THE IMPACT OF HERITABLE AND NON-HERITABLE FACTORS ON TIME TO RESUMPTION OF CYCLICITY IN POSTPARTUM DAIRY COWS

by

SARAH JOHNSON

(Under the Direction of Jillian F. Bohlen)

ABSTRACT

The postpartum period is a challenging time in the dairy cow's life with production demands often overshadowing the need to resume cyclicity. The objective of this research was to evaluate variables with potential influence on time to resumption of cyclicity in postpartum dairy cattle. The first study focused on the influential variables of metabolites, milk production, visits to the robotic milking system, and anti-Müllerian hormone (AMH) on a robotic dairy and their implications for cyclicity resumption in Holstein cattle. The second study focused on the influential variables of milk production, AMH, and genomics on a conventional dairy and their implications for cyclicity resumption in Jersey cattle and how their fertility compares with Holstein cattle. This study additionally compared cyclicity between Jersey and Holstein cattle. The third study focused on the uterine microbiome in the postpartum period and its diversity, abundance, and differences between Holstein and Jersey cattle.

INDEX WORDS: Cyclicity, Metabolites, Microbiome, Postpartum, Robotic Dairy

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DEDICATION

To Cheerio and 817

Thank you for always making me smile on long days spent on the farm. I hope my current and future work can improve the lives of cows like you.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Endocrinology of the Estrous Cycle in Dairy Cattle

The average estrous cycle length in mature dairy cattle is 21 days with a range of 18 to 24 (Crowe 2022). The estrous cycle can be separated into two phases: the follicular phase and the luteal phase. The follicular phase comprises approximately 20% of the estrous cycle. Beginning at luteolysis and ending at ovulation, the follicular phase is dominated by the follicle and the hormone estrogen (Senger, 2012). The luteal phase comprises approximately 80% of the estrous cycle. Beginning at ovulation and ended at luteolysis, the luteal phase is dominated by the corpus luteum (CL) and the hormone progesterone (Crowe, 2022). Each of these phases can be further divided into two stages. The follicular phase consists of the proestrus stage and the estrus stage, while the luteal phase consists of the metestrus stage and the diestrus stage (Ireland et al., 1980).

Bovine females are dependent on feedback mechanisms of the hypothalamic-pituitary-gonadal (HPG) axis in order to have functional estrous cycles. The estrous cycle is maintained and regulated by the tonic and surge centers of the hypothalamus, the anterior lobe of the pituitary gland, and the ovaries. The primary hormones involved in the HPG axis are gonadotropin-releasing hormone (GnRH), follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone (P4) (Figure 1.1; Crowe et al., 2013). Further modulators of the estrous cycle are the frequencies and

amplitudes of hormones produced, meaning how often pulses occur and the extent to which they are secreted.

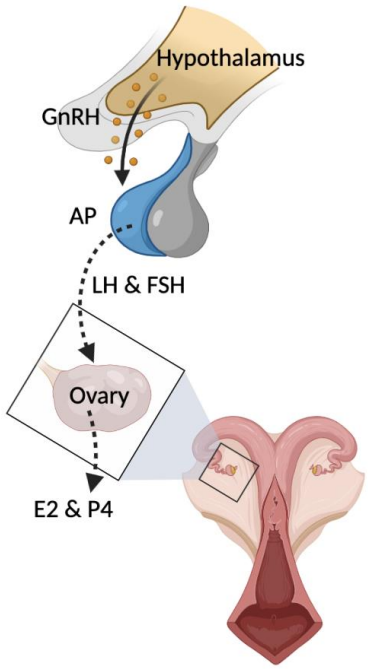

Figure 1.1: Downstream impact of the female hypothalamic-pituitary-gonadal axis.

There are two methods of GnRH secretion from the hypothalamus: pulsatile secretion from the tonic center and surges of secretion from the surge center (Maeda et al., 2010). The tonic center of the hypothalamus is responsive when there are low concentrations of E2 and there is negative feedback from P4. GnRH is released in low, basal quantities with pulsatile secretion throughout the entire estrous cycle from the tonic center. The low levels of progesterone that allow for basal secretion of gonadotropins allow for some follicular development to occur, but the level of follicular development is not sufficient for high levels of estrogen production (Marques et al., 2022). Comparatively, the surge center of the hypothalamus is responsive when there are high concentrations of E2. The surge center elicits the high frequency release of gonadotropins

in a short period of time during the estrous cycle to generate a pre-ovulatory surge necessary for further hormonal production (McDougall et al., 1995).

