratio (HR) 1.34; 95% CI 1.23 to 1.47]. (B) Kaplan-Meier survival curves for time to pregnancy after calving for primiparous cows. The hazard of pregnancy was greater for cows in the VWP60 than the VWP88 treatment (*P* < 0.01, HR 1.38, 95% CI 1.21 to 1.58). (C) Kaplan-Meier survival curves for time to pregnancy after calving for multiparous cows. The hazard of pregnancy was greater for cows in the VWP60 treatment than in the VWP88 treatment (*P* < 0.01; HR 1.31, 95% CI 1.19 to 1.50).

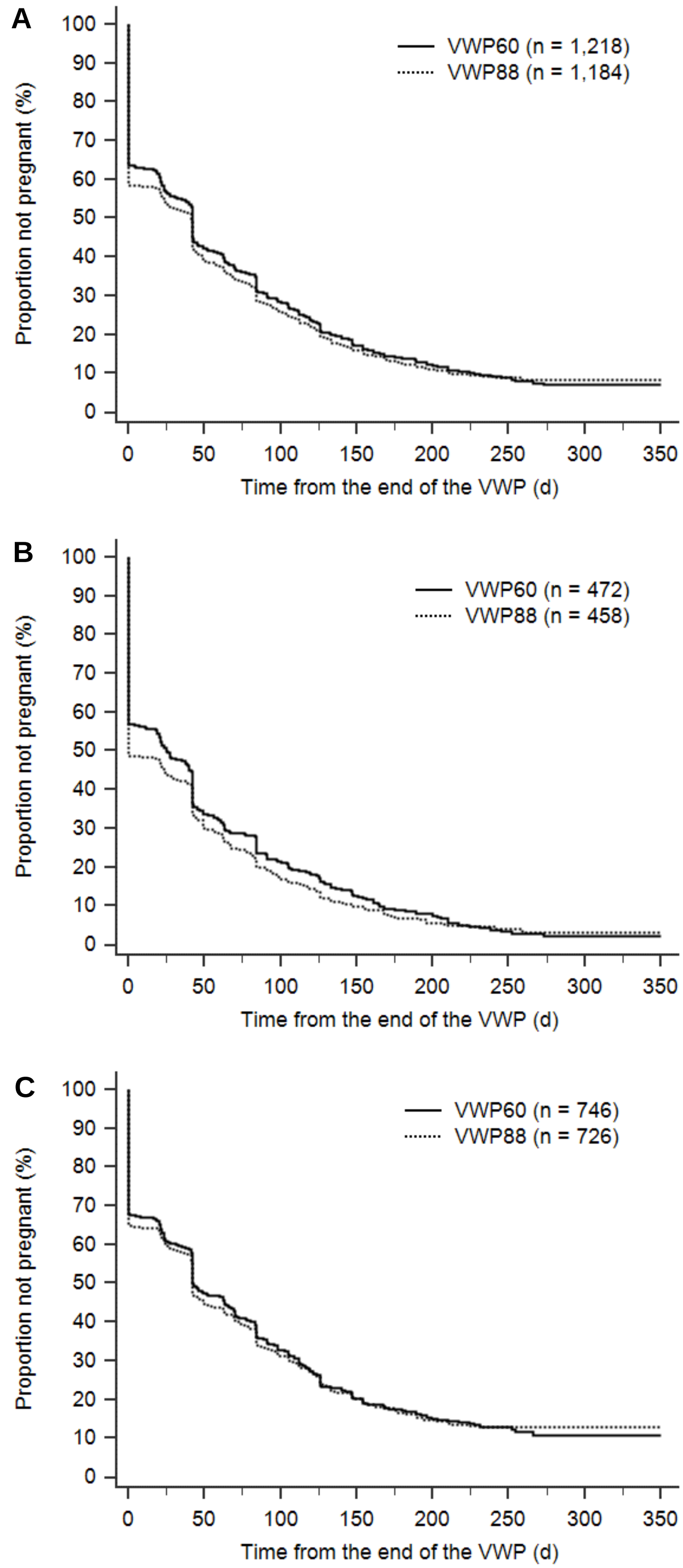
A
B
C
VWP60 (n = 1,218)
VWP88 (n = 1,184)
VWP60 (n = 472)
VWP88 (n = 458)
VWP60 (n = 746)
VWP88 (n = 726)
Proportion not pregnant (%)
Time from the end of the VWP (d)

Figure 3. (A) Kaplan-Meier survival curves for days to pregnancy after the end of the voluntary waiting period (VWP) for each experimental treatment (end of VWP = d 0). The hazard of pregnancy was similar (*P* = 0.30) for cows in the VWP of 60 DIM (VWP60) and VWP of 88 DIM (VWP88) treatments [hazard ratio (HR) 0.96, 95% CI 0.88 to 1.04]. (B) Kaplan-Meier survival curves for days to pregnancy after the end of the VWP for primiparous cows. The hazard of pregnancy was similar for cows in the VWP60 and VWP88 treatment (*P* = 0.23; HR 0.92, 95% CI 0.81 to 1.05). (C) Kaplan-Meier survival curves for days to pregnancy after the end of the VWP for multiparous cows. The hazard of pregnancy was similar for cows in the VWP60 and VWP88 treatments (*P* = 0.73; HR 0.98, 95% CI 0.87 to 1.10).

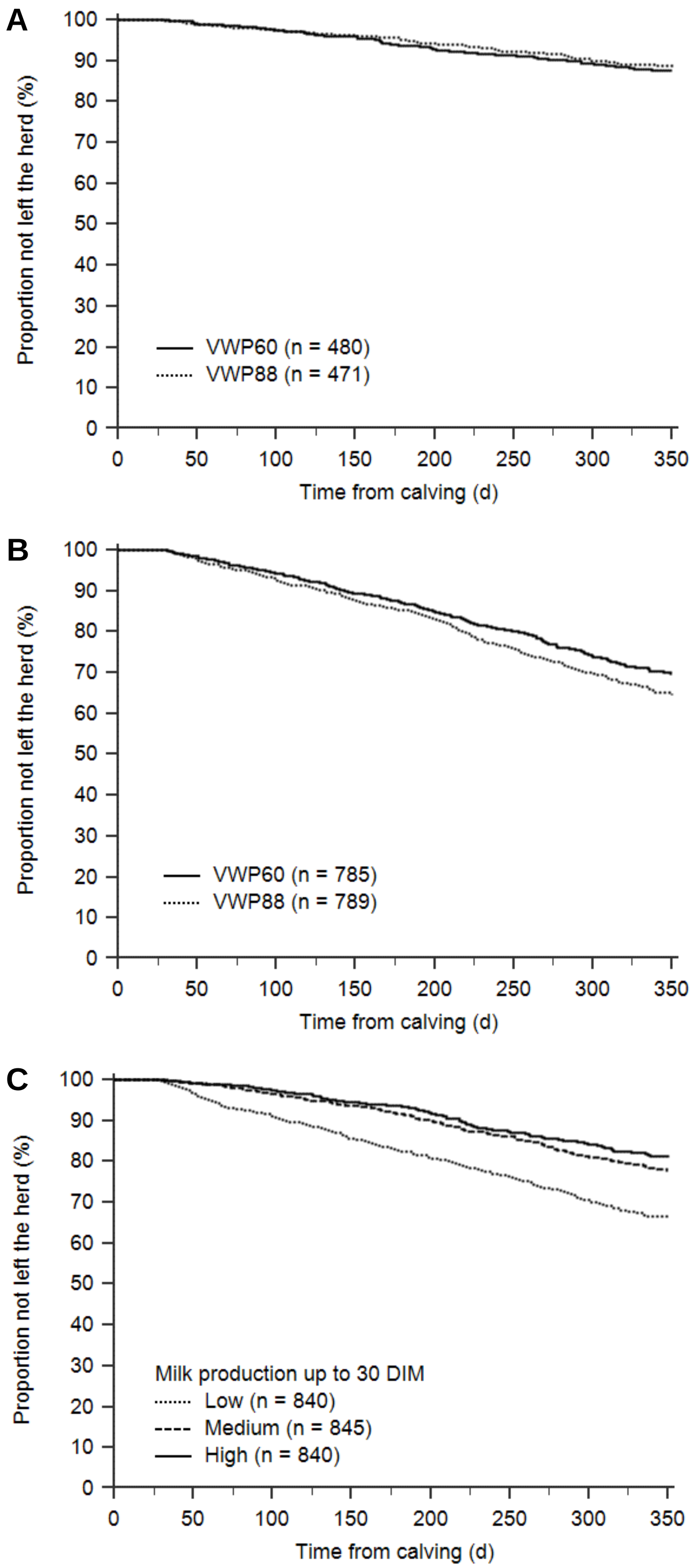
A
Proportion not left the herd (%)
VWP60 (n = 480)
VWP88 (n = 471)
Time from calving (d)
B
Proportion not left the herd (%)
VWP60 (n = 785)
VWP88 (n = 789)
Time from calving (d)
C
Proportion not left the herd (%)
Milk production up to 30 DIM
Low (n = 840)
Medium (n = 845)
High (n = 840)
Time from calving (d)

Figure 4. (A) Kaplan-Meier survival curves for time to herd exit up to 350 d after calving for primiparous cows. The hazard of culling was similar for the voluntary waiting period of 60 DIM (VWP60) and voluntary waiting period of 88 DIM (VWP88) treatments [$P = 0.56$; hazard ratio (HR) 1.12, 95% CI 0.77 to 1.61]. (B) Kaplan-Meier survival curves for time to herd exit up to 350 d after calving for multiparous cows. The hazard of culling was greater for the VWP88 treatment than for the VWP60 treatment ($P = 0.03$; HR 1.21, 95% CI 1.02 to 1.44). (C) Kaplan-Meier survival curves for time to herd exit up to 350 d after calving according to milk yield accumulated up to 30 DIM. The hazard of culling was greater for cows in the low milk yield group than for cows in the medium ($P < 0.01$; HR 1.49, 95% CI 1.29 to 1.72) and high (HR 2.04, 95% CI 1.68 to 2.47) milk yield groups, but was similar for cows in the medium and high milk yield groups (HR 1.18, 95% CI 0.96 to 1.46).

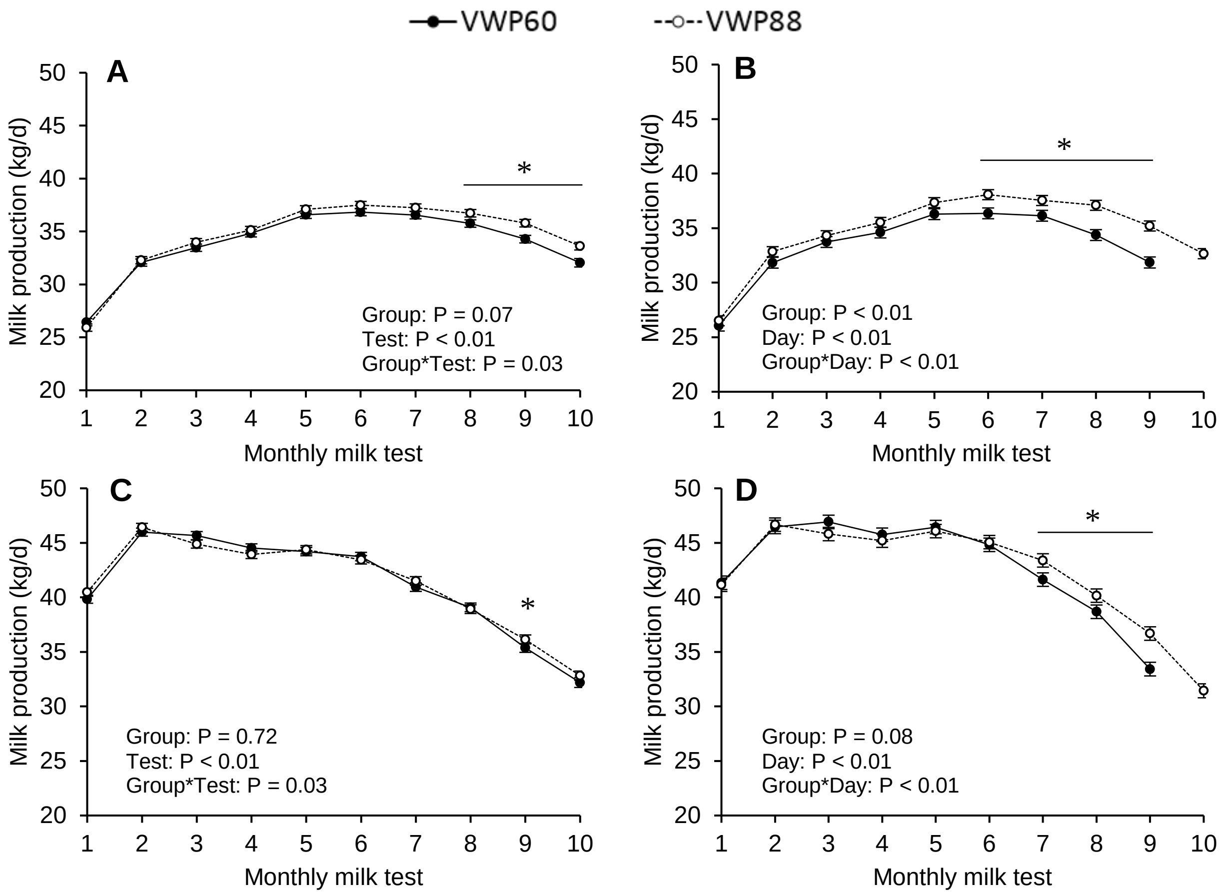

Figure 5. Monthly milk yield (kg/d) for all primiparous cows enrolled in the experiment [A; voluntary waiting period of 60 DIM (VWP60): n = 480; voluntary waiting period of 88 DIM (VWP88): n = 471]; primiparous cows pregnant at first service (B; VWP60: n = 200; VWP88: n = 228); all multiparous cows enrolled in the experiment (C; VWP60: n = 791, VWP88: n = 794); and multiparous cows pregnant at first service (D; VWP60: n = 238, VWP88: n = 247). *Indicates significant difference between the VWP60 and VWP88 treatment based on LSD mean separation test. All values are presented as LSM ± SEM.

Supplemental Table S1. Cutoff points for determination of milk yield tertiles based on accumulated milk yield (kg) up to 30 DIM (MK30)

	Primiparous			Multiparous		
	Low	Medium	High	Low	Medium	High
Farm A	< 733	733 – 836	> 836	< 1,126	1,126 – 1,281	> 1,281
Farm B	< 737	737 – 857	> 857	< 1,049	1,049 – 1,215	> 1,215
Farm C	< 709	709 – 825	> 825	< 1,137	1,137 – 1,309	> 1,309

Supplemental Table S2. Effect of cyclicity status, uterine health, and body condition score before first service on pregnancy per artificial insemination in lactating dairy cows[1]

	P/AI[2] at first service								
	Baseline[3]			Beginning of DO[4]			10 d before TAI[5]		
	Yes	No	*P*-value	Yes	No	*P*-value	Yes	No	*P*-value
P4[6] ≤ 1 ng/mL	44.2 (37.7-50.8)	45.4 (40.3-50.6)	0.75	44.3 (37.1-51.8)	45.2 (40.4-50.1)	0.83	41.7 (30.5-53.9)	45.4 (40.8-50.0)	0.56
PVD[7]	38.3 (26.8-51.3)	48.8 (36.4-61.3)	0.09	38.6 (27.6-50.9)	49.0 (37.7-60.4)	0.09	26.3 (15.7-40.7)	52.6 (40.2-64.7)	<0.01
CYTO[8]	38.0 (24.5-53.7)	45.7 (33.1-59.9)	0.25	37.4 (24.1-52.9)	47.7 (34.1-61.7)	0.11	22.7 (12.5-37.6)	52.5 (41.8-63.0)	<0.01
BCS[9] ≥ 2.75	46.3 (41.4-51.2)	38.4 (29.4-48.2)	0.13	45.9 (41.1-50.8)	39.8 (30.6-49.8)	0.24	47.1 (43.1-51.2)	29.0 (19.9-40.1)	<0.01

[1]All values are presented as LSM and the 95% CI.
[2]P/AI = pregnancies per artificial insemination.
[3]Baseline = 33 ± 3 DIM for voluntary waiting period of 60 days (VWP60) and 33 ± 3 DIM for voluntary waiting period of 88 days (VWP88).
[4]Beginning of Double-Ovsynch protocol = 33 ± 3 DIM for VWP60 and 61 ± 3 DIM for VWP88.
[5]10 d before timed artificial insemination = 50 ± 3 DIM for VWP60 and 78 ± 3 DIM for VWP88.
[6]P4 = cows with progesterone concentration > 1 ng/mL.
[7]PVD = cows with purulent vaginal discharge (metricheck score ≥ 2).
[8]CYTO = cows with cytological endometritis (presence of > 9.5% polymorphonuclear cells at baseline, and > 3.5% PMN at the beginning of the DO protocol and 10 d before TAI).
[9]BCS = cows with body condition score ≥ 2.75.

CHAPTER VII

PROFITABILITY OF DAIRY COWS RECEIVING FIRST SERVICE TIMED ARTIFICIAL INSEMINATION AFTER THE DOUBLE-OVSYNCH PROTOCOL WITH A VOLUNTARY WAITING PERIOD OF 60 OR 88 DAYS.

M. L. Stangaferro, R. Wijma, M. Masello, Mark J. Thomas, and J. O. Giordano.

ABSTRACT

The objective of this study was to evaluate the economic performance of dairy cows managed with a voluntary waiting period (**VWP**) of 60 or 88 d. A secondary objective was estimating variation in profitability under different input pricing scenarios through stochastic Monte Carlo simulations. Lactating Holstein cows from three commercial farms were blocked by parity group and total milk yield in their previous lactation and then randomly assigned to a VWP of 60 (VWP60; n = 1,352) or 88 (VWP88; n = 1,359) d. All cows received timed-artificial insemination (**TAI**) for first service after synchronization of ovulation with the Double-Ovsynch protocol. For second and greater artificial insemination (**AI**), cows received AI after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI. Two analyses were performed: (1) cash flow per cow for the calving interval of the experimental lactation and (2) cash flow per slot occupied by each cow enrolled in the experiment for an 18-mo period after calving in the experimental lactation. Extending the VWP from 60 to 88 d delayed time to pregnancy during lactation (~20 d) and increased the risk of leaving the herd for multiparous cows (hazard ratio = 1.21). As a result, a smaller proportion of multiparous cows calved again and had a subsequent lactation (-6%). The shift in time to pregnancy combined with the herd exit dynamics resulted in longer lactation length for primiparous (+22 d) but not multiparous cows. Longer lactations led to greater milk income over feed cost and cash flow for primiparous but not multiparous cows in the VWP88 group during the experimental lactation. On the other hand, profitability per slot for the 18-mo period was numerically greater (+$68 slot/18 mo) for primiparous cows but numerically reduced (-$85 slot/18 mo) for multiparous cows in the VWP88 treatment. Under variable input pricing conditions generated through stochastic simulations, the longer VWP always increased cash flow per 18 mo for primiparous and reduced cash flow for multiparous cows. In conclusion, extending the duration of the VWP from 60 to 88 d numerically increased profitability of primiparous cows and reduced profitability of multiparous cows. Such effect depended mostly on the herd replacement dynamics and milk production efficiency.

Keywords: voluntary waiting period, profitability, dairy cow, Double-Ovsynch.

INTRODUCTION

Timing of pregnancy during lactation affects the profitability of dairy herds by influencing milk production efficiency, replacement dynamics, reproductive programs costs, and calf revenues (De Vries, 2006a; Inchaisri et al., 2011; Giordano et al., 2012). Dairy farms can optimize timing of pregnancy of lactating cows using reproductive management strategies that result in high pregnancy risk after the end of the voluntary waiting period (**VWP**) and manipulating the duration of the VWP.

In spite of the relevance of the duration of the VWP on timing of pregnancy during lactation and dairy cow profitability, limited data are available about its effect on the profitability of dairy herds. In addition, data available from the few experiments conducted have been inconsistent. For instance, Arbel et al. (2001), using only high producing cows under Israeli conditions, reported an economic advantage when delaying the VWP from 90 to 150 DIM for primiparous, and from 60 to 120 DIM for multiparous cows. Conversely, Gobikrushanth et al. (2014) using a combination of retrospective data analysis and simulation reported that extending the duration of VWP from 60 (range 57-63 DIM) to 83 DIM (range 64 to 121 DIM) during summer in Florida did not affect cow profitability during the experimental lactation, the subsequent lactation, or during a period of six years. Furthermore, a simulation study conducted to represent dairy farms under milk production quota conditions in the Netherlands suggested that a VWP of 6 weeks was optimal, and economic losses increased gradually after extending the VWP beyond 6 weeks (Inchaisri et al., 2011). These inconsistent results, which could be partially explained by differences in study design (e.g., simulation, observational study, or randomized-control experiment), inclusion criteria (e.g., only high yielding cows that did not calve during summer, only cows that calved during summer), or other study-specific conditions (e.g., farm location, quota systems), do not allow decisive conclusions about the effect of the duration of the VWP on dairy herd profitability for cows of all parities, milk production level, and seasons of calving under current U.S. dairy production conditions.