No matter the pattern of secretion, when GnRH is released, it is transported to the anterior lobe of the pituitary (AP) via the hypophyseal portal blood system (Moenter et al., 1992). GnRH is released in minute quantities, making this portal system essential for GnRH to arrive to the AP in sufficient quantities to illicit a downstream response (Casteel and Singh, 2022). Once at the AP, the intended response is the stimulation of FSH and LH release to modulate sex steroid production of E2 and P4 from the ovaries (Casteel and Singh, 2022).

FSH is produced by the AP during both the follicular and luteal phases with the primary responsibility of recruitment and early development of follicles on the ovary from the gonadotrophin sensitive pool (Ginther et al., 1989). Slow pulses of GnRH promote FSH secretion, initiating a follicular wave where recruited follicles will continue to grow and develop or go through the process of atresia. In dairy cattle, only one single follicle typically becomes dominant since they are a monotocous species (Macmillan et al., 2018).

LH is produced by the AP and is fundamental in the growth of follicles by playing a role in establishment of the dominant follicle (McDougall et al., 1995). Steroid hormones modulate LH release by enhancing or suppressing pituitary response to GnRH (Baratta et al., 1994). Increased concentrations of E2 produced from the developing pre-ovulatory follicle increases the sensitivity of the AP to GnRH (Schoenemann et al., 1985). Additionally, the surge center responds to positive feedback of E2. This response

leads to the preovulatory GnRH surge, initiating the LH surge (Stevenson and Pulley 2016).

Both FSH and LH have primary responsibilities in follicular recruitment and development. The primary product of the follicle, E2, is produced by the concerted effort of both follicular thecal and granulosal cells. Once produced, E2 targets all major hypothalamic neuroendocrine and autonomic cellular groups to act on multiple signaling pathways (Kelley et al., 2005). As a result of this, E2 has a differential effect on the hypothalamus, with a negative feedback system during periods of low secretion and positive feedback system during higher levels of secretion. The tonic center is responsive to low levels of E2, and the surge center is responsive to high levels of E2. The increase in E2 secretions as the preovulatory follicles continue to grow increases the sensitivity of the anterior pituitary to GnRH by upregulating the GnRH receptors (Schoenemann et al., 1985). Follicular size is directly and positively correlated with E2 production, and increasing amounts of E2 are produced as follicles grow and develop on the ovary (Perry et al., 2014). In cattle, ideally only one follicle is selected for dominance from each follicular wave.

Following ovulation, follicular cells under the influence of gonadotrophins are transformed into the CL. This transformative process, a stage called metestrus, is a period of time in which neither E2 nor P4 are in high abundance. Following formation of the CL at approximately 5-7 days of the cycle (Senger, 2012), P4 is the dominating hormone and the animal has moved into the stage of diestrus. In the presence of a functional CL, progesterone is able to limit the stimulatory effects of E2 on the hypothalamus (Girmus and Wise, 1992). Follicles are still able to develop but will be

inhibited from reaching ovulation. The estrous cycle and its associated hormones are observed in Figure 1.2.