Thus, the primary objective of this experiment was to compare the profitability of dairy cows managed with a VWP of 60 or 88 days. Productive and reproductive performance as well as herd exit dynamics data from an experiment conducted to compare a VWP of 60 or 88 d (Stangaferro et al., 2017) were used for this study. We evaluated cash flow during the calving interval in which the VWP was extended, and for a fixed period of time (18 mo) after calving in the experimental calving interval. A secondary objective was estimating variation in profitability under different input pricing scenarios through stochastic Monte Carlo simulations. We hypothesized that extending the duration of the VWP from 60 to 88 DIM would increase cash flow during the experimental calving interval, especially in primiparous cows because of their more persistent lactations.

MATERIALS AND METHODS

Farms and Animals

All procedures performed with cows were approved by the Animal Care and Use Committee of Cornell University.

Information about farms, animals, and experimental procedures are described in detail in Stangaferro et al. (2018). Briefly, lactating Holstein cows (n = 2,711) from three commercial dairy farms (A, B and C) located in New York State were enrolled in this experiment from March 2014 to March 2015. All cows remained in the experiment for up to 18 months after their calving date unless they left the herd due to sale or death. Average number of milking cows and daily milk production during the experiment was: 1,034 cows and 39 kg/d in farm A, 1,248 cows and 40 kg/d in farm B, and 793 cows and 40 kg/d in farm C. Cows were housed in free-stall barns with concrete flooring, self-locking headgates, fans and sprinklers in the feedline, and four or six rows of stalls covered with either mattresses with sawdust (farms A and B) or deep sand bedding (farms A, B and C).

Cows in farms B and C were milked thrice daily (approximately every 8 h). Cows in farm A were milked twice daily (approximately every 12 h) until February 2015 and thrice daily thereafter. All farms supplemented cows with recombinant bovine somatotropin (**rbST**; Sometribove zinc, Posilac, Elanco Animal Health, Indianapolis, IN) on a 10 and 11 d schedule until dry-off, beginning at 120 (farm A), 110 (farm B), or 65 (farm C) DIM.

Experimental Procedures and Data Collection

The experiment followed a randomized complete block design using parity (primiparous vs. multiparous) as blocking factor. At 7 ± 3 DIM, cows were blocked by parity and stratified by milk production in the previous lactation (multiparous only), and then were randomly assigned to receive first service through timed-artificial insemination (**TAI**) after a VWP of 60 (**VWP60**; n = 1,352) or 88 (**VWP88**; n = 1,359) d. The total number of cows enrolled in the experiment were 1,153 in farm A, 920 in farm B, and 638 in farm C.

Synchronization of ovulation for first service was performed with the Double-Ovsynch (**DO**) protocol (GnRH-7 d-PGF-3 d-GnRH-7 d-GnRH-7 d-PGF-56 h-GnRH-16 to 20 h-TAI; Souza et al., 2008). Cows were enrolled on a weekly basis (i.e., Fridays on all farms) at 33 ± 3 and 61 ± 3 DIM for the VWP60 and VWP88 treatments, respectively. Thus, cows received TAI at 60 ± 3 and 88 ± 3 DIM in the VWP60 and VWP88 treatments, respectively. For second and greater AI services, cows were inseminated after detection of estrus (**EDAI**) through visual observation (farms A and C) or a combination of visual observation and physical activity monitoring (farm B) using neck-mounted activity tags (DeLaval Activity Meter System, DeLaval International AB, Tumba, Sweden). Cows failing to conceive to a previous insemination and not re-inseminated at a detected estrus 32 ± 3 d after their previous AI, were enrolled in the Ovsynch protocol (**D32-Resynch**; GnRH-7 d-PGF-56 h-GnRH-16 to 20 h-TAI) for resynchronization of ovulation. On farm C, cows without a corpus luteum ≥ 15 mm in diameter at the time of non-pregnancy diagnosis received the Ovsynch protocol with progesterone supplementation [GnRH + CIDR (Controlled Internal Drug Release Insert)-7 d-CIDR removal+PGF-56 h-GnRH-16 to 20 h-TAI] as described in Giordano et al. (2016).

Reproductive events and outcomes, milk production (i.e., monthly test-day milk volume and components as a percentage), and date and reason for herd exit (i.e., sold or died) for the 18 months following calving in the experimental lactation (i.e., lactation in which cows received treatments) were retrieved from the dairy herd management software (DairyComp305, ValleyAg software, Tulare, CA). If a cow completed the experimental lactation before the end of the 18-mo experimental period, data for the dry period and subsequent lactation were also collected to have data available for a total of 18-mo after calving in the experimental lactation. Cows that left the herd due to sale or death, were classified as “do not breed” before 30 DIM, or received their first service TAI outside the DIM range pre-established for their treatment group were excluded from the experiment (87 cows for VWP60 and 99 for VWP88). Therefore, the final number of cows per treatment was 1,265 and 1,260 for the VWP60 and VWP88 treatment, respectively.

Daily Milk and Fat-Corrected Milk Production Estimation

Individual cow daily milk production for the experimental and subsequent lactation was estimated with the MilkBot® model (Ehrlich, 2011) using monthly milk test data from DHIA tests retrieved from the dairy herd management software. MilkBot® is a nonlinear lactation prediction model that predicts individual daily milk yield as a function of DIM and 4 MilkBot® parameters: scale, ramp, offset, and decay. Milkbot® parameters and daily milk yield calculations have been previously described (Ehrlich, 2011; 2013).

Once daily milk yield was estimated, fat-corrected milk yield (**FCM**) was calculated to estimate daily DMI during lactation. Daily milk yield calculated by the MilkBot® model and monthly test milk fat percentage data were used to estimate daily FCM production.

Mathematically,

$$FCM\ (kg/d) = 0.4 \times DMW + 0.15 \times Fat\ \% \times DMW$$

where DMW = daily milk weight (kg/d) estimated by the MilkBot® model and Fat % = fat percentage in milk reported by the DHIA in monthly tests. Because daily milk fat percentage data was not available and could not be estimated with MilkBot®, data for a specific test was repeated each day during the 15 d before and after a test.

Body Weight Estimation

Body weight was modeled daily for every cow using the Korver function as previously described in van Arendonk (1985):

$$BW\ (kg) = A \times \{1 - [1 - (B/A)^{1/3}] \times \exp(-C \times AGE)\}^3 - (P1/P2) \times DIM \times \exp(1 - DIM/P2) + P3^3 \times DPC^3$$

where AGE = cow age in days, A = mature live weight (kg), B = birth weight (kg), C = growth rate, P1 = maximum decrease in live weight during lactation, P2 = time during lactation with minimum live weight, P3 = pregnancy parameter, and DPC = number of days after conception minus 50 (being 0 for the first 50 days of gestation). Data used for average mature live weight (723 kg) and birth weight (40 kg) was retrieved from farm C because it was not available for the other two farms. Parameters C, P1, P2, and P3 were 0.004, 30, 60, and 0.0187 for primiparous cows, and 0.006, 50, 80, and 0.0187 for multiparous cows based on Kalantari et al. (2010).

Dry Matter Intake Estimation

Daily DMI for individual cows was estimated using NRC (2001) equations. During lactation, DMI was estimated as a function of FCM, BW, and week of lactation (DIM/7), as follows:

$$\text{DMI (kg/d)} = 0.372 \times \text{FCM} + 0.0968 \times \text{BW}^{0.75} \times \{1 - \exp[-0.192 \times (\text{DIM}/7 + 3.67)]\}$$

During the dry period, DMI was estimated as a percentage of BW, which was calculated using the following equation:

$$\text{DMI (\% of BW)} = 1.97 - 0.75 \times \exp^{0.16 \times t}$$

where t = days pregnant minus 280.

Values for Input Costs and Revenues

Prices for inputs and outputs were selected to replicate economic conditions representative of the New York State dairy industry during most of the experimental period, which spanned from March 2014 to August 2016. Milk price was the average monthly price reported by the Dairy Market Watch report (Cornell Cooperative Extension of Chautauqua County, 2017) from March 2014 to August 2016. The weighted average accounting for milk class (class I to IV) price and usage was $0.41 per kg ($18.53 per hundredweight).

Feed cost was set at $0.29 per kg for a lactating cow diet, and $0.22 per kg for a dry and close-up cow diet based on data from the New York Farm Dairy Farm Business Summary from 2014 to 2015 (Knoblauch et al., 2015; 2016).

Newborn calves were given a market value of $170 for females (Progressive Dairyman, 2017), $85 for males (i.e., half of the female value), and $0 for stillborn calves. A value of $85 per calf was used for male-female twins assuming that females were freemartins.

Among reproductive program costs, GnRH ($1.60 per dose) and PGF ($2.10 per dose) treatments were calculated based on the market value of 2 commercially available products in New York whereas the cost of intravaginal progesterone releasing devices was set at $10.60 per device based on the cost of the only commercially available product (Eazi-Breed CIDR Cattle Insert, Zoetis, Madison, NJ). Labor cost to apply hormonal treatments was set at $0.25 per injection assuming 60 injections per h and $0.75 per CIDR application assuming application of 20 CIDR devices per h. The cost of a unit of semen was set $10 per dose and the cost to inseminate a cow at $1 per insemination assuming 15 inseminations per h and $15 per h for labor. Pregnancy testing was set at $2.75 per examination based on a cost of $110 per h and 40 cows tested per h. Estrus detection cost was calculated based a total of 2 h of detection per day at $15 per h for labor divided by the number of cows in the herd, which resulted on an average of $0.028 per cow/d.

For replacement costs calculations, the market value of a replacement heifer was set at $1,925 (USDA Economic Research Service, 2017) and the value of beef for cows sold was set at $1.32 per kg based on the average price for “good” and “lean” culled cows during the study period (Empire Livestock Marketing, 2016).

Cost of rbST supplementation was $7 per dose based on the price of the only commercially available product in the US (Posilac, Elanco Animal Health, Indianapolis, IN) and the labor cost needed to apply the treatments ($0.25 per injection, assuming 60 injections per h and $15 per h of labor).

Finally, other operating expenses not accounted for by feeding cost, rbST supplementation, reproductive cost, and replacement cost were set at $3 based on data from accrual operating costs reported in the Dairy Farm Business Summary from 2014 to 2015

(Knoblauch et al., 2015; 2016). The following items were included in the calculation: hired labor, professional nutritional services, machine repairs, rent and lease, fuel, oil and grease, veterinary and medicine, milk marketing, bedding, milking supplies, utilities and other professional fees.

Calculation of Economic Outcomes

Two main outcomes were calculated: (1) cash flow per cow for the experimental lactation (i.e., the lactation in which the experimental treatments were applied) and subsequent dry period if the cow completed this lactation. For cows that did not leave the herd and calved again, this outcome represents cash flow per calving interval; (2) cash flow per slot for each cow enrolled in the experiment for 18-mo after calving in the experimental lactation. This calculation was conducted to represent the dynamics of a commercial herd, whereby cows that leave the herd are replaced by a first lactation cow. Therefore, every slot (i.e., stall or unit of space) at the dairy was filled and herd size remained constant. The rationale for this methodology was to allow comparing the effect of treatments for a fixed period of time and the choice of 18-mo was to provide sufficient time for cows to complete their experimental lactation, dry period, and generate cash flow data for up to ~150 DIM for the lactation after cows received the experimental treatments. Calculations were based on the assumption that cows in the VWP60 treatment could get pregnant as early as 57 DIM, would have a gestation of ~280 d, and a dry period of ~50 to 60 d. Additionally, we further analyzed the effect of the VWP treatment on revenues and expenses during the 18-mo period after calving but only for cows pregnant at first TAI service in the experimental lactation that did not leave the herd up to the end of the 18-mo period after calving. This analysis aimed to explore the effect of achieving pregnancy at 60 ± 3 or 88 ± 3 DIM without confounding due to the herd exit dynamics.

For the calculation of cash flow per cow in the experimental lactation and dry period, all revenues (milk sales and calf value) and expenses (feed cost, replacement, reproductive cost, rbST supplementation cost, and other operating cost) were calculated for individual cows for the calving interval or up to the day of herd exit for cows sold and cows that died.

For the calculation of cash flow per slot, we represented the dynamics of a dairy herd during an 18-mo period based on the assumption that each slot at the dairy was occupied at all times to maintain herd size. Therefore, for cows that reached the end of the experiment (i.e., did not leave the herd) costs and revenues were calculated for their experimental lactation (including dry period) and the following lactation up to a total of 18 mo. Conversely, every cow that left the herd before the end of the 18-mo period after calving in the experimental lactation was immediately replaced by a first lactation cow randomly selected from the data set for the same farm and treatment group so that the slot remained occupied with a representative cow. Daily costs and revenues for the replacement cow were used to fill the slot up to the end of the 18-mo period. If the replacement left the herd before completion of the 18-mo period, another heifer was randomly selected to replace it. As a result, each slot occupied at the beginning of the experiment remained occupied for 18 mo as it would be observed in commercial dairy farms.

Total cash flow and cash flow per day of CI or per day of the 18-mo period after calving was estimated using the following equation:

$$\text{Cash flow} = \text{IOFC} + \text{Calf value} - \text{Replac} - \text{Repro} - \text{rbST} - \text{OE}$$

where IOFC = daily income over feed cost, Replac = cost of replacing cows that left the herd due to sale or death, Repro = cost of applying reproductive management programs, rbST = cost of supplementing cows with rbST, and OE = operating expenses.

Specifically, each item was calculated as follows.

Daily income over feed cost (**IOFC**) was calculated by subtracting feed cost (i.e., DMI kg x DM cost per kg) from milk revenue (i.e., milk volume in kg x milk price per kg) during lactation and the dry period (milk revenue = $0).

Calf value for cows that became pregnant and started a new lactation was the value of the newborn calf. Calves born dead received a value of $0 whereas calves born alive received market value based on gender.

Replacement costs for every cow that left the herd due to sale or death was assessed on the day the cow left the herd assuming that a replacement heifer replaced the cow immediately. This cost was estimated by subtracting the market value of a replacement heifer from the salvage value of the cow sold and the value of the calf born from the replacement. Thus, the cash cost of replacing a cow was equal to the market value of the replacement heifer minus the salvage value of the replaced cow and the value of the calf born. The salvage value for cows sold was calculated based on their estimated BW on the date of sale times the average beef price. Cows that left the herd due to death were assigned a salvage value of $0. The value of the calf born from a replacement heifer was set at $123 per calf to represent the average calf value for first lactation cows enrolled in this experiment (accounting for male, female, stillborn and twins).

Reproductive cost for each AI service for each cow was the aggregation of the cost of hormonal treatments, insemination, and detection of estrus during lactation, pregnancy testing, and labor associated with each activity. Reproductive cost was calculated for the experimental and for the 18-mo since calving in the experimental lactation (including experimental lactation and subsequent lactation).

The cost of rbST supplementation for the experimental lactation and for the 18-mo period after calving in the experimental lactation was calculated for each cow by multiplying the total number of rbST treatments by the cost per treatment (including hormone and labor cost).

The total value for operating expenses was calculated for each cow by multiplying the daily expense by the number of days in lactation and dry period for the experimental lactation, and by 540 d for the calculation of cash flow per slot per 18-mo (18 mo = 540 d).

Sensitivity Analysis for Input Costs and Value of Outputs

Two stochastic Monte Carlo simulation models (one for primiparous and one for multiparous cows) were developed using @Risk version 7.5 (Palisade Corporation, Ithaca, NY) to estimate differences between treatments in cash flow for the 18-mo period under varying market conditions for the most relevant input costs. Outcomes of interest were the distribution of differences in cash flow between the VWP60 and VWP88 treatments and the contribution of individual input costs to the total variation in cash flow differences.

Reproductive performance, production outcomes, and data for herd exit dynamics from our experiment were used as fixed inputs (Supplemental Table S1). Stochasticity in every iteration of the simulation was introduced for milk price, feeding cost, replacement heifers, calf value, beef price, and reproductive costs (cost for a first TAI service and cost of second a greater services at detected estrus or TAI), and rbST cost. Values for each parameter are presented in Supplemental Table S2. Monthly milk prices were collected from 2010 to 2017 from the USDA Agricultural Marketing Service (2017). National average dairy feed cost data and beef price of sold cows were obtained from the University of Wisconsin Dairy Marketing and Risk Management Program for the same period (Gould, 2017a,b). Market value data from 2010 to 2017 for replacement heifers were collected from the USDA Economic Research Service (2017). For calf value, reproductive cost, and rbST price, values were based on a 15% increment and reduction from the average price calculated for this experiment. For example, average calf value was calculated based on the price collected from 2014 to 2016 ($170 for females and $85 per male) times the proportion of males, females, stillborn, and twins calved during the 18-mo period (average calf value = $127.8 per calf born). A range of 15% smaller and greater price was then applied (minimum calf value = $108.7; maximum calf value = $147.0). Average reproductive cost for first service TAI and second and greater services through EDAI or TAI and rbST cost were those observed in the current experiment. Distributions used in the simulations for variables with historical data available (i.e., milk price, feed cost, cost of a replacement heifer, and beef price) were fitted using the BestFit function of @Risk. This function selects the best fitting distribution based on the lowest value for the Aikaike Information Criteria (**AIC**). Specifically, milk price was fitted with a beta distribution, feed cost of a lactating cow diet and a dry cow diet with uniform distributions, replacement heifer cost was fitted with a triangular distribution, and beef price was fitted with a triangular distribution. For input variables with a minimum, most likely, and a maximum value (calf value, reproductive cost, and rbST price), a pert distribution was used to emphasize the most likely value over the minimum and maximum values (McArt and Oetzel, 2015). Input values for each distribution are presented in Supplemental Table S2.

Simulations were run and recorded for 10,000 iterations with replacement. For each iteration, the difference in cash flow between the VWP60 and VWP88 treatments (output) was calculated as follows:

Difference in cash flow ($ per slot/18-mo) = Cash flow VWP88 – Cash flow VWP60

Statistical Analysis

All data were analyzed by parity group because of the well-described and expected differences in performance and profitability between primiparous and multiparous cows.

Dichotomous outcomes [pregnancies per AI (**P/AI**) at first and at second and greater services, proportion of cows non-pregnant at 350 DIM, proportion of cows that were sold, died, or left the herd (sold plus dead), and proportion of cows that calved again after the end of the experimental lactation], were analyzed by logistic regression using the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Time to event data (time to pregnancy and time to herd exit) were analyzed with Cox's proportional hazards models using the PHREG procedure of SAS. A cow was considered pregnant if pregnancy was maintained up to 150 d of gestation. Continuous quantitative outcomes [lactation length, days dry, calving interval (**CI**), milk yield, DMI, incomes (milk income, calf value, IOFC), expenses (replacement cost, reproductive cost, rbST cost, and other operating cost), and cash flow] were analyzed by ANOVA using the MIXED procedure of SAS.

The effect of treatment (VWP60 and VWP88) was offered as explanatory variable to all models and farm was included as a random effect. For reproductive performance and herd exit dynamics outcomes, the effect of milk yield accumulated up to 30 DIM (**MK30**; low, medium, and high based on tertiles from observed data), and the treatment by MK30 interaction were offered to the models. In addition, season of first service (cold: September 21 to June 20; warm: June 21 to September 20; only for first service), and the treatment by season interaction were also offered to the model for P/AI at first service. Results for the effect of MK30 and season of first AI were presented elsewhere (Stangaferro et al., 2017) and are not included in this manuscript except for data for CI and proportion of cows that calved again. For outcomes included in profitability analyses (milk production, DMI, all incomes and expenses, and cash flow), the effect of treatment and season of calving (cold: September 21 to June 20; warm: June 21 to September 20) were offered to the models.

For all linear regression models, assumptions of normality and homoscedasticity of variance were evaluated with normal probability plots (normal Q-Q plot) and plots of residuals versus predicted values. For logistic regression models, goodness of fit was evaluated to test for overdispersion. Graphical examination of the log(-log(survival probability)) function obtained from the PROC LIFETEST of SAS was used to assess the proportional hazard assumption for time to event data. The final model for each outcome of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Aikaike Information Criterion. Treatment was forced in all models. When appropriate, the Least Significant Different (**LSD**) post hoc mean separation test was used to determine differences between Least Square Means (**LSM**).

All proportions reported were generated using the FREQ procedure of SAS, whereas quantitative outcomes were reported as LSM ± SEM. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, while P-values > 0.05 and ≤ 0.10 were considered a tendency.

RESULTS

Reproductive Performance and Herd Exit Dynamics in the Experimental Lactation

Pregnancy per AI at first service was greater ($P < 0.01$) for primiparous cows in the VWP88 (55.3%) than the VWP60 (46.0%) treatment, but similar ($P = 0.16$) between treatments for multiparous cows (Table 1). For second and greater AI services, P/AI was greater ($P = 0.04$) for primiparous cows in the VWP60 (43.6%) than in the VWP88 (37.2%) treatment, but no difference ($P = 0.68$) was observed within the multiparous cow group (Table 1). The hazard of pregnancy during lactation was lower ($P < 0.01$) for cows in the VWP88 than the VWP60 treatment both in the primiparous ($P < 0.01$) and multiparous cow group ($P < 0.01$). Conversely, the proportion of nonpregnant cows at 350 DIM did not differ between treatments neither for primiparous ($P = 0.51$) nor for multiparous cows ($P = 0.20$; Table 1).

Primiparous cows in the VWP60 and VWP88 treatment had similar hazard of leaving the herd ($P = 0.56$), and by 350 DIM a similar ($P = 0.58$) total proportion of cows left the herd. On the other hand, multiparous cows in the VWP88 treatment had a greater ($P = 0.03$) hazard of leaving the herd and a greater ($P = 0.03$) proportion of cows left the herd by 350 DIM primarily because a greater ($P = 0.04$) proportion of cows were sold.