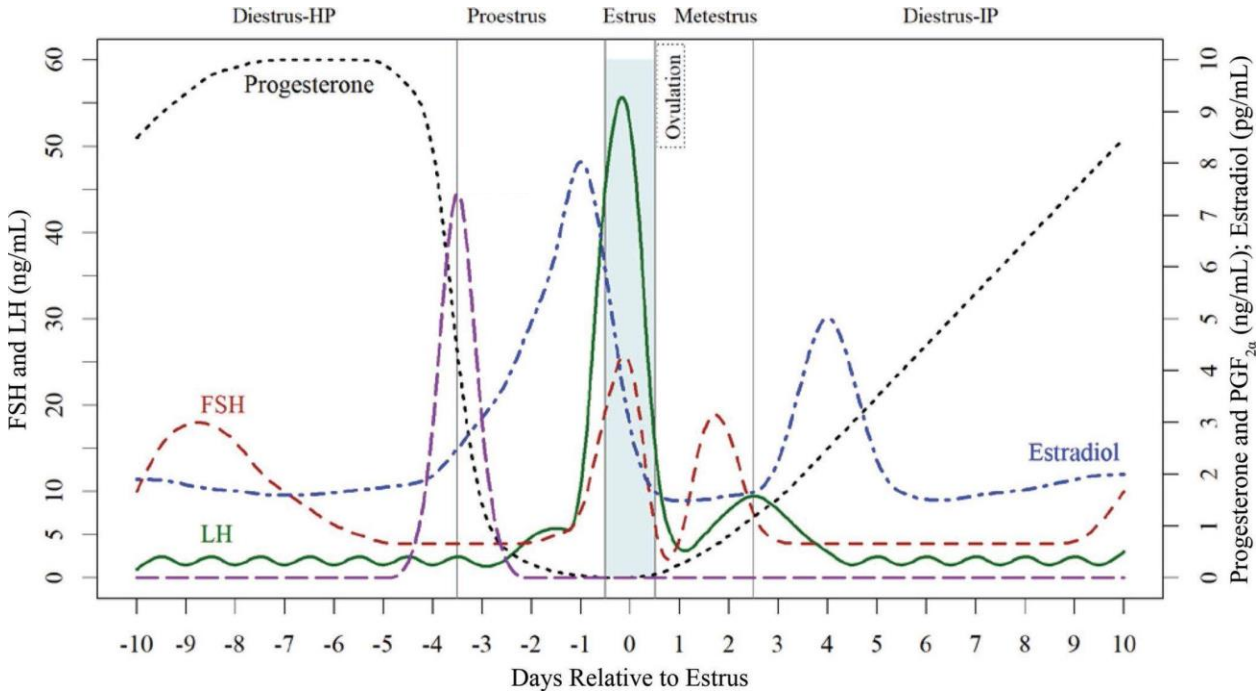

Figure 1.2: Example estrous cycle depicting patterns of secretion of FSH, LH, Progesterone, and Estradiol and plausible, representative concentrations of each hormone. Included are the four stages of the estrous cycle. (Toledo-Alvarado et al., 2018)

The Follicular Phase

The follicular phase is the portion of the estrous cycle in which antral follicles undergo a four stage maturation process known as folliculogenesis in preparation for ovulation. These stages are recruitment, selection, dominance, and atresia. FSH stimulates each follicular wave to begin growing a cohort of follicles towards of achieving dominance (Adams et al., 1992).

This stimulation causes antral follicles on the ovary to undergo the first step, recruitment, a process where a cohort of follicles begins to grow and secrete E2, with most of their fates being degeneration via atresia (Ginther et al., 1989). The cohort begins to emerge during days 1-2 of the estrous cycle in the proestrus stage. The follicles that do not undergo atresia go through the second step of folliculogenesis, a process known as selection, in which follicles are selected to proceed forward in development with a chance to become the dominant follicle (Senger, 2012). A single follicle begins to produce more E2 and inhibin than the others. Together, E2 feeds back positively to allow elevated LH levels for final maturation, and inhibin feeds negatively back to the anterior pituitary. Inhibin negatively regulates the production and secretion of FSH (Xu et al., 2020) which shunts follicular growth and results in eventual atresia. The singular follicle that did not undergo atresia during selection continues to grow to ovulatory size, becoming the dominant follicle, in the third step of folliculogenesis known as dominance.

Estrus is the stage when the female is receptive to mating prior to ovulation. The hormonal driver of this receptivity in females is E2 and is directly correlated with follicular domination and growth. Naturally coupled close with the timing of estrus is ovulation. Resulting from this E2 endocrine influence, ovulation in is considered spontaneous in nature; therefore, the process of ovulation is not dependent on copulation in cattle (Fricke and Wiltbank, 2022). Understanding the endocrine environments that influence the timing of ovulation in female cattle is incredibly important for successful reproductive programs that utilize artificial insemination (AI) as both the male and female gametes have finite lifespans. A preovulatory LH surge is necessary to initiate the hormonal cascade for ovulation to occur. The preovulatory LH surge leads to an increase

in prostaglandins, causing a release of lysosomal enzymes and contraction of ovarian smooth muscle. These actions in turn cause the follicular wall to weaken and increase pressure on the follicle with ovulation being the final event (Senger 2012). The mean duration of estrus between cows and heifers was found to be 11.9 hours with heifers having significantly longer estrus (Hall et al., 1959). In a previous study by Walker et al., mean ovulation time relative to first mounting behavior was 27.6 ± 5.4 h and was not different between animals given exogenous hormones to induce ovulation and those who ovulated spontaneously (Walker et al., 1996).