Lactation length was greater for the VWP88 treatment for primiparous ($P < 0.01$) but not for multiparous cows ($P = 0.47$) when all cows were included in the analyses (i.e., cows that completed the experimental lactation and cows that left the herd). When only cows that completed the experimental lactation were included in the analysis, lactation length was longer ($P < 0.01$) for the VWP88 treatment in the primiparous and multiparous group. No differences were observed in the duration of the dry period for primiparous ($P = 0.41$) and multiparous ($P = 0.21$) cows.

Calving interval was affected ($P < 0.01$) by treatment, whereby cows in the VWP88 treatment had longer CI than cows in the VWP60 treatment (Table 1). Calving interval was also affected by MK30 for both primiparous ($P = 0.03$) and multiparous ($P < 0.01$) cows because cows in the high MK30 group had longer CI than cows in the medium and low MK30 group (primiparous: high = 397 ± 7.7 d, medium = 385 ± 7.7 d, low = 388 ± 7.7 d; multiparous: high = 404 ± 6.2 d, medium = 391 ± 6.2 d, low = 386 ± 6.4 d).

For primiparous cows, the proportion of cows that calved after the experimental lactation was similar ($P = 0.37$) between treatments, but it was greater ($P < 0.01$) for cows in the high (87.4%; 319) and medium (88.4%; 313) than in the low MK30 group (80.0%; 319). For multiparous cows, the proportion of cows that calved after the experimental lactation was greater ($P = 0.01$) for the VWP60 treatment, and greater ($P < 0.01$) for cows in the high (64.3%; 521) and medium (61.3%; 532) than in the low MK30 group (48.5%; 521).

Performance and Economic Outcomes during the Experimental Lactation

For primiparous cows, extending the duration of the VWP from 60 to 88 d resulted in greater ($P < 0.01$) total milk yield and income, greater ($P < 0.01$) total DMI and feed cost during the milking period, and daily DMI and feed cost during the dry period (Table 2). No differences ($P > 0.10$) were observed for daily milk yield and income, daily DMI and feed cost during the milking period, and total DMI and feed cost during the dry period (Table 2). In addition, primiparous cows that calved during the warm season had lower ($P < 0.01$) daily milk yield (warm: 33.0 ± 0.9 kg/d; cold: 34.2 ± 0.9 kg/d), and income (warm: 13.5 ± 0.4 \$/d; cold: 14.0 ± 0.4 \$/d) than cows that calved in the cold season. Cows that calved during the warm season also had lower ($P = 0.01$) daily DMI (warm: 23.5 ± 0.3 kg/d; cold: 23.9 ± 0.3 kg/d) and daily feed cost (warm: 6.75 ± 0.10 \$/d; cold: 6.89 ± 0.09 \$/d).

For multiparous cows, the only difference between treatments was for daily DMI (*P* = 0.02) and daily feed cost (*P* = 0.02) during the dry period (Table 2). There was, however, an effect of calving season because multiparous cows that calved during the warm season had lower (*P* = 0.01) daily milk yield (warm: 39.9 ± 0.6 kg/d; cold: 40.9 ± 0.6 kg/d) and daily milk income (warm: 16.4 ± 0.3 $/d; cold: 16.8 ± 0.2 $/d). Cows that calved during the warm season also tended (*P* = 0.08) to have lower daily DMI (warm: 26.5 ± 0.2 kg/d; cold: 26.7 ± 0.1 kg/d) and daily feed cost (warm: 7.68 ± 0.04; cold: 7.60 ± 0.04 $/d) than cows that calved during the cold season.

The effect of VWP treatment on revenues and expenses during the experimental lactation is presented in Table 3. For primiparous cows, IOFC, rbST cost, and operating expenses were greater (*P* < 0.01) for cows in the VWP88 treatment. rbST cost was greater because the number of injections received by cows in the VWP88 group (24.5 ± 3.0) was greater (P < 0.01) than for cows in the VWP60 group. Moreover, cows in the VWP88 group tended (*P* = 0.08) to have greater cash flow ($89.7 per lactation) than cows in the VWP60 group. No differences between treatments were observed for calf value (*P* = 0.32), replacement cost (*P* = 0.60), reproductive cost (*P* = 0.27), and cash flow per day of CI (*P* = 0.42).

For multiparous cows, there was no effect of treatment on IOFC (*P* = 0.60), calf value (*P* = 0.43), rbST cost (*P* = 0.32), operating expenses (*P* = 0.76), cash flow (*P* = 0.62) and cash flow per day of CI (*P* = 0.16; Table 3). Conversely, replacement cost tended (*P* = 0.06) to be greater for the VWP88 treatment and reproductive cost was greater (*P* < 0.01) for the VWP60 treatment (Table 3). No difference was observed for rbST cost because the number of rbST injections received was similar (*P* = 0.32) for both treatments (20.6 ± 3.0 and 21.1 ± 3.0 for VWP60 and VWP88, respectively).

Season of calving did not affect ($P > 0.10$) any of the parameters evaluated during the experimental lactation for primiparous or multiparous cows.

Performance and Economic Outcomes for 18 month after Calving in the Experimental Lactation

The effect of treatments on milk yield, milk income, DMI, and feed cost for 18-mo after calving in the experimental lactation is presented in Table 4. All parameters evaluated did not differ ($P > 0.10$) for cows in the VWP60 and VWP88 treatments for the primiparous and multiparous group. In contrast, season of calving affected performance and economic outcomes of multiparous cows whereby, cows that calved during the cold season in the experimental lactation produced more milk ($P = 0.02$; cold: 20,297 ± 404; warm: 20,069 ± 409 kg/cow), generated greater milk income ($P = 0.02$; cold: 8,320 ± 166; warm: 8,176 ± 170 \$/cow), had greater DMI ($P = 0.05$; cold: 13,239 ± 164; warm: 13,177 ± 168 kg/cow), and greater feed cost ($P = 0.05$; cold: 3,804 ± 47; warm: 3,769 ± 48 \$/cow) during the milking period than cows that calved during the warm season.

The effect of treatment on revenues and expenses during the 18-mo period after calving is presented in Table 5. For the primiparous group, cows in the VWP60 treatment tended ($P = 0.07$) to have greater replacement cost, had greater ($P < 0.01$) reproductive cost, and lower ($P < 0.01$) rbST cost than cows in the VWP88 treatment. No differences were observed for the rest of the parameters evaluated ($P > 0.10$). For multiparous cows, reproductive cost was greater ($P < 0.01$) for cows in the VWP60 treatment. Coversely, rbST cost was lower ($P < 0.01$) for cows in the VWP60 group because they received fewer ($P < 0.01$) injections (30.3 ± 3.5 and 31.1 ± 3.6 for the VWP60 and VWP88 group, respectively. No treatment effect was observed for the rest of the parameters (P > 0.10) evaluated.

Season of calving only affected IOFC for multiparous cows (P = 0.02; cold: 4,401 ± 131; warm: 4,286 ± 134 \$/cow).

Further, we analyzed the effect of the VWP treatment on revenues and expenses during the 18-mo period after calving but only for cows pregnant at first TAI service in the experimental lactation that did not leave the herd up to the end of the 18-mo period after calving. For primiparous cows, a greater (P < 0.01) proportion of cows in the VWP88 group (41%; n = 480) compared with the VWP60 (31%; n = 480) met the eligibility criteria. From them, cows in the VWP88 treatment tended to have greater IOFC (~127 \$/cow; P = 0.10), greater calf value (~10 \$/cow; P = 0.07), and greater cash flow total (~138 \$/slot/18-mo; P = 0.08) and per day (~0.25 \$/cow/d; Table 6). No effect of treatment was observed for the rest of the parameters evaluated (P > 0.10).

Multiparous cows pregnant at first TAI service in the experimental lactation that did not leave the herd up to the end of the 18-mo period after calving represented 19% (n = 785) and 17% (n = 789) of the total multiparous cows enrolled in the VWP60 and VWP88 group, respectively (P = 0.37).No effect of treatment on any of the parameters evaluated was observed for multiparous cows pregnant at first service (Table 6). Only season of calving affected IOFC (P = 0.04; cold: 4,904 ± 157; warm: 4,680 ± 167 \$/slot/18-mo), cash flow (P = 0.04; cold: 3,222 ± 136; warm: 3,001 ± 148 \$/slot/18-mo), and cash flow per day (P = 0.04; cold: 6.97 ± 0.25; warm: 5.56 ± 0.27 \$/slot/18-mo).

Sensitivity Analysis

For primiparous cows, differences in cash flow under diverse pricing scenarios were always in favor of the VWP88 group. Cash flow differences ranged from a minimum of $0.9 to a maximum of $114.4 per slot/18-mo. The average difference in cash flow after 10,000 iterations was $50.4 per slot/18-mo (95% CI: $18.2 to $88.7 per slot/18-mo). The cost of a replacement heifer was the main contributor to the total variance explaining ~65% of the total variation in cash flow differences. Beef price for cows sold was the second most important factor and explained another ~27% of the total variation. Therefore, these two factors combined explained ~92% of the total variation in cash flow differences.

For multiparous cows, differences in cash flow after 10,000 iterations were always in favor of the VWP60 group, with a mean of $-56.6 per slot/18-mo (95% CI: $-89.5 to $-27.3 per slot/18-mo), and a minimum and maximum of $-89.5 and -27.3 per slot/18-mo, respectively. The contribution to variance was led by the cost of a replacement heifer (47.4%) followed by beef price (45.2%), and both combined represented ~93% of the total variation in cash flow differences.

DISCUSSION

Achieving pregnancy at a range of DIM that optimizes profitability for a majority of dairy cows depends upon (1) the duration of the VWP and (2) reducing the number and variation of days to pregnancy after cows become eligible for pregnancy. In this study, we evaluated the effect of extending the VWP from 60 to 88 DIM on cow profitability accounting for the multiple interactions among reproductive performance, productivity, and herd replacement dynamics. In agreement with previous studies (Van Amburgh et al., 1997; Arbel et al., 2001; Gobikrushant et al., 2014), the greatest effect of extending the VWP on reproductive performance was shifting timing of pregnancy towards later lactation. For primiparous cows in the VWP88 group, pregnancy was delayed in spite of greater P/AI to first service. Cows with shorter VWP fully compensated for reduced first service P/AI through earlier and more opportunities for re-insemination. A direct consequence of delayed pregnancy in the primiparous cow group was longer lactations (for all cows or only cows not culled) and extended calving intervals. For the multiparous group, overall time to pregnancy was also delayed. Nevertheless, we observed longer calving interval and longer overall lactation length for non-culled cows only. This was because another consequence of delayed first service was a greater proportion of cows removed from the herd. Delayed pregnancy and greater culling pressure in older cows (De Vries et al., 2010; Pinedo et al., 2010) likely contributed to the greater proportion of cows culled in this group. As more cows left the herd earlier, their shorter lactations resulted in smaller differences between groups for lactation length.

Total cash flow for the calving interval of the experimental lactation was greater for primiparous cows in the VWP88 group because extended lactations resulted in a substantial IOFC increment not offset by greater rbST cost and OE (all affected by lactation length). On the other hand, parameters that did not depend on lactation length such as calf value, replacement cost, and the cost of implementing reproductive programs were similar because there were either no or negligible differences between groups for calves born, replacement dynamics, and number of AI services. Unlike total cash flow for the calving interval, cash flow per day was similar likely because of the high persistency of lactation of primiparous cows (Stanton et al., 1992; Scott et al., 1996; Van Amburgh et al., 1997) which resulted in high IOFC in late lactation. Collectively, our data suggested that for primiparous cows extending lactation length by delaying first service increased total cash flow per calving interval. This effect was primarily due to more productive days because cash flow per day was similar due to the high milk production persistency of primiparous cows. This is particularly relevant when considering extrapolation of our results to other farms and conditions because in our experiment all cows received rbST supplementation, which is known to increase lactation persistency (Peel and Bauman, 1987; Chalupa and Galligan, 1989; Bauman and Vernon, 1993). Without rbST supplementation it is likely that the differences observed would have been reduced and favored the group with shorter VWP.

Unlike for primiparous cows, the lack of difference in total cash flow per calving interval or per day of the calving interval for the multiparous cow group was a reflection of the similar lactation length for the VWP treatments when all cows enrolled (i.e., culled and not culled) were accounted for. Additional monetary differences associated with the replacement dynamics and reproductive performance balanced overall cash flow differences between treatments. For example, the small numerical increment in IOFC and calf value, along with the reduction in reproductive program cost for cows in VWP88 offset the minor extra cost of rbST and OE. Nevertheless, that was insufficient to compensate for greater replacement cost. Greater culling pressure for multiparous cows in the extended VWP treatment not only limited the potential IOFC benefits of longer lactation, but also negatively affected cash flow by increasing replacement cost. Others have also documented major contributions of replacement cost to differences in overall cash flow of cows managed with different VWP duration (Schmidt, 1989; Incahisri et al., 2011; Gobikrushant et al., 2014). Taken together, our results for multiparous cows and those from previous experiments emphasize the importance of considering the complex interactions between reproductive outcomes, productive performance, and the herd exit dynamics when evaluating the economic consequences of manipulating the duration of the VWP for dairy herds.

Although many studies reported the economic value of reproductive management strategies for dairy cattle (Groenendaal et al., 2004; Tenhagen et al., 2004; Meadows et al., 2005; Olynk and Wolf, 2009; Giordano et al., 2011, 2012), specific criteria, or methods to measure dairy cow profitability have not been clearly established. For this study, we included evaluations of cash flow for the calving interval of individual cows and for an 18-mo period after calving in the experimental lactation for each slot filled at the beginning of the study. The latter analysis was used because evaluating profitability for a single calving interval does not account for the effect of timing of pregnancy on the subsequent lactation of cows (Arbel et al., 2001; Giordano et al., 2012; Gobikrushant et al., 2014). In addition, evaluating slots rather than cows allows accounting for the performance of the replacement that filled a slot after another cow left the herd. This is relevant because replacement cows not only have different IOFC but also different pregnancy and herd exit risk than the cow they replaced (DeLorenzo et al., 1992; Hadley et al., 2006; Dhuyvetter et al., 2007) . Thus, the analysis per slot per 18-mo may better represent the reality of dairy farms that maintain a constant herd size by immediately replacing cows that leave the herd in an attempt to maximize profitability per unit of time (De Vries, 2004; 2006). A caveat of the analysis used in our study is that only a portion of the productive lifetime of cows was included hence, future research should consider monitoring cow performance for the total expected lifetime of cows.

The lack of difference between treatments in total cash flow for primiparous cows reflected the compensation for additional productive days in the experimental calving interval by more productive days early after calving in the subsequent lactation (i.e., second lactation). The

$173 greater IOFC due to ~20 d longer experimental lactations for cows in the VWP88 treatment was fully compensated by a similar number of productive days in the subsequent lactation. This was because dry period length was similar between groups and IOFC per day was of approximately the same magnitude at the end of the experimental lactation than in the early part of the subsequent lactation. Additional compensation between the extra cost of rbST supplementation for the VWP88 treatment (had more days in later lactation) and greater cost of the reproductive program in the VWP60 treatment (due to more AI services), made replacement cost the greatest contributor to differences between treatments. Unexpectedly, a slightly greater proportion of second parity cows (first parity at the time enrollment) in the VWP60 treatment left the herd early in the subsequent lactation increasing replacement cost for the 18 mo period.

Minor monetary differences and compensation among items included in the analysis also explained the lack of differences in total cash flow for the 18-mo period for multiparous cows. Nevertheless, most items evaluated trended in favor of the shorter VWP treatment. This was expected because of the greater replacement cost in the experimental lactation and the lesser benefit of extended lactations on IOFC for multiparous cows in the VWP88 treatment. Lesser persistent lactations for multiparous cows (Stanton et al., 1992; Scott et al., 1996; Van Amburgh et al., 1997) favor profitability in early lactation more dramatically in this group than for cows transitioning from their first to their second lactation. Overall, our results disagree with some previous studies that recommended longer VWP for multiparous cows because of expected increases in profitability (Van Amburgh et al., 1997; Arbel et al., 2001). Nevertheless, these studies included assumptions about herd replacement dynamics, multiple exclusion criteria (i.e., enrolled only second lactation cows or cows that did not calve during summer months and only cows with above average milk production), and different methods to calculate profitability (e.g., per cows instead of per slot) which could explain the contradictory results.

The lack of significance for differences in cash flow in spite of the large number of cows included in the experiment was likely a reflection of the major variation in profitability per slot, ranging from $-2,503 to $5,599 (data not shown). This major disparity in cash flow was explained primarily by large differences in replacement costs between slots in which the cow did not leave the herd during the 18 mo period (i.e., replacement cost was $0) versus those in which cows were replaced one or more times. Indeed, an interesting observation from the stochastic analysis was that two of the main factors that determine total replacement cost (i.e., replacement heifer price and beef price) were the major contributors to differences in cash flow between VWP treatments. The effect of the replacement dynamics on the cow population in the subsequent lactation also affected results because more replacements entering the herd in the subsequent lactation for the VWP60 treatment resulted in a greater proportion of first parity cows filling up slots by the end of the 18-mo period. This may have explained, at least in part, why more DIM of the 18 mo period filled with cows in their second lactation did not translate into major IOFC benefits in the subsequent lactation for primiparous cows in the VWP60 treatment.

The duration of the VWP has a significant effect on cows that become pregnant to first service, in particular if cows are submitted to first service with all TAI programs. As P/AI to first service increase, lactation length and the calving interval are locked for a greater proportion of cows in the herd. Our analysis for cows that became pregnant at first service only was meant to provide insights about the effect of timing of pregnancy during lactation on cow profitability. It also allowed visualizing, albeit indirectly, the effect of herd exit dynamics on profitability because only cows that did not leave the herd during the 18 mo period after calving were included in the analysis. Greater cash flow for primiparous cows inseminated at 88 rather than 60 DIM is in line with the concept that high producing cows with persistent lactations benefit by pregnancy closer to 100 than 50 DIM (De Vries, 2006; Inchaisri et al., 2011). On the other hand, the opposite was observed for multiparous cows suggesting that through increased IOFC, pregnancy at 60 DIM was more profitable than at 88 DIM. As the monetary differences for most items were very similar, IOFC was the main contributor to cash flow differences between treatments. This was due to the substantial difference in IOFC between early and late lactation for multiparous cows. Our results are in agreement with previous studies that recommended shorter calving intervals to maximize milk production and profitability of dairy cows (Olds et al., 1979; Olentacu et al., 1981; Dijkhuizen et al., 1985; Holmann et al., 1984; Schmidt, 1989). The similar trends for differences between treatments when only cows pregnant to first service and all cows in the study were evaluated, provides additional evidence that the VWP duration and time to pregnancy that maximize cow profitability can be different for primiparous and multiparous cows.

Results from the stochastic analysis for both primiparous and multiparous cows confirmed that the direction of differences between treatments remained in spite of variable prices for major inputs and sources of revenues. Moreover, after 10,000 simulations of different pricing scenarios, in no case results favored the VWP60 treatment for primiparous cows or the VWP88 treatment for multiparous cows. Therefore, the relative benefit of an 88 d VWP for primiparous and a 60 d VWP for multiparous cows as compared to the alternative in this study should be expected under a wide range of economic conditions. Interestingly, the major contributors (>90%) to differences between treatments were factors related to the herd replacement dynamics (i.e., replacement heifer price and beef price). Thus, culling policies as well as current and future prices for replacements and the salvage value of cows sold are important considerations when defining the duration of the VWP for a particular dairy herd. In addition, the agreement for results of the stochastic analysis and the main findings of this study emphasized the relevance of accounting for the herd exit dynamics when comparing the profitability of different VWP durations.

CONCLUSION

We conclude that dairy farms using aggressive reproductive management programs that result in high P/AI to first service might benefit by extending the duration of the VWP beyond 60 d for primiparous cows. First insemination at around 88 DIM may result in greater profitability per slot per unit of time. On the other hand, a VWP in the range of 60 rather than 88 DIM might be preferred for multiparous cows. In addition, our data strongly suggested that decisions about VWP duration for dairy cows should not be made without careful consideration of the complex interactions between replacement dynamics and the productive and reproductive performance of dairy cows in multiple lactations.

Finally, this study also emphasized the need to standardize the methods used to calculate the effect of reproductive management strategies on cow and herd profitability if decision-making of on-farm practices is to be based on this type of research.

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Table 1. Effect of extending the duration of the voluntary waiting period from 60 to 88 DIM on reproductive performance and herd exit dynamics during the experimental lactation

	Primiparous			Multiparous		
	VWP60[1]	VWP88[2]	*P*-value	VWP60	VWP88	*P*-value
P/AI[3] 1st service, % (n)	46.0 (463)	55.3 (453)	<0.01	36.2 (726)	39.9 (698)	0.16
P/AI 2nd and greater AI, % (n)	43.6 (540)	37.2 (494)	0.04	31.5 (1,204)	30.6 (1,015)	0.68
Time to pregnancy after calving						
Hazard Ratio (CI)	*REF*	0.72 (0.63-0.83)	<0.01	*REF*	0.76 (0.67-0.84)	<0.01
Mean, d	115	135	-	142	163	-
Median, d	85	91	-	104	132	-
% NP at 350 DIM[4], % (n)	2.3 (480)	2.9 (471)	0.51	11.0 (785)	13.0 (789)	0.20
Time to exit the herd[5]						
Hazard Ratio (CI)	*REF*	0.89 (0.62-1.30)	0.56	*REF*	1.21 (1.02-1.44)	0.03
Mean, d	324	332	-	302	295	-
Median, d	-	-	-	-	-	-
% Exit the herd, % (n)	8.6 (480)	7.8 (471)	0.58	29.9 (785)	35.1 (789)	0.03
Sold, % (n)	7.6 (480)	7.2 (471)	0.78	25.5 (785)	30.1 (789)	0.04
Died, % (n)	1.8 (480)	1.2 (471)	0.45	4.1 (785)	4.6 (789)	0.87
Lact. lenght						
All cows, d	319.4 ± 14.0	341.3 ± 14.0	<0.01	300.7 ± 13.7	305.0 ± 13.7	0.47
Not culled, d	333.0 ± 12.3	354.4 ± 12.4	<0.01	336.2 ± 12.3	356.5 ± 12.3	<0.01
Days dry, d	53.9 ± 2.7	53.6 ± 2.7	0.41	55.7 ±4.5	57.3 ± 4.5	0.21
Calving Interval, d	380.4 ± 7.4	399.1 ± 7.4	<0.01	384.0 ± 6.0	403.5 ± 6.0	<0.01
% Cows calved again, % (n)	86.5 (480)	84.6 (471)	0.37	61.2 (785)	55.0 (789)	0.01

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]P/AI = pregnancies per artificial insemination.
[4]% NP at 350 DIM = percentage of cows non-pregnant at 350 DIM.
[5]Time to exit the herd = time (d) from calving until exit the herd because sold or died.

Table 2. Effect of extending the duration of the voluntary waiting period from 60 to 88 DIM on milk production, milk income, dry matter intake and feed cost during the experimental lactation and dry period

	Primiparous				Multiparous			
	VWP60[1] (n = 480)	VWP88[2] (n = 471)	Diff[3]	*P*-value[4]	VWP60 (n = 785)	VWP88 (n = 789)	Diff	*P*-value
Milk (kg/cow)								
Total	10,972 ± 725	11,805 ± 726	833	<0.01	12,378 ± 721	12,536 ± 721	158	0.53
Daily	33.37 ± 0.91	33.77 ± 0.91	0.40	0.27	40.61 ± 0.62	40.21 ± 0.62	-0.40	0.25
Milk income ($/cow)								
Total	4,499 ± 297	4,840 ± 298	341	<0.01	5,075 ± 295	5,140 ± 295	65	0.53
Daily	13.68 ± 0.37	13.85 ± 0.37	0.17	0.27	16.65 ± 0.25	16.48 ± 0.25	-0.17	0.25
Dry matter intake (kg/cow)								
Milking (*total)*	7,695 ± 407	8,281 ± 407	586	<0.01	8,132 ± 424	8,277 ± 424	145	0.36
Milking *(daily)*	23.60 ± 0.33	23.84 ± 0.33	0.24	0.11	26.62 ± 0.14	26.56 ± 0.14	-0.06	0.64
Dry *(total)*	705.2 ± 32.6	715.6 ± 32.6	10.4	0.32	754.3 ± 60.0	777.5 ± 60.1	23	0.12
Dry *(daily)*	13.18 ± 0.02	13.27 ± 0.02	0.09	<0.01	13.58 ± 0.02	13.64 ± 0.02	0.06	0.02
Feed Cost ($/cow)								
Milking (*total)*	2,211 ± 117	2,379 ± 117	168	<0.01	2,337 ± 122	2,378 ± 122	41	0.36
Milking *(daily)*	6.78 ± 0.10	6.85 ± 0.10	0.07	0.11	7.65 ± 0.04	7.63 ± 0.04	-0.02	0.64
Dry *(total)*	155.5 ± 7.2	157.8 ± 7.2	2.3	0.32	165.6 ± 13.2	171.4 ± 13.2	6	0.12
Dry *(daily)*	2.90 ± 0.01	2.92 ± 0.01	0.02	<0.01	2.99 ± 0.01	3.00 ± 0.01	0.01	0.02

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]Diff = difference between VWP88 and VWP60 (Diff = VWP88-VWP60).
[4]The effect of season of calving is described in the text.

Table 3. Effect of extending the duration of the voluntary waiting period from 60 to 88 DIM on revenues and expenses during the experimental lactation

	Primiparous				Multiparous			
	VWP60[1] (n = 480)	VWP88[2] (n = 471)	Diff[3]	*P*-value[4]	VWP60 (n = 785)	VWP88 (n = 789)	Diff	*P*-value
	$/cow				$/cow			
Milk income over feed cost	2,156 ± 175	2,329 ± 175	173	<0.01	2,635 ± 183	2,665 ± 182	30	0.60
Calf value	99.27 ± 6.06	103.46 ± 6.08	4.19	0.32	71.68 ± 2.55	68.84 ± 2.54	-2.84	0.43
Replacement cost	143.1 ± 50.9	156.3 ± 51.1	13.2	0.60	390.5 ± 20.0	440.1 ± 19.9	49.6	0.06
Reproductive cost	60.31 ± 3.62	58.21 ± 3.62	-2.10	0.27	63.73 ± 1.94	58.69 ± 1.94	-5.04	<0.01
rbST cost	161.4 ± 21.8	177.4 ± 21.8	16.0	<0.01	148.9 ± 21.9	152.7 ± 21.9	3.8	0.32
Other operating expenses	1,023 ± 45	1,083 ± 45	60	<0.01	941.0 ± 35.6	946.2 ± 35.6	5.2	0.76
Cash flow	859.2 ± 160.6	948.9 ± 160.7	89.7	0.08	1,164 ± 130	1,137 ± 130	-27	0.62
Cash flow per day of CI	1.62 ± 0.57	1.84 ± 0.57	0.22	0.42	2.24 ± 0.41	1.81 ± 0.41	-0.43	0.16

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]Diff = difference between VWP88 and VWP60 (Diff = VWP88-VWP60).
[4]The effect of season of calving is described in the text.

Table 4. Effect of extending the duration of the voluntary waiting period from 60 to 88 DIM on milk production, milk income, dry matter intake and feed cost during 18 mo after calving in the experimental lactation

	Primiparous				Multiparous			
	VWP60[1] (n = 480)	VWP88[2] (n = 471)	Diff[3]	*P*-value[4]	VWP60 (n = 785)	VWP88 (n = 789)	Diff	*P*-value
Milk (kg/slot)	18,185 ± 378	18,227 ± 378	42	0.79	20,166 ± 409	20,069 ± 409	-97	0.49
Milk income ($/slot)	7,456 ± 155	7,473 ± 155	17	0.79	8,268 ± 168	8,228 ± 168	-40	0.49
Dry matter intake (kg/slot)								
Milking	12,226 ± 120	12,303 ± 120	77	0.21	13,165 ± 166	13,191 ± 166	26	0.66
Dry	693.8 ± 33.9	703.1 ± 34.0	9.3	0.41	723.1 ± 49.8	728.9 ± 49.9	5.8	0.71
Feed Cost ($/slot)								
Milking	3,512 ± 34	3,535 ± 34	23	0.21	3,783 ± 48	3,790 ± 48	7	0.66
Dry	153.0 ± 7.5	155.0 ± 7.5	2.0	0.41	159.4 ± 11.0	160.7 ± 11.0	1.3	0.71

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]Diff = difference between VWP88 and VWP60 (Diff = VWP88-VWP60).
[4]The effect of season of calving is described in the text.

Table 5. Effect of the extending duration of the voluntary waiting period from 60 to 88 DIM on revenues and expenses during 18 mo after calving in the experimental lactation

	Primiparous				Multiparous			
	VWP60[1] (n = 480)	VWP88[2] (n = 471)	Diff[3]	*P*-value[4]	VWP60 (n = 785)	VWP88 (n = 789)	Diff	*P*-value
	$/slot				$/slot			
Milk income over feed cost	3,806 ± 119	3,803 ± 119	-3	0.95	4,363 ± 132	4,324 ± 132	-39	0.38
Calf value	100.5 ± 3.5	102.9 ± 3.5	2.4	0.59	80.78 ± 2.52	77.98 ± 2.52	-2.80	0.43
Replacement cost	327.3 ± 52.1	259.0 ± 52.3	-68.3	0.07	624.9 ± 31.4	673.6 ± 31.3	48.7	0.16
Reproductive cost	97.58 ± 1.38	91.28 ± 1.40	-6.30	<0.01	104.14 ± 1.95	93.63 ± 1.95	-10.51	<0.01
bST cost	215.9 ± 23.6	221.6 ± 23.6	5.7	<0.01	219.4 ± 25.6	224.9 ± 25.6	5.5	<0.01
Other operating expenses	1,512	1,512	-	-	1,512	1,512	-	-
Cash flow	1,756 ± 148	1,824 ± 148	68	0.32	2,006 ± 124	1,921 ± 124	-85	0.19
Cash flow per day	3.25 ± 0.28	3.37 ± 0.28	0.12	0.32	3.71 ± 0.23	3.56 ± 0.23	-0.15	0.19

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]Diff = difference between VWP88 and VWP60 (Diff = VWP88-VWP60).
[4]The effect of season of calving is described in the text.

Table 6. Effect of extending the duration of the voluntary waiting period from 60 to 88 DIM on revenues and expenses during 18 mo after calving in the experimental lactation for cows pregnant at first service that did not leave the herd

	Primiparous				Multiparous			
	VWP60[1]	VWP88[2]	Diff[3]	*P*-value[4]	VWP60	VWP88	Diff	*P*-value
	$/slot				$/slot			
Milk income over feed cost	3,906 ± 145	4,033 ± 143	127	0.10	4,854 ± 161	4,730 ± 161	-124	0.23
Calf value	118.6 ± 4.5	128.5 ± 4.1	9.9	0.06	118.1 ± 3.9	125.0 ± 4.0	6.9	0.22
Reproductive cost	82.41 ± 1.89	81.32 ± 1.76	-1.09	0.60	83.97 ± 2.14	79.94 ± 2.15	-4.03	0.11
bST cost	210.6 ± 24.5	211.3 ± 24.5	0.7	0.19	209.5 ± 26.9	208.0 ± 26.9	-1.5	0.21
Other operating expenses	1,512	1,512	-	-	1,512	1,512	-	-
Cash flow	2,218 ± 119	2,356 ± 116	138	0.08	3,167 ± 141	3,056 ± 141	-111	0.28
Cash flow per day	4.11 ± 0.22	4.36 ± 0.22	0.25	0.08	5.87 ± 0.26	5.66 ± 0.26	-0.21	0.28

[1]VWP60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]VWP88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]Diff = difference between VWP88 and VWP60 (Diff = VWP88-VWP60).
[4]The effect of season of calving is described in the text.

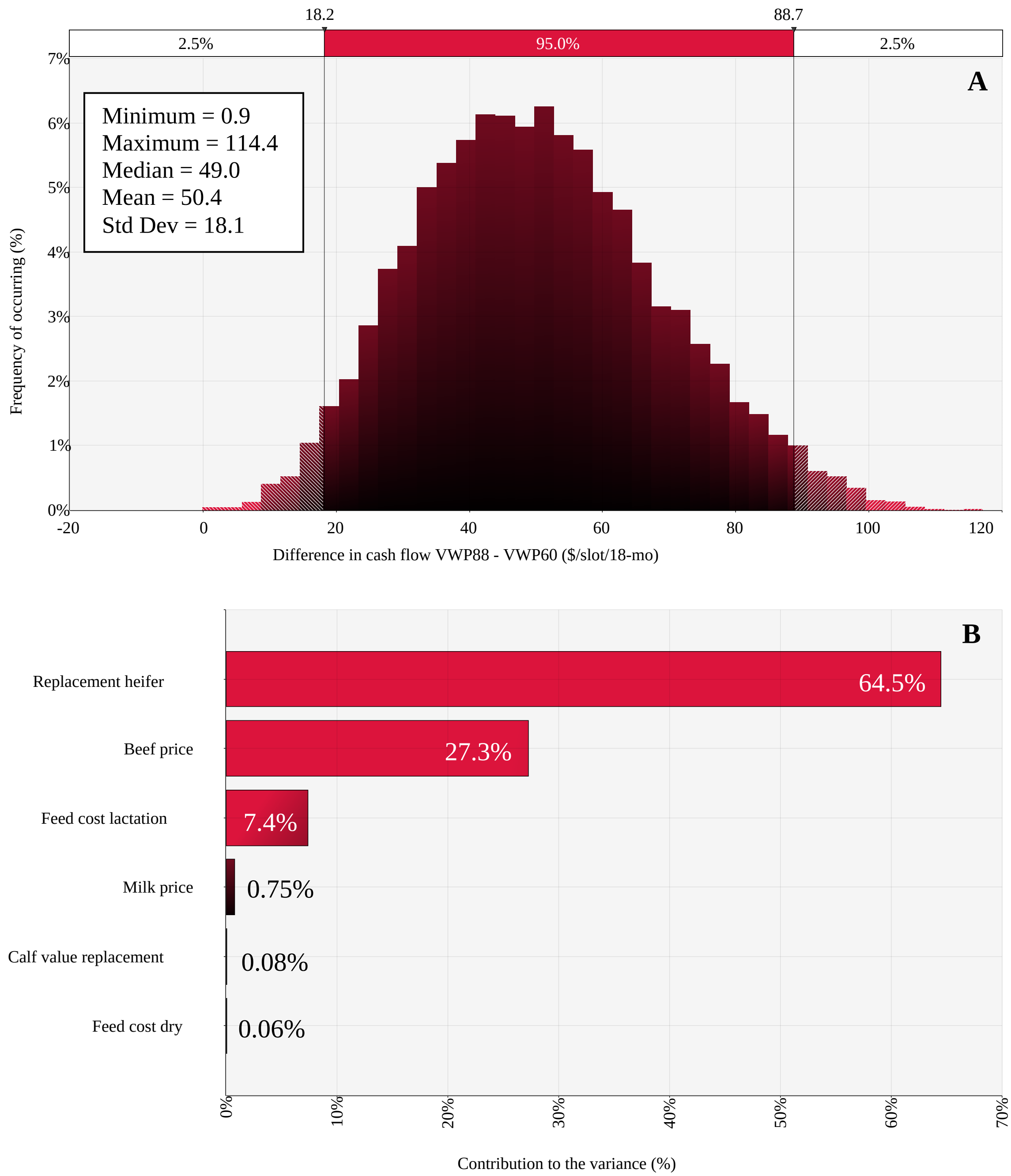

Figure 1. (A) Relative frequency distribution for differences in cash flow ($/slot per18 mo) for primiparous cows in the VWP88 and VWP60 treatments for 10,000 iterations of stochastic simulation. Differences in cash flow were calculated by subtracting cash flow for the VWP60 from the VWP88 treatment. (B)Tornado graph depicting the contribution to variance in percentage for non-fixed variables included in the stochastic simulation model.

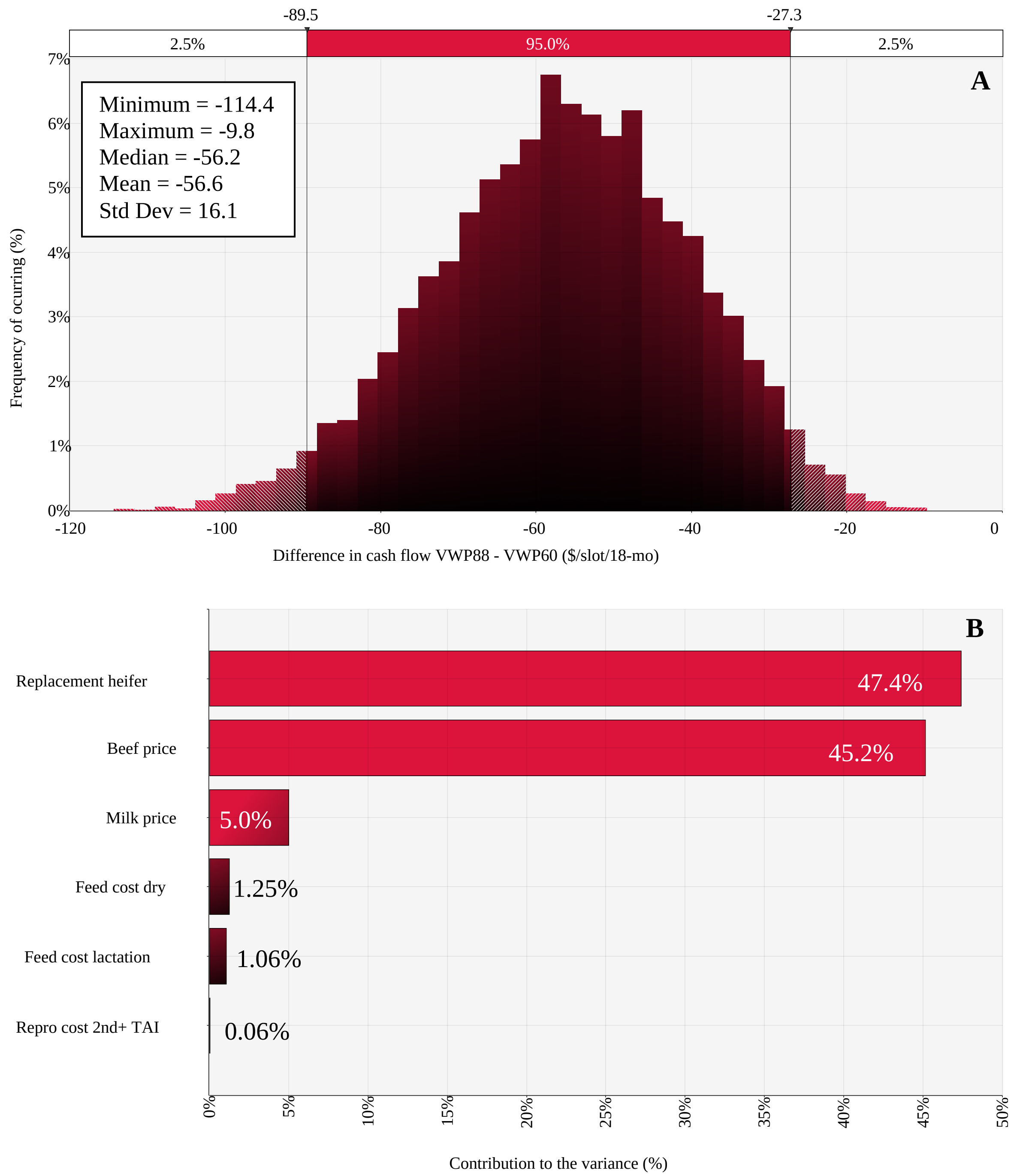

Figure 2. (A) Relative frequency distribution for differences in cash flow ($/slot per18 mo) for multiparous cows in the VWP88 and VWP60 treatments for 10,000 iterations of stochastic simulation. Differences in cash flow were calculated by subtracting cash flow for the VWP60 from the VWP88 treatment. (A)Tornado graph depicting the contribution to variance in percentage for non-fixed variables included in the stochastic simulation model.

Supplemental Table S1. Model fixed inputs to estimate cash flow for 18 mo after calving in the experimental lactation[1]

Item	Primiparous		Multiparous	
	VWP60	VWP88	VWP60	VWP88
Milk production (kg/slot)	18,115	18,227	20,166	20,069
DMI milking period (kg/slot)	12,226	12,303	13,165	13,191
DMI dry period (kg/slot)	611	603	557	520
Cows calved during the study (%)	80.2	82.0	63.2	61.0
Cows sold (%)	27.3	23.4	48.9	53.8
Body weight of cows sold (kg)	671	678	696	699
Cows dead (%)	5.0	3.4	10.8	11.2
Number of 1st services per slot[2] (n)	2.0	1.9	3.0	2.9
Number of 2nd and greater EDAI services per slot[3] (n)	1.2	1.2	2.2	2.2
Number of 2nd and greater TAI servicies per slot[4] (n)	1.1	1.0	2.1	1.7
Number of rbST doses per slot (n)	28.9	29.6	29.5	30.4
Other operating costs per slot ($)	1512	1512	1512	1512

[1]Reproductive performance, production outcomes, and data for herd exit dynamics from our experiment were used as fixed inputs.
[2]Number of first services per slot accounting for experimental lactation, subsequent lactation (if any), and replacement heifer (if any).
[3]Number of 2nd and greater EDAI services per slot accounting for experimental lactation, subsequent lactation (if any), and replacement heifer (if any).
[4]Number of 2nd and greater TAI services per slot accounting for experimental lactation, subsequent lactation (if any), and replacement heifer (if any).

Supplemental Table S2. Model stochastic inputs to estimate cash flow for 18 mo after calving in the experimental lactation.