The Luteal Phase

After the dominant follicle has ovulated, the ruptured blood vessels of the follicular walls following ovulation create a bloody body known as the corpus hemorrhagicum. This process is driven by LH as it is responsible for transforming the remaining follicular tissue into functional luteal tissue, which will eventually become the corpus luteum (CL) (Alila et al., 1984). The remaining follicular tissue following ovulation consists of granulosa cells and thecal cells, which are transformed into large luteal cells and small luteal cells, respectively (Alila et al., 1984). Between day 3 and 5 following ovulation the CL begins to increase in size and in P4 production and become less bloody in appearance. The CL secretes progesterone from both luteal cell types and continues to grow until its maximum size is achieved and growth and hormone production plateaus. This formation of a normal, functional CL is necessary for normal cyclicity and pregnancy maintenance (Nancarrow, 1994, Zavy, 1994).

If pregnancy is not achieved, the uterus begins to secrete PGF2a episodically, causing regression of the CL in a process known as luteolysis. (Schams and Berisha,

2004). In the process of luteinization, receptors for PGF2a increase to reach maximum numbers per large luteal cell (Wiltbank et al., 1995). When luteolysis occurs, progesterone concentration decreases and is closely associated with a reduction in blood flow to the CL (Niswender et al., 1976) and a reduction in the steroidogenic capacity of luteal cells (McGuire et al., 1994). The sustained low level of progesterone allows re-cyclicity and eventual ovulation of a dominant follicle emerging from a group of follicles that has undergone selection.

Non-Heritable Influences on Cyclicity

Metabolites

It is important to minimize nutritional risk factors by maintaining proper body condition score in the dry period and early postpartum. Poor nutrition during the periparturient period can have a negative impact on reproductive efficiency as a consequence of the negative energy balance (NEB) and metabolic stress associated with nutrients being partitioned toward lactation (Table 1.1; Roche, 2006).

Reproductive process	Target to be achieved	Risk factors affecting targets
Normal uterine involution	50 DIM	Dystocia RFM Uterine infection
Resumption of ovulation	90% of cows by 42 DIM	Loss of > 0.5 BSC Low feed intake Uterine health
High oestrus detection	85% per cycle	Infrequent checks Sub-oestrus High milk yield
High conception rate to AI	50% per breeding	Excess BCS loss Prior uterine issue(s) Low P4 days 4-7 of pregnancy

Table 1.1. The postpartum reproductive targets to be met to obtain high reproductive efficiency and the associated key risk factors affecting these targets (Roche, 2006).

Nutrition requirements may not be met due to rations that are inadequately formulated or rations are offered in insufficient quantities. This NEB has negative implications for the oocyte (Leroy et al., 2006), the ovary (Llewellyn et al., 2007), and the uterus (Butler 2001), potentially impacting fertility in the postpartum cow. In the postpartum period, growth hormone concentrations are increased, inducing lipolysis and suppressing peripheral tissue insulin responsiveness (Roche 2006). Increased growth hormone in circulation sends the body into a catabolic state, causing a reduction of BCS and decrease in weight in cattle (Lucy 2003). In an attempt to overcome NEB in the periparturient period, fat mobilization is commonly utilized via lipolysis to support high energy demand that is not entirely met through the diet (Rodríguez et al., 2020). These mechanisms have implications for concentrations of metabolites such as glucose, NEFA, and insulin in circulation.

Glucose is an essential nutrient and is necessary for cells and tissues such as the brain and erythrocytes to have energy to function (Aschenbach et al., 2010). Glucose is a simple carbohydrate that plays a vital role in regulation of endocrine mechanisms controlling homeorhesis (Figure 1.3; Lucy et al., 2014). In the rumen and gastrointestinal tract, glucose is rapidly fermented to VFA and enters circulation before being resynthesized in the liver from propionate, amino acids, and glycerol in gluconeogenesis (Lucy et al., 2014).