Item	Distribution[1]	Measure/parameters[2]
Milk price ($/kg)	Beta	$\alpha1$ = 2.27; $\alpha1$ = 6.12; min = 0.32; max = 0.58
Feed cost - lactating cow diet ($/kg)	Uniform	min = 0.21; max = 0.44
Feed cost - dry cow diet ($/kg)	Uniform	min = 0.16; max = 0.33
Calf value ($/newborn calf)	Pert	min = 108.7; most likely = 127.8; max = 147.0
Replacement heifer ($/heifer)	Triangular	min = 1,300; most likely = 1,350; max = 2,210
Beef price ($/kg)	Triangular	min = 0.9; most likely = 1.6; max = 2.4
Repro cost 1st service TAI ($/AI)	Pert	min = 22.0; most likely = 25.9; max = 29.7
Repro cost 2nd and greater EDAI ($/AI)	Pert	min = 11.7; most likely = 13.8; max = 15.8
Repro cost 2nd and greater TAI ($/AI)	Pert	min = 16.8; most likely = 19.8; max = 22.8
rbST price ($/dose)	Pert	min = 6.0; most likely = 7.0; max = 8.1

[1]Distribution of variable with historical data (milk price, feed cost, cost of a replacement heifer, and beef price) were fitted using @Risk's BestFit function. Input variables with a minimum, most likely and a maximum values (calf value, reproductive cost and rbST price), a pert distribution was fit.
[2]Parameters specified for each distribution fitted.

CHAPTER VIII

REPRODUCTIVE PERFORMANCE AND HERD EXIT DYNAMICS OF LACTATING DAIRY COWS MANAGED FOR FIRST SERVICE WITH THE PRESYNCH-OVSYNCH OR DOUBLE-OVSYNCH PROTOCOL AND DIFFERENT DURATION OF THE VOLUNTARY WAITING PERIOD

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ABSTRACT

The objective of this experiment was to evaluate the reproductive performance and herd exit dynamics of dairy cows managed for first service with programs varying in method of submission for insemination and voluntary waiting period (**VWP**) duration. Holstein cows from a commercial farm in New York were randomly allocated to receive timed AI (**TAI**) after the Double-Ovsynch protocol (GnRH, 7 d later PGF2α, 3 d later GnRH, 7 d later GnRH, 7 d later PGF2α, 56 h later GnRH, and 16 to 18 h later TAI) at 60 ± 3 DIM (**DO60** = 458), TAI after DO at 88 ± 3 DIM (**DO88** = 462), or a combination of AI at detected estrus (starting at 50 ± 3 DIM) and TAI with the Presynch-Ovsynch protocol (PGF2α, 14 d later PGF2α, 12 d later GnRH, 7 d later PGF2α, 56 h later GnRH, and 16 to 18 h later TAI; **PSOv** = 450). Subsequent AI services were conducted at detected estrus or the Ovsynch protocol (32 ± 3 d after AI GnRH, 7 d later PGF2α, 56 h later GnRH, and 16 to 18 h later TAI) for cows not re-inseminated at detected estrus. In a subgroup of cows, cyclicity (based on P4 concentration), uterine health (vaginal discharge and uterine cytology) and BCS were evaluated at baseline (DO60 and DO88 = 33±3

DIM; PSOv = 34±3 DIM), beginning of the synchronization protocol (DO60 = 33±3 DIM; DO88 = 61±3 DIM; PSOv = 34±3 DIM); and -5 (PSOv) or -10 d (DO) of the VWP end (DO60 = 50±3 DIM; DO88 = 78±3 DIM; PSOv = 45±3 DIM). Effects of treatments were assessed using SAS with multivariable statistical methods relevant for each outcome variable. Cows in the DO88 treatment had delayed time to pregnancy during lactation (hazard ratio: DO60 vs. DO88 = 1.53, 95% CI: 1.32 to 1.78; PSOv vs. DO88 = 1.37, 95% CI: 1.19 to 1.61) and, within multiparous cows, the DO88 and PSOv treatments had greater risk of leaving the herd than cows in the DO60 treatment (hazard ratio: DO88 vs. DO60 = 1.49, 95% CI: 1.11 to 2.00; PSOv vs. DO60 = 1.39, 95% CI: 1.03 to 1.85). Cows in the DO88 treatment had improved uterine health, greater BCS, and reduced incidence of anovulation than cows in DO60 and PSOv, however, overall pregnancy per AI (**P/AI**) 39 ± 3 d after AI was similar for the three treatment groups. In summary, reproductive management strategies that led to similar average DIM to the first service (~60 d) through a combination of AI at estrus with TAI (PSOv) or all TAI (DO60) resulted in reduced time to pregnancy after calving when compared with an all TAI program (DO88) with a VWP of 88 d. Within the multiparous cow group, those that received all TAI with a VWP duration of 60 d were less likely to leave the herd than cows in the other treatments.

Keywords: voluntary waiting period, Double-Ovsynch, Presynch-Ovsynch, reproductive performance, dairy cow.

INTRODUCTION

Reproductive management strategies incorporating synchronization of estrus and ovulation for first service have been widely adopted by the dairy industry in recent years

(Caraviello et al., 2006; Ferguson and Skidmore, 2013; Wiltbank and Pursley, 2014). These strategies reduce the number of and the variation for days to the first service (Pursley et al., 1997; Miller et al., 2007). More recently, fertility programs were developed to maximize pregnancy per AI (**P/AI**) by improving synchrony of ovulation and optimizing the endocrine environment before timed AI (**TAI**) (Moreira et al., 2001; Bello et al., 2006; Souza et al., 2008). Thus, incorporating synchronization of estrus and ovulation protocols for first service allows better control of the timing of pregnancy during lactation through immediate insemination after the end of the voluntary waiting period (**VWP**; Chebel and Santos, 2010; Machado et al., 2017), similar or greater P/AI than inseminations at detected estrus (Chebel and Santos, 2010; Fricke et al., 2014; Santos et al., 2017), or both.

Two of the most widely used strategies to submit lactating dairy cows for first service include: (1) combining insemination after detection of estrus and TAI and (2) submission of all cows for TAI (Caraviello et al., 2006; Ferguson and Skidmore, 2013; Scott, 2016). Among combined strategies, a common approach consists of synchronizing estrus so that a substantial proportion of cows receive AI immediately after the end of the VWP. Thereafter, cows not detected in estrus receive TAI through synchronization of ovulation. Indeed, PGF2α-based presynchronization protocols such as Presynch-Ovsynch are used by many dairy farms because up to ~ 50 to 70% of cows may be inseminated at estrus after the PGF2α treatments of the protocol (Chebel et al., 2006; Fricke et al., 2014; Giordano et al., 2016). This strategy drastically reduces the number of cows submitted to TAI thereby, the number of hormonal treatments and associated labor. A caveat of combining AI at estrus with TAI through the Presynch-Ovsynch protocol is the wide range of DIM to the first service and the fact that P/AI may be reduced as compared with using all TAI (Gumen et al., 2012; Fricke et al., 2014; Borchardt et al., 2016). In contrast, GnRH-based presynchronization protocols such as Double-Ovsynch may be more suitable for all TAI. These protocols do not promote estrus expression but through resolution of anovulation in most cows (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013) and improved synchrony of ovulation increase first service P/AI when compared with PGF2α-based presynchronization protocols such as Presynch-Ovsynch, especially in primiparous cows (Souza et al., 2008; Herlihy et al., 2012; Borchardt et al., 2017). Thus, for farms aiming to reduce variation for days to the first service while maximizing P/AI, a GnRH-based protocol such as Double-Ovsynch may be superior to a combined program using a PGF2α-based protocol like Presynch-Ovsynch.

Several experiments compared P/AI and whole lactation reproductive performance after submission to the first service with combined or all TAI programs (Chebel and Santos, 2010; Fricke et al., 2014; Machado et al., 2017). Although differences in P/AI were reported, time to pregnancy during lactation was similar for both strategies. In all cases, however, cows received all TAI after synchronization with the Presynch-Ovsynch protocol which does not maximize P/AI to the first service, in particular for primiparous cows (Souza et al., 2008; Herlihy et al., 2012; Borchardt et al., 2017). Therefore, it remains to be determined whether using a GnRH-based protocol like Double-Ovsynch, which is known to lead to greater first service P/AI than PGF2α-based protocols, results in better reproductive performance than a combined approach (i.e., at estrus and TAI) to submit cows to first service such as the Presynch-Ovsynch protocol.

Another important aspect of first service management strategies that may affect time to pregnancy during lactation is duration of the VWP. Extra days of VWP may help reduce the proportion of cows with uterine disease around the time of insemination (Gautam et al., 2009; Sheldon et al., 2009; LeBlanc, 2014), allow more cows to resume hormone secretion patterns that promote ovulation and resumption of ovarian cyclicity (Butler, 2003; Kawashima et al., 2012; Cheong et al., 2015), and provide more time for cows to recover BCS, all of which have been strongly associated with positive first service outcomes (Souza et al., 2008; Herlihy et al., 2012; Carvalho et al., 2014a). The potential benefits of extending the VWP on first service P/AI may be more easily realized with all TAI programs using GnRH-based synchronization of ovulation protocols because of the narrow range of DIM to the first service and the potential to obtain P/AI in the 45 to 55% range (Souza et al., 2008; Giordano et al., 2013; Carvalho et al., 2014b). Nevertheless, it is unknown if the expected increase in P/AI to the first service obtained by extending the VWP would compensate for the additional pregnancies generated earlier using programs with shorter VWP.

Thus, we hypothesized that cows managed with shorter VWP and two different methods of submission for first service (i.e., combination of insemination at estrus and TAI or all TAI) would have reduced time to pregnancy after calving in spite of reduced P/AI to the first service. We expected that earlier re-insemination of cows with shorter VWP would compensate for the increment in P/AI in cows with longer VWP. Therefore, we conducted an experiment with the main objective of investigating the effect of submitting lactating dairy cows for first service with three different management strategies on time to pregnancy and herd exit dynamics. Cows received first service after detection of estrus or TAI during the Presynch-Ovsynch protocol with VWP of 50 ± 3 DIM or received TAI after the Double-Ovsynch protocol with VWP of 60 ± 3 or 88 ± 3 DIM. Secondary objectives included determination of cyclicity status, uterine health, and BCS before first service.

MATERIALS AND METHODS

All procedures performed with cows were approved by the Animal Care and Use Committee of Cornell University.

Animals and Management

Lactating Holstein cows (n = 1,370) from a commercial farm in New York State (Cayuga County) were enrolled in this experiment from March, 2014 to December, 2014. Cows were housed in free-stall barns with six rows of stalls, concrete flooring, fans and sprinklers in the feedline, and self-locking headgates in the feedline. Stalls surfaces were covered with either deep sand bedding or mattresses covered with sawdust. Cows assigned to the three management strategies evaluated were commingled during the experiment. Cows were milked thrice daily at ~8 h intervals and received recombinant bovine somatotropin (**rbST**; Sometribove zinc, Posilac, Elanco Animal Health, Indianapolis, IN) following a 10 and 11 d schedule beginning at 110 DIM until dry-off. During the experiment average number of milking cows in the herd was 1,417 with milk production of 40 kg/day (data obtained with the ECON\ID command in DairyComp305, ValleyAg Software, Tulare, CA).

Experimental Design

The experiment followed a complete randomized block design with parity (i.e., primiparous vs. multiparous) as the blocking factor. At 7 ± 3 DIM, cows were blocked by parity and stratified based on milk production in the previous lactation (multiparous cows only). Thereafter, cows were randomly allocated to receive first service TAI after the Double-Ovsynch protocol (**DO**; GnRH,7 d later PGF2α, 3 d later GnRH, 7 d later GnRH, 7 d later PGF2α, 56 h later GnRH, and 16 to 18 h later TAI) at 60 ± 3 DIM (**DO60** = 458), TAI after DO at 88 ± 3 DIM (**DO88** = 462), or a combination of insemination after detected estrus and TAI with the Presynch-Ovsynch protocol (**PSOv** = 450; PGF2α, 14 d later PGF2α, 12 d later GnRH, 7 d later PGF2α, 56 h later GnRH, and 16 to 18 h later TAI). Cows in the PSOv treatment received AI at detected estrus (**EDAI**) after the second PGF2α treatment given at 50 ± 3 DIM. Cows not inseminated at estrus that completed the Presynch-Ovsynch protocol received TAI at 72 ± 3 DIM (Figure 1). Cows failing to conceive to an insemination were re-inseminated after detection of estrus through a combination of physical activity monitoring using neck-mounted physical activity monitoring tags (DeLaval Activity Meter System, DeLaval International AB, Tumba, Sweden) and visual observation by farm personnel. Every morning, the dairy herd management software (DairyComp305) generated a list of cows for insemination based on estrus alerts generated by the activity monitoring system. Cows not re-inseminated at estrus and confirmed non-pregnant 39 ± 3 d after a previous AI service received TAI after resynchronization of ovulation with the Ovsynch protocol (GnRH-7 d-PGF2α-56 h-GnRH-16 to 18 h-TAI) initiated 32 ± 3 d after AI (7 d before non-pregnancy diagnosis).

For all synchronization of ovulation protocols GnRH treatments consisted of 100 µg of Gonadorelin diacetate tetrahydrate given i.m. (Fertagyl, Merck Animal Health, Madison, NJ), whereas PGF2α treatments consisted of 500 µg of Cloprostenol sodium given i.m. (Estrumate, Merck Animal Health, Submitt, NJ).

Cows that were sold, died, or were classified as do not breed by farm personnel before 30 DIM were excluded from the experiment. Cows that received first AI outside the DIM range specified for their respective treatment were also excluded. A total of 68 cows (DO60, n = 22;

DO88, n = 33; PSOV, n = 13) were excluded. Therefore, the total number of cows with data available after 30 DIM for each treatment was 436 for DO60, 429 for DO88, and 437 for PSOv.

Blood Sampling and Determination of Circulating Progesterone Concentrations

Blood samples were collected from a subgroup of cows (n = 463; DO60 = 165, DO88 = 142, and PSOv = 156) by puncture of the coccygeal vein or artery using 8-mL heparinized evacuated tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were immediately placed into crushed ice until transported to the laboratory. Samples were centrifuged at 2,000 × *g* for 20 min at 4 °C in a refrigerated centrifuge. Plasma was harvested and transferred into 3 storage tubes and stored at -20 °C until assayed.

The sampling schedule is presented in Figure 1. Samples were collected at different time points to represent the following stages for each treatment: baseline (26 ± 3 and 33 ± 3 DIM for DO60 and DO88, and 27 ± 3 and 34 ± 3 DIM for PSOv), beginning of the synchronization protocol (26 ± 3 and 33 ± 3 DIM for DO60; 54 ± 3 and 61 ± 3 DIM for DO88; 27 ± 3 and 34 ± 3 DIM for PSOv); and finally -5 (i.e., for Presynch-Ovsynch) or -10 d (i.e., for Double-Ovsynch) of the VWP end (50 ± 3 DIM DO60; 78 ± 3 DIM for DO88; 45 ± 3 DIM for PSOv). At baseline and the beginning of the synchronization protocols cows were classified as cyclic if at least one of the samples collected seven days apart had a P4 concentration ≥ 1 ng/mL. Minor adjustments to sampling time points (e.g., baseline at 27 and 34 DIM instead of 26 and 33 DIM) were necessary for the PSOv treatment because of the different days of the week at which cows received hormonal treatments.

Plasma progesterone (**P4**) concentrations were determined in duplicates using a commercial solid-phase, no-extraction radioimmunoassay (ImmuChem Coated Tube, MP

Biomedicals, Costa Mesa, CA). Control samples with low (0.3 ng/mL) and high (5.8 ng/mL) P4 concentration were included at the beginning and end of each assay (n = 4 assays) to assess precision. Average detection limit for all the assays was 0.1 ng/mL. Average intra-assay CV for the low-concentration sample was 11.4% whereas the inter-assay CV was 19.9%. For the high-concentration sample the average intra-assay CV was 5.6% whereas the inter-assay CV was 7.9%. The presence of a functional CL was defined as circulating P4 concentration ≥ 1 ng/mL.

Body Condition Scoring

Body condition score was recorded every time a blood sample was collected. A scale of 1 (emaciated) to 5 (fat) with increments of 0.25 was used (Edmonson et al., 1989). Data for BCS were dichotomized using a threshold of 2.75 units (high ≥ 2.75, low < 2.75).

Uterine Health Examinations

Uterine health status was determined by evaluation of vaginal discharge and uterine cytology samples at baseline (DO60 and DO88 = 33 ± 3 DIM; PSOv = 34 ± 3 DIM), beginning of the synchronization protocol (DO60 = 33 ± 3 DIM; DO88 = 61 ± 3 DIM; PSOv = 34 ± 3 DIM); and -5 (i.e., for Presynch-Ovsynch) or -10 d (i.e., for Double-Ovsynch) of the VWP end (DO60 = 50 ± 3 DIM; DO88 = 78 ± 3 DIM; PSOv = 45 ± 3 DIM). Discharge present in the vagina was exteriorized using the Metricheck device and classified according to McDougall et al. (2007) on a scale of 0 to 5 (0 = no discharge, 1 = clear mucus, 2 = clear mucus with flecks of pus, 3 = mucopurulent but < 50% pus, 4 = mucopurulent with > 50% pus, and 5 = foul-smelling discharge). For subsequent analysis, vaginal discharge score was dichotomized, defining purulent vaginal discharge (**PVD**) as a Metricheck score > 2 (LeBlanc et al., 2002).

The percentage of polymorphonuclear cells (**PMN**) present in uterine cytology samples collected through the cytobrush technique was determined as described by Madoz et al. (2013). A stainless steel gun attached to a sterile brush (Medscand Cytobrush Plus GT, CooperSurgical Inc., Trumbull, CT) was used to collect samples. Once collected, samples were air-dried and stained using the Dip Quick kit (Jorgensen Laboratories Inc, Loveland, CO). All slides were evaluated at 400X magnification by a single observer. Percentage PMN was calculated by averaging two counts of 100 cells each from two different locations on each slide. If the difference between the two counts was greater than 10 percentage points, a third count was performed. The final percentage was calculated as the average of the three counts.

Pregnancy Testing

Pregnancy testing was conducted by transrectal ultrasonography (Ibex Pro, Loveland, CO) of the reproductive tract 39 ± 3 d after AI. Reconfirmation of pregnancy after the initial examination was performed by transrectal ultrasonography 95 ± 3 d after AI. A cow was considered to have undergone pregnancy loss after the initial examination if found nonpregnant at reconfirmation or if re-inseminated at estrus before reconfirmation of pregnancy.

Daily Milk Production

Monthly milk test data retrieved from the dairy management software (DairyComp305) was used to calculate daily milk production for every cow in the study using the MilkBot® model (Ehrlich, 2011). This model estimated four parameters (scale, ramp, offset, and decay), that in combination with DIM allowed predicting daily milk production. Milk production tertiles (low, medium, and high) were estimated for primiparous and multiparous cows enrolled in the experiment using accumulated milk production up to 30 DIM (**MK30**). Thresholds for MK30 tertiles were as follow: primiparous (low ≤ 747 kg; medium = 748 to 866 kg; high ≥ 867 kg); multiparous (low ≤ 1,061 kg; medium = 1,062 to 1,217 kg; and high ≥ 1,218 kg)

Statistical Analysis

Sample size estimations were conducted using the sample size calculation option of WinPepi version 11.54 (Abramson, 2011). A total of 374 cows per group were needed to detect a hazard ratio for pregnancy of 1.25, assuming a proportion of non-pregnant cows (probability of survival) at 350 DIM of 15%, probability of type I error rate of 5%, and a power of 80%. Additional cows (~20%) were enrolled in the experiment to compensate for cow losses beyond the control of the researchers.

Statistical analyses for binary outcomes [i.e., P/AI, pregnancy loss, proportion of cows AI at estrus, proportion of cows sold, died, or left the herd (sold plus dead), proportion of cows with P4 ≥ 1 ng/mL, PVD, and BCS ≥ 2.75] were performed using logistic regression with the GLIMMIX procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). For quantitative outcomes (i.e., inter-service interval) ANOVA was performed with the MIXED procedure of SAS. Assumptions of normality of residuals, linear relationship, and homoscedasticity of variance were tested by evaluating the normal probability plot (normal Q-Q plot) and plotting residuals versus predicted values. Poisson regression with the GENMOD procedure of SAS was used to analyze count data (i.e., PMN number and total number of inseminations). Goodness of fit of all models was evaluated with the Pearson Chi-Square test. Cox's proportional hazards regression with the PHREG procedure of SAS was used for the analysis of time to event data (i.e., time to pregnancy and herd exit). Cows were right censored if they left the herd due to sale or death before 350 DIM and if not pregnant at 350 DIM for the analysis of time to pregnancy and time to herd exit after calving. Likewise, cows were right censored if left the herd due to sale or death or were not pregnant by 250 d after the end of the VWP for the analysis of time to pregnancy after the end of the VWP. The proportional hazard assumption for time to event data analysis was evaluated by graphical examination of the log(-log(survival probability)) function obtained from the PROC LIFETEST of SAS. According to this analysis, the assumption of proportional hazards was met for all models. Kaplan-Meier survival curves generated with the survival analysis option of MedCalc (version 17.2; MedCalc Software bvba, Ostend, Belgium) were used to illustrate time to pregnancy and herd exit. The proportion of cows pregnant at 91 DIM, when all cows received at least one insemination (i.e., end of DIM range to the first service in the DO88 treatment), was calculated including all cows enrolled in the study by 30 DIM (similar to survival analysis for time to pregnancy after calving) and analyzed using logistic regression with the GLIMMIX procedure of SAS.

Treatment (DO60, DO88, and PSOv), parity (primiparous vs. multiparous), and the treatment by parity interaction were offered as explanatory variables to all models. For outcomes related to first AI, the effect of MK30 (low, medium, and high), season of insemination (cold: September 21 to June 20; warm: June 21 to September 20), treatment by MK30 interaction, and treatment by season interaction were also offered to the models. Season of first insemination was not included in the models to evaluate cyclicity status, uterine health, body condition score, and time to event outcomes. The final model for each outcome of interest was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Aikaike Information Criterion. Treatment and parity were forced in all models. When appropriate, the Least Significant Different (**LSD**) post hoc mean separation test was used to determine differences between Least Square Means (**LSM**).

All proportions reported are LSM generated using the LSMEANS option of PROC GLIMMIX of SAS, whereas quantitative outcomes were reported as LSM ± SEM. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, while P-values > 0.05 and ≤ 0.10 were considered a tendency.

RESULTS

Time to Pregnancy after Calving and after the End of the VWP

The hazard of pregnancy after calving was affected by treatment ($P < 0.01$), whereby it was greater for the DO60 than DO88 treatment (HR 1.53, 95% CI: 1.32 to 1.78; Figure 2), and for the PSOv than DO88 treatment (HR 1.37, 95% CI: 1.19 to 1.61; Figure 2). No differences were observed between the DO60 and PSOv treatments (HR 1.12, 95% CI: 0.96 to 1.30). Median and mean days to pregnancy were 90 and 123 for DO60, 116 and 150 for DO88, and 96 and 126 for PSOv. The proportion of cows pregnant at 91 DIM, when all cows received at least one service, was also greater ($P < 0.01$) for the DO60 (51.0%; n = 436) and PSOv (48.3%; n = 437) than for the DO88 (39.1%; n = 429) treatment. Primiparous cows had greater ($P < 0.01$) hazard of pregnancy than multiparous cows (HR 1.52, 95% CI: 1.33 to 1.72). Median and mean days to pregnancy were 89 and 116 for primiparous cows and 112 and 146 for multiparous cows. The hazard of pregnancy was not affected by MK30 ($P = 0.18$), and there was not a treatment by parity interaction ($P = 0.92$), or a treatment by MK30 interaction ($P = 0.58$). At 350 DIM, the proportion of nonpregnant cows was similar ($P = 0.19$) for the three treatments (DO60 = 8.2%, DO88 = 11.4%, PSOv = 11.7%).

When the hazard of pregnancy was calculated after the end of the VWP for each management strategy, there was also an effect of treatment (*P* = 0.01; Figure 3). In this case, however, cows in the DO60 treatment had greater hazard of pregnancy than cows in PSOv (HR 1.25, 95% CI: 1.08 to 1.45) and no differences were observed between the DO60 and DO88 treatments (HR 1.04, 95% CI: 0.89 to 1.20). The hazard of pregnancy was also greater for the DO88 than the PSOv treatment (HR 1.20, 95% CI: 1.03 to 1.40). Median and mean days from the end of the VWP to pregnancy were 28 and 63 for DO60, 27 and 62 for DO88, and 46 and 76 for PSOv. There was no effect of MK30 (*P* = 0.26), and no treatment by parity (*P* = 0.43) or treatment by MK30 interaction (*P* = 0.27) for the hazard of pregnancy after the end of the VWP.

Pregnancies per Artificial Insemination and Pregnancy Loss after First AI

Pregnancies per AI 39 ± 3 d after first service were similar (*P* = 0.12) when the three treatments were compared (Table 1), but were greater (*P* < 0.01) for primiparous (49.8%; n = 481) than multiparous (35.2%; n = 747) cows. For the PSOv treatment, 65% of the cows received AI at estrus after the second PGF2α treatment of Presynch-Ovsynch whereas the rest of the cows received TAI at 72 ± 3 DIM. Average DIM at first service for all cows in PSOv was 61 d (56 d for cows inseminated at estrus and 72 d for cows that received TAI). There was ~7 percentage points difference in P/AI between cows inseminated at estrus (34.8%, n = 270) and cows that received TAI (41.4%, n = 145) within the PSOv group, but it was not statistically significant (*P* = 0.19). There was no effect of MK30 (*P* = 0.65) or season (*P* = 0.79), and no treatment by parity (*P* = 0.70), treatment by MK30 (*P* = 0.58), or treatment by season interaction (*P* = 0.57) on P/AI.

At the time of reconfirmation of pregnancy 95 ± 3 d after AI, there was no effect (P = 0.14; Table 1) of treatment on P/AI but there was an effect of parity (P < 0.01), whereby primiparous cows (46.4%; n = 476) had greater P/AI than multiparous cows (31.5%; n = 737). There was no effect of MK30 (P = 0.11) and season (P = 0.36), and there was not a treatment by parity (P = 0.48), treatment by MK30 (P = 0.85), or treatment by season interaction (P = 0.61) for P/AI 95 ± 3 d after AI.

Pregnancy loss was not affected by treatment (P = 0.76) or parity (P = 0.33) and there was no treatment by parity interaction (P = 0.59; Table 1). In addition, pregnancy loss was not affected by MK30 or season, and there was not a treatment by MK30 or treatment by season interaction (P > 0.10).

Reproductive Outcomes for Second and Greater Inseminations

The proportion of cows inseminated at estrus for second and greater AI was greater (P < 0.01) for cows in the PSOv than the DO60 and DO88 treatments (Table 2), and for multiparous (75.2%; n = 1,301) than primiparous (70.3%; n = 563) cows (P = 0.03). The inter-service interval was similar (P = 0.69; Table 2) among treatments, and the overall P/AI for inseminations at estrus and TAI combined were not affected by treatment (P = 0.16; Table 2), but were affected by parity (P < 0.01) because primiparous cows had greater P/AI (38.3%; n = 563) than multiparous cows (28.3%; n = 1,301). Finally, the average total number of AI services up to 350 DIM was similar between treatments (P = 0.87; Table 2) but, it was greater (P < 0.01) for multiparous than primiparous cows (2.9 vs. 2.3 inseminations, respectively).

Cyclicity Status, Uterine Health, and Body Condition Score before First Service

Cyclicity status, uterine health, and body condition score before first service are presented in Table 3. The proportion of cyclic cows at baseline tended (*P* = 0.08) to be greater for primiparous (75.9%; n = 172) than multiparous (68.0%; n = 291) cows. In addition, there was an effect of MK30, whereby the proportion of cows cyclic was greater (*P* = 0.02) for the low (74.6%; n = 155) and medium (77.2%; n = 154) than for the high (63.7%; n = 154) MK30 group. When compared at the beginning of the synchronization protocol for each treatment, there was a greater (*P* < 0.01) proportion of cyclic cows in the DO88 than in for the DO60 and PSOv treatments (Table 3). A tendency (P = 0.07) for an effect of parity was also detected [primiparous = 81.7% (n = 172); multiparous = 74.6 % (n = 291)]. No effect of MK30 (*P* = 0.14) and no treatment by parity interaction (*P* = 0.97) was observed at this time-point. At -5 or -10 d before the end of the VWP, the proportion of cyclic cows was greater for the DO60 and DO88 than the PSOv treatment, but no effect of parity (*P* = 0.34) or MK30 (*P* = 0.21) was observed. Also, there was not a treatment by parity interaction (*P* = 0.57).

There was no effect of treatment, parity, or MK30, and no treatment by parity interaction for the proportion of cows with PVD at any of the time points evaluated (*P* > 0.10; Table 3).

The PMN percentage observed in uterine cytology samples was similar between treatments at the beginning of the synchronization protocol for each treatment (*P* = 0.31), but it was less (*P* = 0.03) at -5 to -10 d before the end of the VWP end for the DO88 than the DO60 and PSOv treatments (Table 3). No effect of parity and MK30 was detected, and there was no treatment by parity interaction at any of the time-points analyzed (*P* > 0.10).

At baseline, the proportion of cows with BCS ≥ 2.75 was greater (*P* < 0.01) for primiparous (95.4%; n = 172) than multiparous (72.9%; n = 291) cows. At the beginning of the synchronization protocol, there was an effect of treatment (*P* = 0.02) and parity (*P* < 0.01), whereby the proportion of cows with BCS ≥ 2.75 was greater for cows in the DO88 than in the DO60 and PSOv treatments (Table 3). A greater (*P* < 0.01) proportion of primiparous (96.4%; n = 172) than multiparous (78.7%; n = 291) cows had BCS ≥ 2.75. At -5 or -10 d before the end of the VWP, the proportion of cows with BCS ≥ 2.75 was greater (*P* = 0.03) for the DO88 than the PSOv treatment, whereas the DO60 treatment was similar to the other two treatments. There was also an effect of parity (*P* < 0.01) and MK30 (*P* = 0.01) on the proportion of cows with BCS ≥ 2.75. A greater proportion of primiparous (96.6%; n = /169) than multiparous cows (79.5%; n = 290), and a greater proportion of cows with low (95.4%; n = 236) than medium (89.3%; n = 233) and high (87.0%; n = 201) milk production had BCS ≥ 2.75.

Herd Exit Pattern

The effect of treatment on time to herd exit during the experiment was analyzed separately for each parity group because of the well-known differences in herd exit dynamics between primiparous and multiparous cows. In this experiment, the hazard of culling was, as expected, greater (*P* < 0.01) for multiparous than primiparous cows (HR = 2.41, 95% CI: 1.89 to 3.08). For primiparous cows, the hazard of culling was similar (*P* = 0.22) between treatments (DO60 vs. DO88 = HR 1.12, 95% CI: 0.64 to 1.96; DO60 vs. PSOv = HR 0.72, 95% CI: 0.43 to 1.20; DO88 vs. PSOv = 0.64, 95% CI: 0.38 to 1.09; Figure 4A). Mean days from calving to herd exit were 318 for DO60, 328 for DO88, and 323 for PSOv. For multiparous cows there was an effect of treatment (*P* = 0.02), whereby cows in DO88 and PSOv treatments had greater hazard of culling than cows in the DO60 treatment (DO88 vs. DO60 = HR 1.49, 95% CI: 1.11 to 2.00; PSOv vs. DO60 = HR 1.39, 95% CI: 1.03 to 1.85; Figure 4B). No difference was observed between the DO88 and PSOv treatments (HR 1.07, 95% CI: 0.82 to 1.41). Mean days from calving to exit from the herd were 299 for DO60, 294 for DO88, and 290 for PSOv. In both parity groups, milk production affected ($P < 0.01$) the hazard of culling because cows in the low MK30 group had greater hazard of culling than cows in the medium (HR 2.08, 95% CI: 1.62 to 2.67) and high (HR 2.17, 95% CI: 1.67 to 2.78) MK30 group (Figure 4C). Mean days from calving to herd exit were 285 for low MK30, 323 for medium MK30, and 320 for high MK30.

As expected, the proportion of cows sold was greater ($P < 0.01$) for multiparous (31.9%; n = 801) than primiparous (13.9%; n = 502) cows. For the primiparous group, the proportion of cows sold was similar between treatments ($P = 0.65$), but for the multiparous group it was greater ($P = 0.04$) for DO88 than DO60 (Table 4). No differences were observed for PSOv (31.6%) and the other two treatments. The proportion of cows that died did not differ within the primiparous ($P = 0.31$) and multiparous cow group ($P = 0.20$; Table 4). Consequently, the total proportion of cows that left the herd during the experiment (sold plus died) was similar among treatment groups within primiparous ($P = 0.36$), but it was greater for the DO88 than the DO60 treatment within the multiparous cow group (Table 4).

DISCUSSION

In this experiment, we investigated the effect of different management strategies for first service and VWP duration on time to pregnancy during lactation in dairy cows. The strategies compared, which represented typical programs for first service in commercial dairy farms (Caraviello et al., 2006; Ferguson and Skidmore, 2013; Wiltbank and Pursley, 2014), varied in method of submission to insemination, synchronization protocol, and VWP duration. Our data supported the hypothesis that submission of cows to the first service after a shorter VWP, either after all TAI (DO60) or a combination of insemination at estrus and TAI (PSOv) reduced time to pregnancy during lactation. Our results for time to pregnancy are in agreement with previous studies, which reported reduced time to pregnancy after calving in spite of lower P/AI to the first service in cows with shorter rather than extended VWP (Tenhagen et al., 2003; Gobikrushanth et al., 2014).

Results for time to pregnancy were consistent across parity groups regardless of differences in P/AI and hazard of pregnancy between parities. These data suggest that extending the VWP without a substantial gain in P/AI to compensate for pregnancies generated earlier in lactation when a shorter VWP program is used, would likely delay overall time to pregnancy. The effect of shorter VWP was also evident for cows in the PSOv treatment, which, in spite of a 7-percentage point numerically lower P/AI to the first service than cows in the DO88 treatment, had reduced time to pregnancy. Collectively, data from the current experiment suggest that earlier opportunities for re-insemination when cows are managed with a shorter VWP (i.e., 28 d for all TAI and 38 d for combined approach) compensate for an overall reduction in P/AI to the first service of up to 7-percentange points.

A 10 d shorter VWP for the combined strategy with PSOv resulted in similar time to pregnancy after calving to the DO60 treatment which entailed use of 100% TAI. This could be explained by the combined effect of (1) reasonable P/AI (i.e., 34.8%) for the 65% of cows inseminated at estrus after Presynch and (2) similar P/AI for TAI services after completion of Presynch-Ovsynch (i.e., 41.4%) and Double-Ovsynch at 60 DIM (i.e., 41.8%). These data suggest that submitting cows for insemination at estrus and TAI with the Presynch-Ovsynch protocol may result in similar reproductive performance to using all TAI with a program like Double-Ovsynch when average DIM at first service are the same (e.g., 60 d in our experiment).

Certainly, the success of combined programs may be more dependent upon the ability of farm personnel to accurately detect and inseminate cows in estrus and the success of cows that receive TAI. Poor detection of estrus results in greater average DIM to the first service because a majority of cows receive TAI at later DIM, whereas low P/AI increases the proportion of cows that need multiple inseminations at later DIM to conceive.

Unlike the results observed using calving as reference point, when time to pregnancy was evaluated after the end of the VWP, the hazard of pregnancy and survival curves reflected the benefit of all TAI. This finding was likely related to differences in the pattern of submission to the first service and resulting P/AI because, unlike all TAI programs, combining insemination of cows at estrus and TAI with Presynch-Ovsynch results in a wider range of days to the first service. Although up to 50% or more of the cows may be inseminated at estrus within a week of the VWP end, the rest of the cows receive TAI 20 to 24 d after the end of the VWP depending on the interval (i.e., 10 to 14 d) from Presynch to Ovsynch (Chebel et al., 2006; Stevenson et al., 2012; Giordano et al., 2016). Poor P/AI for inseminations at estrus are also problematic because cows not pregnant after first service at estrus have an additional delay to conception. Collectively, our results suggest that both shorter VWP and the greatest possible P/AI for inseminations at estrus are critical to achieve the same or better reproductive performance with programs that combine AI at estrus and TAI than all TAI programs. This is because the bimodal distribution of AI services generated with combined programs leads to a wider range of DIM at first service and the additional delay to achieve pregnancy caused by failure to conceive after first service at detected estrus.

As expected, a longer VWP allowed a greater proportion of cows in the DO88 treatment to resolve anovulation and have better body condition at the beginning of the synchronization protocol. Conversely, differences in cyclicity status and BCS at -5 or -10 d before the end of the VWP were only observed between the DO88 and the PSOv treatments. These differences or lack thereof were likely because of the well-known effect of GnRH-based protocols on reducing anovulation (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013) and because of the greater DIM difference at this time point between the DO88 and PSOv treatments which allowed cows in the DO88 treatment to recover more body condition than cows in PSOv. Cows in the DO88 treatment also had better uterine health (PMN percent only) immediately before the end of the VWP supporting the notion that uterine inflammation after calving is reduced over time (Gautam et al., 2009; Sheldon et al., 2009; LeBlanc, 2014; Vieira-Neto et al., 2014).

The detrimental effects of anovulation (Santos et al., 2004; Ribeiro et al., 2016; Bruinjé et al., 2017), poor uterine health (Gilbert et al., 2005; Barlund et al., 2008; Dubuc et al., 2010), and suboptimal BCS (Souza et al., 2007; Souza et al., 2008; Carvalho et al., 2014a) on first service P/AI have been extensively documented and were the basis for our expectation of greater P/AI for cows with 88 d VWP. Nevertheless, the gain in P/AI due to the extended VWP was insufficient to compensate for the greater number of pregnancies created up to 91 DIM (i.e., end DIM range to the first service in the DO88 treatment) by the programs with shorter VWP. Under the conditions of our current experiment, the overall proportion of pregnant cows after first service (accounting for cows that left the herd before first service and pregnancy losses) in the DO88 treatment should have been at least 9 to 11 percentage points greater to compensate for the longer VWP compared with the PSOv and DO60 groups, respectively. Achieving the P/AI needed to compensate for a 28 d extension of the VWP, as in our experiment, may be possible as fertility programs such as Double-Ovsynch continue evolving to further increase P/AI (Giordano et al., 2013; Carvalho et al., 2015; Wiltbank et al., 2015). In addition, other improvements in dairy herd management that may contribute to improved uterine health, earlier resumption of cyclicity, and recovery of body reserves may be used in combination with extended VWP and fertility programs to maximize P/AI to first service.

Our experiment was not designed to and was underpowered to detect statistically significant differences in P/AI to the first service as observed between the three treatments. Interestingly, P/AI was similar for the DO60 and DO88 treatments suggesting that the cyclicity status, uterine health (PMN percent only), and body condition score differences observed after extending the VWP from 60 to 88 DIM were insufficient to substantially increase P/AI. We speculate that the lack of difference was, at least in part, due to the high proportion of cows properly synchronized with Double-Ovsynch regardless of DIM at the beginning of the protocol and the resolution of anovulation in most cows treated with the Double-Ovsynch protocol (Souza et al., 2008; Herlihy et al., 2012; Ayres et al., 2013). Further, the differences in proportion of cows with poor uterine health (PMN percent only) between the DO60 and DO88 treatments and the effect of improved uterine health on P/AI were also insufficient to generate overall differences in P/AI. Interestingly, we observed a numerical difference of ~6-percentage points ($P = 0.38$) for P/AI in favor of the DO88 treatment for primiparous cows but similar P/AI for multiparous (data not shown) suggesting that only primiparous cows may benefit from the VWP extension. Although data for primiparous cows in this experiment was underpowered to detect a statistical difference in spite of a 6-percentage point difference in P/AI, it is an agreement with a larger data set for an experiment conducted in three commercial farms in which we observed a statistically significant increase in P/AI for primiparous (DO60 = 46.2% and DO88 = 55.0%) but not multiparous cows with the extended VWP (DO60 = 36.2% and DO88 = 40.1%; Stangaferro et al., 2017). Further investigation is necessary to elucidate the effect of parity on P/AI after extending the VWP in lactating dairy cows.

Differences in rate of pregnancy establishment during lactation not only affect the hazard of pregnancy, but also the herd exit dynamics because pregnancy status along with milk production level and number of lactations (data from this experiment) are major drivers of culling in dairy herds (Rajala-Schultz and Gröhn, 1999; De Vries et al., 2010; Pinedo et al., 2010). Cows with fewer lactations, pregnant cows, and cows with higher milk production level are less likely to be removed from a herd. In our experiment, this was evident as we observed differences in the herd exit dynamics among treatments for multiparous cows only. Cows in the DO88 and PSOv treatments had a greater hazard of herd exit than cows in the DO60 treatment and the proportion of cows sold was greater for multiparous cows in the DO88 than the DO60 treatment. At least in part, these differences were explained by the effect of extended VWP on pregnancy establishment and herd exit dynamics because extending the VWP resulted in delayed pregnancy. Nevertheless, the effect of treatments on timing of pregnancy cannot fully explain our observations for multiparous cows in the PSOv treatment because the hazard of pregnancy after calving in these cows was similar to cows in the DO60 treatment.

The effect of time to pregnancy during lactation on individual cow and dairy herd profitability is affected by a complex interplay of several factors including but not limited to income over feed cost, reproductive management costs, fixed operating costs, and replacement costs (De Vries, 2006; Inchaisri et al., 2011; Giordano et al., 2012). Moreover, these parameters are affected by multiple others factors that should be accounted for when considering the effects of first service management strategies on profitability. Therefore, the observed differences in reproductive performance and herd exit dynamics reported in this experiment likely had a complex effect on profitability that was not evaluated in sufficient detail to make inferences about economic differences among the strategies compared. A detailed evaluation of the effects on profitability of the management strategies compared will be presented in a separate manuscript.

CONCLUSION

Data from the present experiment demonstrated that reproductive management strategies that resulted in average DIM to the first service of ~60 d through a combination of AI at estrus with TAI (PSOv) or all TAI (DO60) resulted in same hazard of pregnancy and overall time to pregnancy after calving. These two strategies; however, reduced time to pregnancy during lactation when compared with an all TAI program (DO88) with a VWP of 88 d. Most of the reduction in time to pregnancy for the groups with shorter VWP was explained by earlier opportunities for re-insemination. The observed improvement in cyclicity status, uterine health (PMN percent only), and BCS for cows with 88 d VWP was insufficient to generate the number of pregnancies at first service needed to compensate for delayed first insemination.

Beyond time to pregnancy, the first service management strategies tested in this experiment led to differences in the hazard of herd exit which were also affected by parity and milk production level. Cows with low milk production level regardless of treatment were more likely to leave the herd, and within the multiparous cow group, cows that received all TAI with a VWP duration of 60 d were less likely to leave the herd than cows in the other treatments. Therefore, data from this experiment suggest that the effect of first service management strategies on dairy herd performance depends upon complex interactions between the pattern of insemination, pregnancy per AI, and herd exit dynamics, all of which may vary for primiparous and multiparous cows.