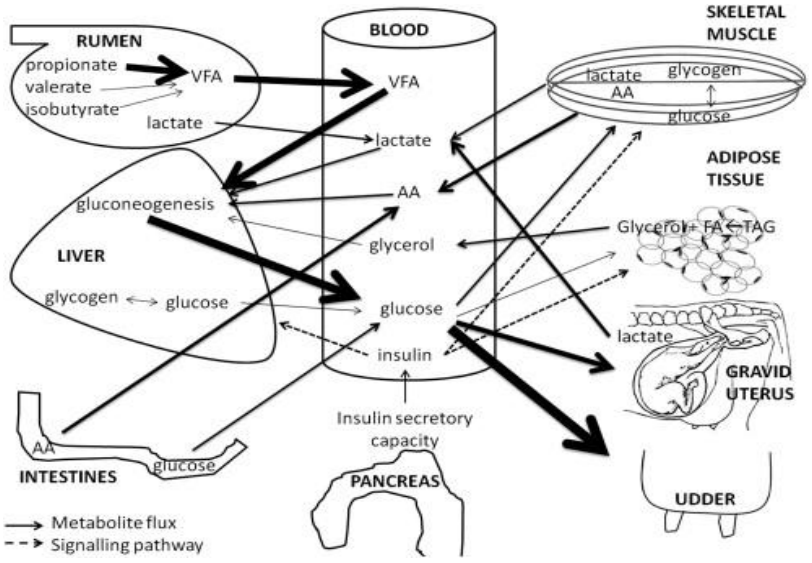

Figure 1.3: A schematic overview of the glucose metabolism in dairy cows where thickness of the arrow indicates the importance of the metabolite or tissue (Koster and Opsomer, 2013).

Glucose metabolism is regulated by secretion of insulin by the pancreas (Koster and Opsomer, 2013). Glucose is essential for lactose synthesis, with seventy-two grams of glucose required to produce one kilogram of milk recognizing most of this glucose is converted directly into lactose (Bell, 1995). With milk production increasing postpartum, glucose in circulation decreases to meet the needs of the mammary gland. The early postpartum cow experiences a series of homeorhetic mechanisms, including altering metabolism of body tissues, so nutrients can be prioritized for synthesis of milk in the mammary gland (Table 1.2) (Bauman and Currie, 1980).

Physiological Function	Metabolic Changes	Tissues Involved
Milk synthesis	Increased use of nutrients	Mammary
Lipid metabolism	Increased lipolysis Decreased lipogenesis	Adipose tissue
Glucose metabolism	Increased gluconeogenesis Increased glycogenolysis	Liver
	Decreased use of glucose and increased use of lipids	Body tissues in general
Protein metabolism	Mobilization of protein reserves	Muscle and other body tissue
Mineral metabolism	Increased absorption and mobilization of calcium	Kidney, liver, gut, and bone

Table 1.2: A partial list of the metabolic changes associated with lactogenesis in ruminants. Adapted from Bauman and Currie 1980.

While glucose is vital for milk production, it also has an influence on future reproductive performance. The demand for glucose in lactose synthesis leaves less glucose available to other tissues, including those involved in postpartum reproduction (Wathes et al., 2011; Green et al., 2012). Previous studies have shown that cows who become pregnant after first insemination had greater blood glucose concentrations on the day of calving and for the first 3 weeks postpartum compared to cows who did not successfully become pregnant (Garverick et al., 2013). This relationship between blood glucose concentration on day of calving and immediately thereafter improved future reproductive performance in both cows in confinement operations (Garverick et al., 2013) and also in cows on pasture-based systems (Moore et al., 2004). Instances of infection can cause this limit in glucose uptake by the ovary to preserve glucose for the immune system (Kaewlamun et al., 2020). Low blood glucose could compromise

metabolic processes in ovarian cells, including the oocyte that is glucose dependent for its energy, meaning difficulty with successful reproduction.

Glucose has a reciprocal relationship with NEFAs realizing that cows can break down triglycerides within adipose tissue to yield glycerol for energy and NEFA products, which are utilized for energy less effectively. Therefore, increasing glucose supply in circulation decreases the need to break down triglycerides, thus reducing circulating NEFAs. NEFAs provide a large portion of the body's energy and are utilized primarily as a marker of NEB in the lactating cow. Triglycerides can be broken down for use as an energy source and hydrolyzed into NEFA and glycerol (Oikawa et al., 2017). NEFA is mobilized to the liver and utilized in two pathways for energy: becoming acetyl-CoA for the tricarboxylic acid cycle for energy and re-esterified as triglyceride and secreted as low-density lipoprotein to be used as energy (Oikawa et al., 2017). In milk production, fat is formed by attaching glycerol to fatty acids synthesized from acetic acid. The fatty acids used originate from feed or are released from adipose tissue as NEFAs (Guliński 2021).

Dairy cattle are able to mobilize body fat from adipose tissue in an effort to maintain themselves when they are under metabolic stress. The rate of body tissue mobilization to maintain energy balance is influenced by dry matter intake, energy stores measured by BCS, and energy output measured by milk production. Cows enter a NEB postpartum and the body transitions to increasing usage of fatty acids as the main source of energy to preserved glucose stores for lactose synthesis (Wathes et al., 2013). The BCS of cows when they calve in is highly influential to their metabolic state and risk of disease. Overweight cows with a BCS of 4 or higher on a 5 point scale are at extreme risk

for disease while underweight cows with a BCS below 2.5 are at a much lower risk but will have poor production and reproduction (Guliński 2021). In a previous study, obese cows who lost BCS postpartum due to NEB had higher levels of NEFA in circulation than cows who maintained BCS (Wang et al., 2019).