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Table 1. Pregnancies per AI and pregnancy loss for lactating dairy cows managed for first service with the Double-Ovsynch or Presynch-Ovsynch protocol and different duration of the voluntary waiting period.

	DO60[1]	DO88[2]	PSOv[3]	*P*-value[4]		
		% (n)		Trt	Parity	T x P[5]
P/AI[6] 39 ± 3 d after AI	43.3 (416)	45.5 (397)	38.4 (415)	0.12	<0.01	0.70
P/AI 95 ± 3 d after AI	40.0 (410)	41.3 (392)	34.7 (411)	0.14	<0.01	0.48
Pregnancy loss	5.9 (168)	7.1 (170)	8.0 (150)	0.76	0.33	0.59

[1]DO60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]DO88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]PSOv = first service at detected estrus after the second PGF2α treatment of Presynch-Ovsynch given at 50 ± 3 DIM or TAI at 72 ± 3 DIM after completion of the Presynch-Ovsynch protocol.
[4]Effect of additional explanatory variables are described in the text.
[5]T x P = treatment by parity interaction.
[6]P/AI = pregnancies per artificial insemination.

Table 2. Effect of first service with the Double-Ovsynch or Presynch-Ovsynch protocol and different duration of the voluntary waiting period on pregnancy per AI after second and greater AI services, interval between AI services, and total number of inseminations up to 350 DIM.

	DO60[1]	DO88[2]	PSOv[3]	*P*-value[4]		
		% (n)		Trt	Parity	T x P[5]
EDAI[6]	67.9[a] (624)	70.2[a] (594)	79.3[b] (646)	<0.01	0.03	0.15
Inter-service interval[7]	32.7 ± 0.3	32.4 ± 0.3	32.8 ± 0.3	0.69	0.01	0.79
P/AI[8]	35.2 (624)	30.2 (594)	34.1 (646)	0.16	<0.01	0.28
Total number of services[9]	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	0.87	<0.01	0.83

[a-b]Different superscripts within a row indicate significant differences ($P \leq 0.05$).
[1]DO60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.
[2]DO88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.
[3]PSOv = first service at detected estrus after the second PGF2α treatment of Presynch-Ovsynch given at 50 ± 3 DIM or TAI at 72 ± 3 DIM after completion of the Presynch-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.
[4]Effect of additional explanatory variables are described in the text.
[5]T x P = treatment by parity interaction.
[6]EDAI = cows inseminated at detected estrus.
[7]Inter-service interval = interval (days) between two consecutive AI services.
[8]P/AI = pregnancies per artificial insemination.
[9]Total number of services = total number of AI services received up to 350 DIM.

Table 3. Cyclicity status, uterine health, and body condition score for lactating dairy cows that received first service AI after the Double-Ovsynch or Presynch-Ovsynch protocol and different duration of the voluntary waiting period.

	Treatment			*P*-value		
	DO60[1]	DO88[2]	PSOv[3]	Trt	Parity	T x P[4]
Cyclic[5], % (n)						
Baseline	70.9 (165)	71.8 (142)	73.7 (156)	0.88	0.08	0.95
Beginning of SP[6]	70.9[a] (165)	87.5[b] (142)	73.6[a] (156)	<0.01	0.07	0.97
-5 or -10 d of VWP end[7]	95.2[a] (164)	95.0[a] (142)	83.1[b] (155)	<0.01	0.34	0.57
PVD[8], % (n)						
Baseline	19.6 (53)	18.7 (50)	17.7 (82)	0.96	0.33	0.99
Beginning of SP	19.6 (53)	20.3 (51)	17.6 (82)	0.93	0.40	0.99
-5 or -10 d of VWP end	22.6 (53)	14.0 (50)	12.4 (81)	0.26	0.23	0.47
PMN[9], (%)						
Baseline	13.0 ± 2.9	15.9 ± 3.0	13.0 ± 2.2	0.56	0.74	0.62
Beginning of SP	13.0 ± 2.9	8.2 ± 2.5	13.0 ± 2.2	0.31	0.76	0.42
-5 or -10 d of VWP end[7]	8.8 ± 2.3[a]	3.5 ± 1.7[b]	9.2 ± 1.8[a]	0.03	0.38	0.25
BCS[10] ≥ 2.75, % (n)						
Baseline	88.5 (165)	88.7 (142)	87.2 (156)	0.87	<0.01	0.79
Beginning of SP	88.3[a] (165)	95.1[b] (142)	87.0[a] (156)	0.02	<0.01	0.97
-5 or -10 d of VWP end[7]	90.3[ab] (164)	94.7[b] (142)	87.2[a] (153)	0.03	<0.01	-

[a-b]Different superscripts within a row indicate significant differences ($P \leq 0.05$).
[1]DO60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol.
[2]DO88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol.
[3]PSOv = first service at detected estrus after the second PGF2α treatment of Presynch-Ovsynch given at 50 ± 3 DIM or TAI at 72 ± 3 DIM after completion of the Presynch-Ovsynch protocol.
[4]T x P = treatment by parity interaction.
[5]Cyclic = cows with progesterone concentration > 1 ng/mL.
[6]Beginning of SP = samples collected at the beginning of the synchronization protocol.
[7] -10 d of VWP end = 50 ± 3 DIM for DO60 and 78 ± 3 DIM for DO88; -5 d of VWP end = 45 ± 3 DIM for PSOv.
[8]PVD = cows with purulent vaginal discharge (metricheck score ≥ 2).
[9]PMN = percentage polymorphonuclear cells in uterine cytology sample.
[10]BCS = body condition score.

Table 4. Proportion of cows sold, dead, and total proportion that left the herd (sold and dead) for lactating dairy cows that received first service AI after the Double-Ovsynch or Presynch-Ovsynch protocol and different duration of the voluntary waiting period.

	Primiparous				Multiparous			
	DO60[1]	DO88[2]	PSOv[3]	*P*-value	DO60	DO88	PSOv	*P*-value
Sold, % (n/n)	14.3 (168)	12.1 (166)	15.5 (168)	0.65	26.9[a] (268)	37.1[b] (264)	32.0[ab] (269)	0.04
Died, % (n/n)	1.8 (168)	1.8 (166)	4.2 (168)	0.31	2.2 (268)	2.7 (264)	4.8 (269)	0.20
Left herd, % (n/n)	16.1 (168)	13.9 (166)	19.6 (168)	0.36	29.1[a] (268)	40.2[b] (264)	36.8[ab] (269)	0.02

[a-b]Different superscripts within a row indicate significant differences ($P \leq 0.05$) within the same parity.
[1]DO60 = first service timed AI at 60 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.
[2]DO88 = first service timed AI at 88 ± 3 DIM after the Double-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.
[3]PSOv = first service at detected estrus after the second PGF2α treatment of Presynch-Ovsynch given at 50 ± 3 DIM or TAI at 72 ± 3 DIM after completion of the Presynch-Ovsynch protocol. Second and greater services after detection of estrus or the Ovsynch protocol initiated 32 ± 3 d after AI.

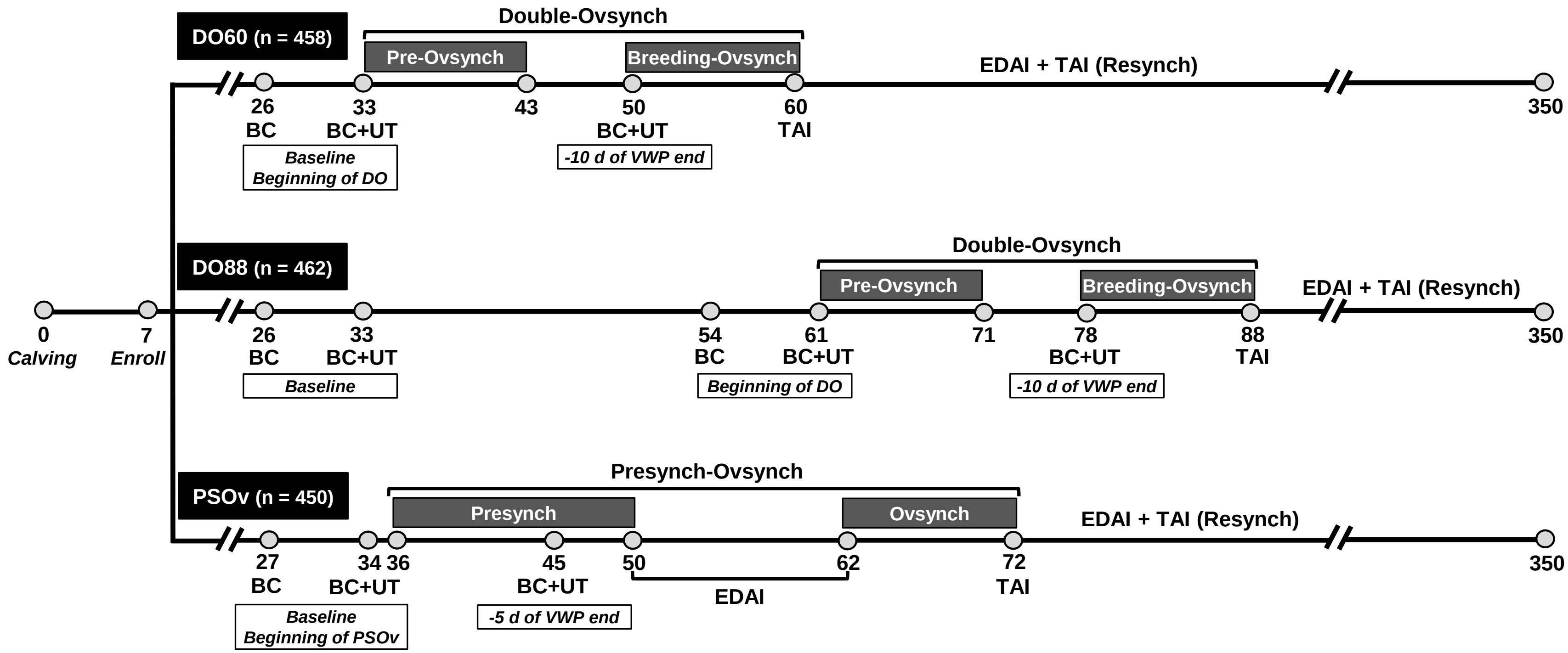

Figure 1. Schematic representation of experimental procedures. Cows were randomly allocated to receive first service timed AI (TAI) after the Double-Ovsynch protocol (DO) at 60 ± 3 DIM (**DO60** = 458), TAI after DO at 88 ± 3 DIM (**DO88** = 462), or a combination of insemination after detected estrus and TAI with the Presynch-Ovsynch protocol (**PSOv** = 450). Cows in the PSOv treatment received AI at detected estrus (EDAI) after the second PGF2α treatment given at 50 ± 3 DIM or TAI at 72 ± 3 DIM. Subsequent AI services occurred through EDAI or TAI after resynchronization of ovulation with the Ovsynch protocol (Resynch). Cyclicity (progesterone concentration in circulation), uterine health (**UT**; vaginal discharge and uterine cytology), and BCS were determined at baseline (DO60 and DO88 = 33 ± 3 DIM; PSOv = 34 ± 3 DIM), beginning of the synchronization protocol (DO60 = 33 ± 3 DIM; DO88 = 61 ± 3 DIM; PSOv = 34 ± 3), and -5 (i.e., for PSOv) or -10 d (i.e., for DO) of the VWP end (DO60 = 50 ± 3 DIM; DO88 = 78 ± 3 DIM; PSOv = 45 ± 3 DIM). For determination of cyclicity status at baseline and beginning of DO, samples were collected 7 d apart and cows were classified as cyclic if at least one of the samples had a P4 concentration ≥ 1 ng/mL. BC = blood collection.

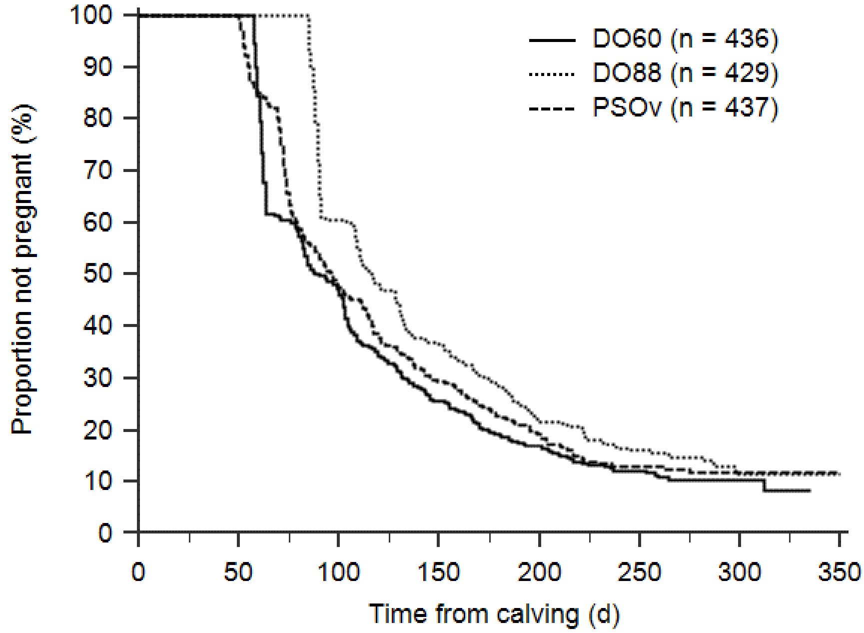

Figure 2. Kaplan-Meier survival curves for time to pregnancy after calving. The hazard of pregnancy was greater (*P* < 0.01) for cows in the DO60 than the DO88 treatment (HR 1.53, 95% CI: 1.32 to 1.78) and for cows in the PSOv than the DO88 treatment (HR 1.37, 95% CI: 1.19 to 1.61). No differences were observed between cows in the DO60 and PSOv treatments (HR 1.12, 95% CI: 0.96 to 1.30).

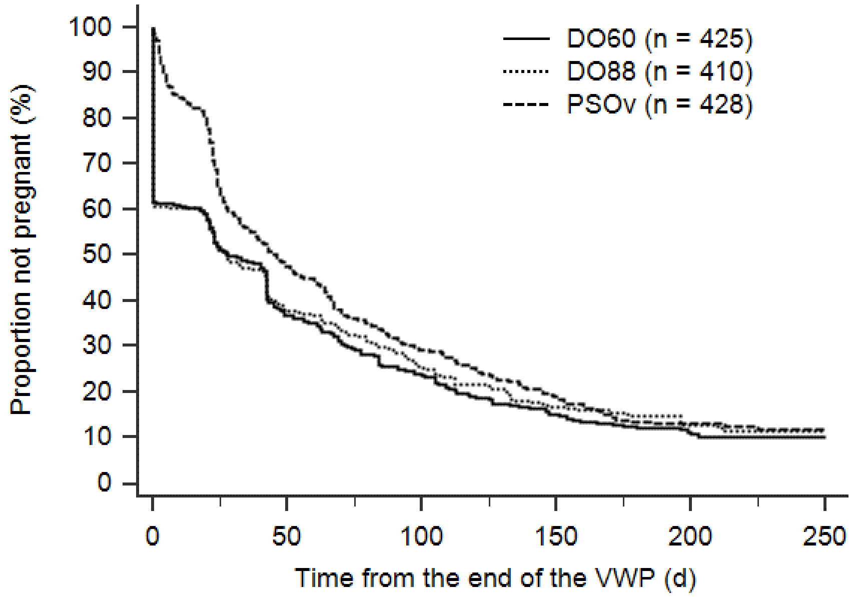

Figure 3. Kaplan-Meier survival curves for days to pregnancy after the end of the voluntary waiting period (end of VWP = Day 0). The hazard of pregnancy was affected by treatment group (P = 0.01), whereby cows in the DO60 treatment had greater hazard of pregnancy than cows in the PSOv treatment (HR 1.25, 95% CI 1.08 to 1.45) and no differences were observed between the DO60 and DO88 treatments (HR 1.04, 95% CI: 0.89 to 1.20). The hazard of pregnancy was also greater for the DO88 than the PSOv treatment (HR 1.20, 95% CI: 1.03 to 1.40).

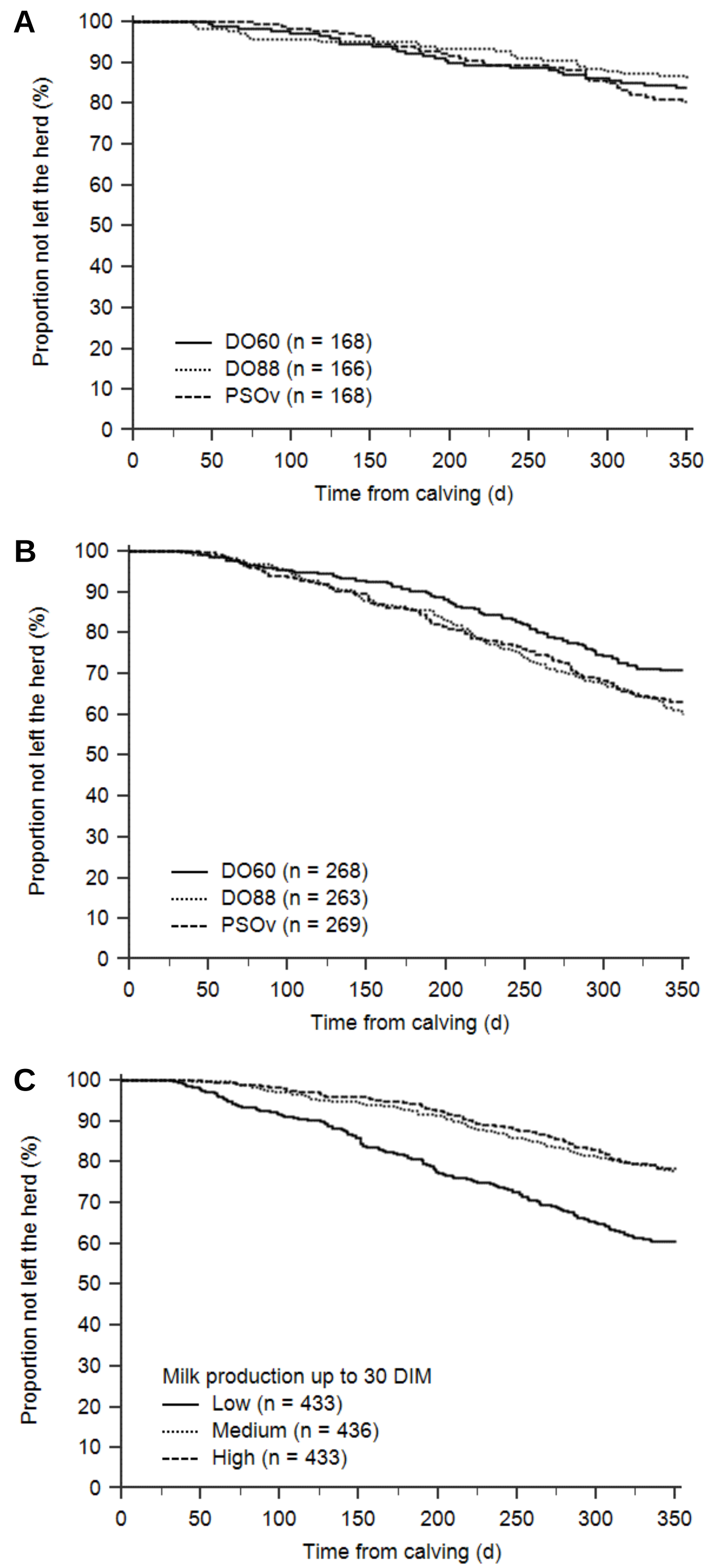
A
Proportion not left the herd (%)
Time from calving (d)
DO60 (n = 168)
DO88 (n = 166)
PSOv (n = 168)
B
Proportion not left the herd (%)
Time from calving (d)
DO60 (n = 268)
DO88 (n = 263)
PSOv (n = 269)
C
Proportion not left the herd (%)
Time from calving (d)
Milk production up to 30 DIM
Low (n = 433)
Medium (n = 436)
High (n = 433)

Figure 4. Kaplan-Meier survival curves for time to herd exit after calving for primiparous cows. The hazard of culling was similar (P = 0.22) between treatments (DO60 vs. DO88 = HR 1.12, 95% CI 0.64 to 1.96; DO60 vs. PSOv = HR 0.72, 95% CI 0.43 to 1.20; DO88 vs. PSOv = 0.64, 95% CI 0.38 to 1.09). (B) Kaplan-Meier survival curves for time to herd exit after calving for multiparous cows. The hazard of culling was affected by treatment (P = 0.02), whereby cows in the DO88 and PSOv treatments had greater hazard of culling than cows in the DO60 treatments (DO88 vs. DO60 = HR 1.49, 95% CI 1.11 to 2.00; PSOv vs. DO60 = HR 1.39, 95% CI 1.03 to 1.85). No difference was observed between the DO88 and the PSOv treatments (HR 1.07, 95% CI 0.82 to 1.41). (C) Kaplan-Meier survival curves for time to herd exit after calving according to milk production accumulated up to 30 DIM (MK30). The hazard of culling was affected by MK30, whereby cows in the low milk production group had greater hazard of culling than cows in the medium (HR 2.08, 95% CI 1.62 to 2.67) and high (HR 2.17, 95% CI 1.67 to 2.78) MK30 group.