Elevated plasma NEFA levels are characteristic of ketosis, the most common metabolic disorder in high-performance dairy cows that typically occurs within the first few weeks postpartum (Guliński 2021). The amount of energy and nutrients provided to the postpartum cow is often difficult to match her metabolic demand, which leads to the ketotic event. Clinical symptoms of ketosis include loss of appetite, an acetone odor in the mouth and urine, a drop in milk production, increase in concurrent illness, and poor reproductive performance (Guliński 2021). Cows who were diagnosed with ketosis in the first two weeks postpartum were 20% less likely to achieve pregnancy at first insemination (Walsh et al., 2007).

An in vitro study was conducted to explore the effects of NEFA concentration on proliferation and progesterone production of follicular granulosa cells along with its impact on oocyte development and fertilization. NEFA was found to cause a decline in proliferation of in vitro cultured granulosa cells and that CL growth was stunted during the NEB, resulting in a smaller CL size and therefore progesterone production (Jorritsma et al., 2004). Combined, these effects can cause irregular ovarian cyclicity, causing poor future reproductive performance in cows with a NEB. The presence of NEFA in the maturation stage delayed progression through meiosis and lowered successive fertilization of the oocyte in vitro (Jorritsma et al. 2004). While this study did not explain how high NEFA concentrations impact reproductive performance in the future, it is

believed that the follicles developing during the period of high NEFA and NEB are impacted, with potential for issues to manifest months down the line when those follicles have fully developed.

While in vitro studies are informative, they often miss complexities of dynamics in live animals. An in vivo study by Ospina et al (2010) found increased concentrations of NEFA in postpartum animals was correlated with poor reproductive efficiency. There is a strong association between NEB in the postpartum period and elevated NEFA concentrations which is likely the cause of poor reproductive performance (Herdt, 2000). Cows with elevated NEFA concentrations conceived less frequently at 1st service when compared to cows with low NEFA (Garverick et al., 2013).

In the early postpartum cow, a state of insulin resistance is assumed in which NEFA is elevated, glucose is prevented from being stored and prevented from being used for lipogenesis in adipose tissue (De Koster and Opsomer, 2013). The insulin resistance in this case is when a normal concentration of insulin elicits a decreased biological response in the typically insulin-sensitive adipose tissue and muscle (Kahn 1978). Glucose typically stimulates the release of insulin, with insulin then partitioning glucose toward adipose tissue and muscle. This process is allowed by glucose transporters moving to the cell surface (Fu et al., 2013. In the case of insulin resistance, glucose instead is needed to meet the needs of milk production because mammary gland requires a significant amount of glucose for milk production (50-85% of whole-body glucose consumption) (Drackley et al., 2001; Zhao and Keating, 2007; Lemosquet et al., 2009), where reduced glucose availability would mean reduced milk production. This state of

insulin resistance is vital as it maintains glucose reserves to be used in synthesis of lactose for milk production within the mammary gland.

In addition to insulin's role with glucose, insulin also plays a role in stimulating the liver to increase the expression of growth hormone receptors and to release IGF-1 into circulation (Thissen et al., 1994). The change from a catabolic state to an anabolic state is a key regulator of the reproductive axis (Kawashima et al., 2012). When glucose availability is low, circulating levels of insulin decreases and cows enter a catabolic state meaning they are prioritizing energy for production instead of maintenance. As the cow produces less milk, there is less glucose demand, leading to higher levels of insulin and IGF-1 in circulation. This transition allows the cow to exist in an anabolic state. When in an anabolic state, glucose can again be partitioned toward adipose tissue and muscle for storage. There is a positive association between insulin, IGF1, and the day postpartum that the cow achieves successful resumption of cyclicity (Velazquez et al., 2008). Glucose and insulin were found to be the most likely metabolites to exert an effect on GnRH secretion in the postpartum dairy cow (Bossaert et al., 2008). Therefore, increasing the glucose in circulation in order to increase insulin and IGF1 should cause an earlier resumption of cyclicity postpartum by causing the cascade initiated by GnRH.