SECTION III

GENERAL CONCLUSION

CHAPTER IX

OVERALL CONCLUSIONS AND FUTURE RESEARCH

1. Automated monitoring of dairy cow health

1.1. Overall conclusions

When I started my PhD program in 2013, most studies evaluating sensors for monitoring dairy cow health were done to either validate the system measurements (i.e., ability to monitor the parameter of interest, for example rumination time) or test if changes in sensor data were associated with the occurrence of health disorders (reviewed by Rutten et al., 2013). At that time, scientific studies that evaluated the performance and usefulness of sensor systems to identify cows suffering health issues were scarce or not available. Therefore, we conducted a prospective cohort study in a commercial dairy farm with the main objective of evaluating the performance of an automated health-monitoring system (**AHMS**) based on physical activity and rumination sensors to identify cows with metabolic and digestive disorders (Chapter II), clinical mastitis (Chapter III) and metritis (Chapter IV). This AHMS integrates rumination and activity data in one parameter called Health Index (**HI**) score. Cows with a HI score (0 to 100 arbitrary units) below a certain threshold (86 arbitrary units) are flagged as potentially ill and therefore need clinical examination.

Results from this study showed high sensitivity (**Se**) to identify cows with metabolic and digestive disorders based on HI score. Specifically, Se was 98% for cows suffering displaced abomasum (**DA**), 91% for cows with ketosis, and 89% for cows with indigestion, which resulted in an overall Se of 93% for all metabolic and digestive disorders combined. Moreover, cows with DA and ketosis were identified (flagged) earlier by the AHMS (3.0 and 1.6 days for DA and ketosis, respectively) than through clinical diagnosis by farm personnel, suggesting that there is potential to either implement treatments earlier or implement preventive treatments to either avoid or stop the progression of health disorders. Overall, these results suggest that the combination of rumination time and physical activity could be useful to identify cows suffering from metabolic and digestive disorders in the early postpartum period. Conventional monitoring of metabolic and digestive disorders usually requires performing blood, milk or urine test, as well as physical examination and auscultation to all or most cows at greater risk of developing these disorders. Thus, use of an AHMS like the one used in our research in commercial herds has the potential to reduce the burden associated with monitoring cows to identify metabolic and digestive disorders, reducing labor cost and improving cow time budgets.

On the other hand, the overall ability of the HI score to identify cows with clinical mastitis was moderate (Se = 58%), with substantial variation according to the type of pathogen affecting the mammary gland. For example, the Se for mastitis cases caused by *E. Coli* reached ~80%, while the Se for cases caused by other pathogens such as Gram+ bacteria and *S. aureus* was below 50%. These results were expected because intramammary infections caused by *E. coli* are characterized by a severe inflammatory response and systemic compromise, whereas clinical mastitis caused by Gram+ pathogens and *S. aureus* are usually mild and characterized by changes in the mammary gland and milk; however, cows usually do not present systemic clinical signs. Although we did not have information about the severity of mastitis events, we speculated that cows suffering mild cases of clinical mastitis and with less severe systemic compromise were responsible for the moderate Se observed. Indeed, cows flagged based on HI score (**HI+**) had sudden and dramatic reductions in rumination, activity, and HIS, whereas for cows with clinical mastitis not flagged based on HI score (**HI-**) the parameters monitored behaved more like in healthy cows (i.e., presented minor changes). As a result, we concluded that HI score could be helpful to detect severe cases of clinical mastitis caused by pathogens such as *E. coli*, which have profound systemic effects on the cow. However, other direct and simple methods of mastitis detection based on changes in milk appearance, udder appearance, and consistency (e.g., milk stripping, udder visual inspection, palpation) may be more effective to identify cows with mild cases of clinical mastitis than an AHMS based on rumination and activity only. Thus, it seemed reasonable to suggest that the automated health monitoring systems could be used in combination with other traditional methods of mastitis detection, and it may be a valuable tool for providing further insights about the overall health status of the cow.

Similar to mastitis, the overall Se of the AHMS to identify cows that developed metritis in early lactation was moderate (55%). Interestingly, we did not observe a difference in Se when cows were sorted based on rectal temperature measured on the day of clinical diagnosis. It is important to highlight that the incidence of metritis in our study (32%) was within the upper level reported for lactating dairy cows, likely because the farm standard operating procedures for metritis diagnosis considered cows with mild symptoms of metritis as metritic. Therefore, regardless of the lack of records about severity of metritis, we speculated that the major reason for the moderate Se of HIS could have been related to the wide range of severity of the disorder which, in turn, may differentially affect rumination and activity patterns around clinical diagnosis. Indeed, we observed that cows with metritis flagged based on HI score (HI+) presented dramatic reductions in rumination, activity, and milk production around the day of diagnosis, whereas cows with metritis not flagged based on HI score (HI-) presented similar values for the parameters of interest than healthy cows. Moreover, cows with metritis in the HI+ group were twice more likely to be sold during lactation compared with cows in the HI- group and healthy cows. Thus, we concluded that an AHMS that monitors rumination and physical activity was effective to identify cows with severe cases of metritis; however, it should be used in combination with traditional methods of metritis detection to identify mild cases of metritis. For example, farms could perform a systematic evaluation of uterine health once a week (e.g., for cows between 6 and 12 DIM when most cases are expected) to identify mild cases of metritis, whereas the AHMS could be used to identify cows with significant systemic compromise every day. Using this approach, cows not diagnosed with metritis by the AHMS are examined at set DIM to rule out the presence of the disorder.

The overall performance of the AHMS from 2 to 80 DIM presented high accuracy (~96%) when all disorders of interest (displaced abomasum, ketosis, indigestion, metritis and mastitis) were included in the analysis. This was likely a reflection of the high specificity and negative predictive value (both > 97%), and the considerably greater number of days during which cows were healthy rather than suffering from a health disorder. Of note, the alarm system based on HIS resulted in a low number of false positives (2.4%) outcomes, which is a desired feature for an AHMS. Because it is expected that cows flagged are carefully examined to identify underlying health issues, AHMS should be able to not only flag sick cows, but also not flag (identify) healthy cows that do not need clinical examination.

In summary, the AHMS based on rumination and activity data tested in our study presented high Se to identify cows with metabolic and digestive disorders, but moderate Se to identify cows suffering mastitis or metritis. Severe cases of clinical mastitis and metritis that affect cows systemically are more likely to impact rumination and activity, and consequently, more likely to be identified by the system. The low number of false positives outcomes observed in this study were encouraging and support the on-farm application of these type systems (similar to the one evaluated in our study) to reduce the burden associated with conventional health monitoring programs. Nevertheless, routine screening for health disorders using traditional methods seems necessary to ensure detection of mild cases of clinical mastitis and metritis and cases of metabolic and digestive disorders that do not cause dramatic changes to the parameters monitored by the AHMS.

1.2. Possible future directions for research

In the experiment presented in chapters II, III, and IV of this dissertation, we described the performance of a commercially available AHMS based on rumination and activity data to identify cows suffering health disorders. The results showed high Se to identify cows with metabolic and digestive disorders, but the ability of the system to detect cows with clinical mastitis and metritis was moderate (~55%). Our data suggests that only cows suffering a more severe case of mastitis or metritis were flagged based on HI score. However, a pitfall of our study was the lack of records for disease severity (e.g., mild, moderate, and severe). Thus, future studies could be conducted to determine if the differences observed in Se to detect cows with mastitis and metritis are attributable to the wide range of severity of these disorders in cows. Moreover, studies evaluating the association between sensor data and the severity of health disorders could be helpful to improve the performance of the AHMS.

A first study to test the hypothesis that severity is associated with the degree of change in the parameters of interest may consists of a large prospective cohort study similar to our previous study. For mastitis, all clinical cases would be classified during clinical examination as follows: (1) changes in milk appearance (mild), (2) signs of udder inflammation with or without changes in milk characteristics (moderate), and (3) signs for scores 1 and 2 but combined with systemic signs of illness (severe). Therefore, the analysis of HI score performance could be stratified by severity score with the Se of the AHMS determined for mild, moderate, and severe cases of clinical mastitis.

Despite the fact that rumination and activity patterns of cows with mastitis not identified by HI score (HI-) behaved very similar to healthy cows, another interesting observation was that cows in the HI- group with clinical mastitis had a similar reduction in milk yield around clinical diagnosis than cows with clinical mastitis flagged based on HI score (HI+). For that reason, milk production seems to be a better marker of mild cases of clinical mastitis. Rumination and activity behavior are not affected as dramatically in these cows as to generate an alert based on these two parameters. Therefore, a study with similar design than the study described above could have the following objectives: (1) define thresholds of milk production changes to identify cows with mild, moderate, and severe cases of clinical mastitis, (2) evaluate the performance of those thresholds, (3) compare the performance of milk yield changes against HI score to identify cows with mastitis and different levels of severity, (4) use the data generated to develop a new index that integrates the 3 parameters (milk production, rumination, and activity) along with cow information (e.g., lactation number, days in milk, previous disorders, etc.) using machine learning algorithms, and (5) compare the performance of the new index versus HI score and milk production thresholds. All sensor and non-sensor data would be collected prospectively, whereas the analysis of the AHMS performance and development of new indices would be retrospective. A final prospective study evaluating the three monitoring methods (HI score, milk yield thresholds, and index integrating rumination, activity, and milk yield) could be conducted to compare their performance (objective number 5), using mastitis diagnosis by trained personnel and milk culture as the gold standard method.

As for mastitis, a similar type of study could be conducted to evaluate the ability of the HI score to detect cows with different severity of metritis. In fact, the study can be conducted concomitantly using cows that develop metritis. Cases of metritis would be classified during clinical examination as: mild (watery, foul smelling, pink/brown uterine discharge) and severe (same symptoms combined with systemic signs of illness such as anorexia, depression, decreased rumen motility, and dehydration). After data collection (sensor and non-sensor), a retrospective analysis to evaluate the performance of HI score to detect cows with metritis would be performed as reported in Chapter IV, except that cows would be stratified by the severity of the case.

An important finding of the study presented in Chapter IV of this dissertation was that cows with metritis not flagged by HI score (HI-) presented similar rumination, activity, milk production, reproductive performance, and culling dynamics than healthy cows. Because these cows (HI-) were all treated with antibiotics, it was not possible to determine if the parameters evaluated were similar to those of healthy cows because of a positive response to treatment or the case was too mild to affect cow health and performance hence, they would have likely had spontaneous cure without treatment. Therefore, future research should be directed towards testing if cows suffering mild metritis presenting no changes in sensor-monitored parameters truly need antibiotic treatment for optimal production, reproductive performance and welfare. A randomized control experiment could be conducted using cows diagnosed with mild metritis and no substantial changes in rumination, activity, HI score and milk yield around clinical diagnosis.

At the time of diagnosis, cows would be randomly assigned to a treatment (**TRT**) or non-treatment (**NTRT**) group. Cows in the TRT group would receive antibiotic therapy according product label and industry standards, whereas cows in the NTRT group would not receive antibiotic treatment. Cows from both groups would be examined daily until clinical symptoms disappear (cured). If cows in the NTRT group develop secondary signs of illness as detected by sensors (e.g. reduced rumination time) and clinical examination, they would be immediately treated with supportive therapy for 3 days and moved to a third group (**NTRT-TRT**). Antibiotic treatment in the NTRT-TRT group would be restricted only to cows that do not respond favorably to supportive therapy. Outcomes of interest include productive (e.g., milk yield and milk components), reproductive (e.g., P/AI at first service, time to pregnancy), and culling dynamics (e.g., proportion of cows left the herd, time to culling). Ultimately, this study could help reduce the use of antibiotics in dairy herds without affecting cow well-being and performance.

To optimize the use of resources (both labor and funds), most of the studies proposed could be conducted as part of a large experiment monitoring cows from calving until the end of lactation. Most of the effort and resources, however, would be dedicated to the first 30 DIM when most cases of metritis and clinical mastitis are observed.

2. Impact of extending the postpartum voluntary waiting period on dairy herd performance

2.1. Overall conclusions

Although several studies evaluated the effect of extending the VWP duration on overall cow and herd performance, ambiguous results and multiple experimental design exclusion criteria did not allow drawing definitive conclusions about the effect of this management strategy on the reproductive and lactation performance of dairy cows. Therefore, we conducted a randomized controlled experiment to evaluate the effect of VWP duration of 60 or 88 DIM on (1) reproductive performance, herd exit dynamics, and lactation performance (Chapter VI), (2) uterine health, cyclicity status, BCS, and systemic inflammation before first service (Chapter VI), and (3) cow and herd profitability (Chapter VII).

Based on results of our randomized controlled experiment with ~2,700 cows enrolled in three commercial dairy herds, we concluded that extending the duration of the VWP affected herd reproductive performance, exit dynamics (i.e., cow sales), and profitability. From a reproductive performance standpoint, delaying the VWP resulted in greater P/AI to first service, mostly because of greater P/AI in primiparous cows. A physiological status more conducive to pregnancy characterized by improved uterine health, greater BCS, reduced systemic inflammation, and (to a lesser extent) reduced anovulation before insemination explained the greater P/AI in cows with extended VWP. Despite improved P/AI to first service, extending the VWP resulted in delayed time to pregnancy during lactation, mainly because cows with a short VWP fully compensated for reduced fertility at first service with earlier and more opportunities for re-insemination. As the proportion of nonpregnant cows at 350 DIM was not affected by VWP duration, we also concluded that one of the greatest consequences of longer VWP was shifting timing of pregnancy toward later lactation rather than generating a difference in the proportion of pregnant cows during lactation. Nevertheless, this may have been biased to some degree by the herd exit dynamics because another consequence of delayed first service and pregnancy was increased risk of herd exit, especially in late lactation for multiparous cows. As most cows that leave a herd are nonpregnant, the absence of these cows in late lactation biased the survival analysis for time to pregnancy and proportion of pregnant cows at the end of lactation (i.e., the proportion of cows pregnant at 350 DIM out of the cows present up to 350 DIM).

From an economic standpoint, extending the duration of the VWP from 60 to 88 DIM increased the overall profitability of primiparous cows and reduced the profitability of multiparous cows under fixed or variable (i.e., stochastic analysis) input pricing scenarios. Interestingly, the differences observed depended mostly on the herd replacement dynamics. The opposite effect of extending VWP on profitability, which was expected, may be explained by differences in milk production persistency after peak (i.e., greater for primiparous cows) and contrasting replacement dynamics for primiparous (i.e., more cows sold in the short VWP group) and multiparous (i.e., more cows sold in the long VWP group) cows.

Another relevant and interesting aspect of the economic analyses conducted for this experiment was the potential to introduce biases in the results based on methods used to calculate profitability. For example, substantial variation was observed for profitability calculated per lactation versus a fixed period after calving (i.e., 18 mo after calving in our experiment) and when using different types and number of factors for the calculation (e.g., including or not including fixed costs and calculation of fixed cost). Although the direction of the effect did not change, a wide range of values was obtained through the stochastic analysis of input costs which resulted in either negligible or major differences in profitability. Thus, standardizing the methods used to calculate profitability certainly deserves more attention if decision-making of on-farm practices is to be based on this type of research.

As we were designing and planning the previous experiment, there was an opportunity to add a third experimental treatment at one of the commercial dairies. Therefore, we were able to conduct a unique experiment to investigate the effect of submitting lactating dairy cows for first service with three different management strategies on time to pregnancy and herd exit dynamics (Chapter VIII). We compared a combined approach to submit cows for insemination (i.e., at detected estrus and TAI) using a PGF-based synchronization of estrus and ovulation protocol (i.e., Presynch-Ovsynch) with all TAI programs using a GnRH-based synchronization of ovulation protocol (i.e., Double-Ovsynch) and different VWP duration. Specifically, cows received first service after detection of estrus or TAI during the Presynch-Ovsynch protocol with VWP of 50 ± 3 DIM (**PSOv**) or received TAI after the Double-Ovsynch protocol with VWP of 60 ± 3 (**DO60**) or 88 ± 3 DIM (**DO88**).

Results from this study showed that the combined and all TAI management strategies with average days at first service of ~60 DIM presented similar hazard of pregnancy and overall time to pregnancy during lactation. On the other hand, we also observed that cows with shorter VWP (either in the PSOv or in DO60 group) had reduced time to pregnancy compared with cows managed with all TAI and longer VWP (DO88) because of earlier opportunities for re-insemination in cows with shorter VWP. Similar to the study presented in Chapter VI, the extended VWP shifted timing of pregnancy towards later lactation when compared with programs with shorter VWP regardless of the method of submission to first service (combined method or all TAI). Thus, management programs that reduce DIM at first service through either a combination of AI at detected estrus and TAI or all TAI can reduce time to pregnancy when compared with all TAI programs with longer VWP. This is especially important when the extension of the VWP does not substantially increase first service P/AI, as observed for multiparous cows in our experiment. An economic analysis similar to the one conducted in Chapter VII is crucial to determine the profitability of these management strategies.

2.2. Possible future directions for research

High lactation persistency is one the main drivers of profitability in cows with extended calving intervals. In this regard, the use of recombinant bovine somatotropin (**rbST**) in our experiment played a major role in increasing lactation persistency, improving milk production and feed efficiency after peak milk. Nevertheless, due to changing consumer preferences, rbST use in New York State and the U.S. has decreased dramatically in recent years and may not be used in the future. Therefore, a caveat of our experiment is that the data may better reflect the performance of cows supplemented with rbST, which may not be representative of the majority of dairy cows today and in the future. Thus, new experiments with similar experimental designs than those described in Section II should be conducted to elucidate the effect of VWP duration and different first service management strategies on productive and reproductive performance, herd exit dynamic, and profitability of dairy cows not treated with rbST.

In addition to the studies proposed above, future research in this area should focus on defining the optimal VWP duration for specific sub-groups of cows or individual cows in a herd. Using the vast amount of data collected about cow features and parameters of performance, it may be possible to predict with acceptable accuracy the duration of the VWP that maximizes cow profitability. For example, information usually available for individual cows in dairy herds about cow genetics, age, lactation number, health issues, reproductive events, milk yield, and milk components yield as well as historical data such as previous lactations DIM, days to pregnancy, and days dry could be used. Historical and predicted non-cow data including environmental, economic, and farm management conditions as well as whole herd parameters of performance could also be included as they may contribute to individual cow predictions. Including these multiple sources of variation for reproductive and productive performance may substantially improve predictions as multiple studies documented direct or indirect effects of these parameters on time to pregnancy during lactation, productive performance, and likelihood of herd exit. Therefore, future research should include the following major components/steps: (1) collect historical, current, and predicted data on cow features and parameters of performance, including sensor data (e.g., daily milk weights, DIM at estrus, etc.), (2) develop prediction algorithms using machine learning techniques to identify the timing of pregnancy during lactation that maximizes individual cow profitability, and (3) conduct a randomized controlled experiment to evaluate the profitability of dairy cows managed with either a variable VWP duration based on the algorithm(s) developed in step 2 versus fixed VWP duration according to herd standard operating procedures.

The first step of this study is finding commercial farms with the necessary records and tools for retrospective and prospective data collection. Data may include: cow features (e.g., genetics, age, lactation number, season of calving, etc.), previous and current lactations events and performance (e.g., lactation length, days dry, gestation length, calving ease, health events, daily milk weights and milk components, days open, calving interval, services per pregnancy, number and DIM of estrus events, etc.), herd performance and management (e.g., management strategy for first and subsequent services, rbST use, P/AI at each service by lactation number and

DIM at insemination, time to pregnancy during lactation and after the end of the VWP, culling dynamics, etc.), and economic conditions (milk price, beef price, replacement cost, reproductive program cost, labor cost, etc.). After data collection, an economic analysis of individual animals would be performed to calculate the profitability values that will be used as the main outcome of interest for the algorithms to be developed.

Machine learning techniques would be used to identify the ideal VWP duration of individual animals based on patterns of association of the multiple variables available to the models. The large dataset with all the collected information and calculated profitability for each cow would be divided into three different parts to conduct the following analyses: (1) training dataset for algorithm(s) development (~60% of the data), (2) validation dataset (~20% of the data), and (3) testing dataset (~20% of the data). For each dataset, a representative proportion of cows would be randomly selected to avoid bias.

Finally, once the algorithms are fully developed, a randomized controlled experiment should be conducted to test the hypothesis that managing cows based on individual subgroups of cows VWP would result in increased profitability compared with using a fixed VWP for all cows (or cows within a parity). In this experiment, lactating dairy cows at 30 ± 3 DIM would be blocked by parity and randomly assigned to a TRT or a CON group. Cows in the TRT group would have their VWP duration defined by the algorithm created in step 2 (minimum VWP duration of 60 ± 3 DIM), whereas cows in the CON group would have a fixed VWP of 88 ± 3 DIM for primiparous cows or 60 ± 3 DIM for multiparous cows. The VWP duration selected for CON cows is based on the profitability results presented in Chapter VII. All cows would receive first service TAI after synchronization of ovulation with the Double-Ovsynch protocol. Enrolling cows at 30 DIM would allow collection of cow information during early lactation and have enough time to implement the synchronization of ovulation in cows with the minimum VWP duration within the TRT group or in multiparous cows within the CON group. Data would be collected and analyzed following the same procedures described in Chapter VI and VIII of this dissertation. This experiment would be unique by describing reproductive performance, herd exit dynamics, and profitability of dairy cows managed with a variable VWP duration based on big data analysis of cow features, parameters of performance, herd management, and economic conditions.