The ovary is insulin-dependent, and glucose transporters GLUT1 and GLUT3 allow glucose to permeate the plasma membrane of the ovary (Nishimoto et al., 2006). Glucose uptake by the ovary may become limited resulting from low insulin and lack of responsiveness to insulin in insulin-sensitive tissues (Vernon et al., 1990). Low circulating insulin levels are associated with delayed resumption of cyclicity in the postpartum period (Laskowski et al., 2016). Selecting diet to increase levels of insulin in

circulation in the postpartum period can positively impact reproductive efficiency by increasing energy levels and has been observed to reduce the interval from calving to first estrus postpartum (Gong et al., 2002).

The Uterine Microbiome

The area of microbiome research has evolved extensively in recent years, and with development of new technologies and methodologies for its evaluation, has become an area of extreme interest. Microbiota can be defined as all living microbes within an environment (Berg et al., 2020) while the microbiome can be defined as the genes and genomes of the microbiota and their products within a given environment (Whiteside et al., 2015). Metagenomics refers to culture-independent studies of the collective set of genomes of mixed microbial communities (Machado et al., 2012).

Prior works relied on culture information for determination of bacterial clearing in the postpartum cow. Unfortunately, culture data may not provide enough information to make that determination. Traditional culture methods were only able to study 0.1-15% of naturally occurring microbes as most uterine bacteria can not be cultured (Lamont et al., 2011). The development of sequencing technologies such as 16S rRNA gene sequencing allowed researchers to discover bacterial communities of commensal microbes in low density environments, like the uterine environment of a healthy animal (Santos et al., 2011).

The uterine environment in dairy cattle has historically been considered a clean and sterile environment as the uterus is separated from the vagina by the cervix, both physical barriers designed to protect the uterus. Potential sources of the uterine microbiota are originating from the gut and oral microbiota, ascension of vaginal

bacteria, and/or transmission of bacteria via the seminal microbiota (Altmäe 2018; Baker et al., 2018). Additionally, in the calving process, the cervix dilates causing a breach of the protective physical structures, and the uterine environment is exposed to bacteria and becomes rapidly colonized by a dynamic microbial community (Sheldon et al., 2009).

The bacteria that may infiltrate the uterine environment during the calving process are considered to originate from feces and the environment (Nguyen et al., 2019). With adequate immune response, animals should control and tolerate pathogenic bacteria in the postpartum uterus rather than eliminate it, because the microbiota contains microorganisms that are commensal and symbiotic as well (Karstrup et al., 2017; Moore et al., 2017; Machado et al., 2012). In the periparturient period, the dairy cow's immune system is suppressed, resulting from metabolic stress and/or a negative energy balance associated with a lack of sufficient nutrition to compensate for milk production (Mordak and Anthony, 2016). Between 80-100% of cattle were found to have bacteria in their uterine lumen within the first 2 weeks postpartum, and if the immune system is overwhelmed, uterine disease can occur (Sheldon et al., 2008; Galvão et al., 2019).

Using technological advancements such as 16S rRNA sequencing, healthy animals were found to have greater bacteria richness and diversity than animals with disease (Galvão et al., 2019). *Firmicutes* was found to be the most abundant phylum of the uterine microbiome, showing abundances of 52.3 and 76.7% in the uterine microbiota of healthy cows at around one month postpartum (Machado et al., 2012; Wang et al., 2018). Another study reported that *Proteobacteria* was the most abundant phylum in the uterine microbiota of healthy cows (Santos et al., 2011). A previous study by Moore et al (2017) found the three most abundant phyla of the pregnant uterus to be *Firmicutes*,

Bacteroidetes, and *Proteobacteria*. Some of the microbes that healthy animals harbor are found in animals with uterine disease, implying the immune system plays a role in the extent of overgrowth of pathogenic bacteria (Moore et al., 2017).

There are disease-related alterations in the microbiome in this time period as pathogenic bacteria can suppress existing communities by reducing overall bacterial diversity and colonizing new bacterial populations (Fredericks et al., 2005; Manichanh et al., 2006; Ott et al., 2004). Early research has indicated that animals with puerperal disorders had lower microbial diversity at a genus level than animals who were healthy postpartum. In a study by Kronfeld et all in 2022, 27 of 46 dairy cattle observed had pathological puerperium, including retained placenta, puerperal metritis, clinical metritis, and clinical endometritis. Diseased cattle were positive for seven genera for which healthy cows were negative, but the diseased cattle also lacked twenty genera that were found in healthy cows (Kronfeld et al., 2022). *Escherichia coli, Trueperella pyogenes*, *Fusobacterium necrophorum* and *Prevotella* spp. are well known opportunistic uterine pathogens (Wagener et al., 2015). The most frequently isolated, populous intrauterine bacteria are *Bacillus*, *Streptococcus*, *Enterococcus*, and coagulase-negative *Staphylococci* (Westermann et al., 2010, Werner et al., 2012). Two of the most common uterine pathogens, *Escherichia coli and Trueperella pyogenes*, are known for their potential to cause detrimental effects on fertility by impacting the uterine endometrium and disturbing ovary function (Sheldon et al. 2002, Amos et al., 2014). Uterine disease associated with *Escherichia coli* reduces estrogen secretion from the dominant follicle (Sheldon et al., 2002; Herath et al., 2007) and delays the LH surge and ovulation (Suzuki et al., 2001).

Heritable Influences on Cyclicity

Fertility in dairy cattle has decreased over the past five decades as milk production per cow has increased. The selection intensity for production-based traits has led to metabolic extremes in animals as well as reduced selection pressure on other traits such as fertility. Its decline is likely a result of a complex interaction between genetic, environmental, and managerial factors, making it difficult to pinpoint an exact cause. Homeorhetic mechanisms give priority to milk production over other functions such as reproduction along with cattle experiencing increased metabolic pressure resulting from a negative energy balance associated with high milk yields (Bach 2018). In recent years, there has been a shift in emphasis for selection indices in Holstein-Friesians from predominantly production-based traits to functional nonproduction traits associated with improved health and fertility (Miglior et al., 2005). These genetic traits and their interaction with the environment play an incredibly important role in determining estrus expression and therefore breedability and herd longevity.

Anti-Mullerian Hormone

Anti-Müllerian Hormone (AMH) is considered a potential biological marker of fertility in dairy cattle. AMH plays a vital role in fetal sex differentiation during embryogenesis. The Y chromosome contains a gene called the Sex Determining Region (SRY) and SRY protein controls the embryo's pathway of sexual differentiation. When SRY protein is synthesized, the male reproductive system's development is stimulated. Since the X chromosome does not have this region or protein, female genes will be expressed. As SRY protein stimulates testes development, Sertoli cells in the testes of the

embryo secrete AMH which causes regression of the Müllerian duct (Senger, 2012). This regression allows the male reproductive tract to fully develop.

By 52 days of gestation, the Müllerian ducts in fetal females lose responsiveness to AMH (Vigier et al., 1982) At 100 days of gestation, the fetal female ovary begins differentiating, which is long after the Müllerian ducts regress in the male fetus and long after responsiveness to AMH has been lost in the female fetus (Takahashi et al., 1986). Late in gestation, the granulosa cells of healthy, growing follicles of the ovaries in females begin secreting AMH (Vigier et al., 1982). This lack of responsiveness to AMH's properties in the female allows her to synthesize and secrete AMH without regressing their own reproductive tracts.

With the granulosal cell source point, AMH is considered representative of the ovarian reserve in female dairy cattle as plasma AMH is both positively and highly correlated with antral follicle count (AFC) (Ireland et al., 2008, Riberio et al., 2014). Additionally, AFC was found to be extremely variable between animals, but highly repeatable within animals over time, allowing AMH to be used reliably for phenotyping (Burns et al., 2005). AMH's reliability for utilization is further confirmed with a heritability much higher than other reproductive metrics in cattle ($h^2 = 0.36 \pm 0.03$) (Nawaz et al., 2018). A previous study has shown that when comparing animals with low and high antral follicle counts, fertility is higher in animals with higher AFC (Jimenez-Krassel et al., 2009). Dairy heifers with low AMH concentrations compared with heifers with higher AMH concentrations subsequently had lower pregnancy rates, higher probability of being culled early in their first lactation, and shorter herd longevity (Mossa et al., 2017). A study conducted on both primiparous and multiparous Holstein dairy

cattle showed no correlation between AMH concentrations and parity number or milk production level (Souza et al., 2015).

Daughter Pregnancy Rate (DPR)

Daughter pregnancy rate (DPR) is defined as the percentage of cows eligible to become pregnant in a 21-day period that actually become pregnant (Lima et al., 2020). Phenotypic and genetic trends for milk production and DPR had a negative correlation between the 1960s and 2000s with milk production breeding values increased by about 3,000 kg accompanied by a concurrent decline in fertility (AIPL 2005) (Figure 1.4). The genetic evaluation of DPR was introduced in the United States in 2003 to try to combat the decline in fertility seen with the selection for increased milk production (VanRaden et al., 2004). However, DPR values in 2019 were still severely diminished compared to values in the 1960's (USDA, 2019).

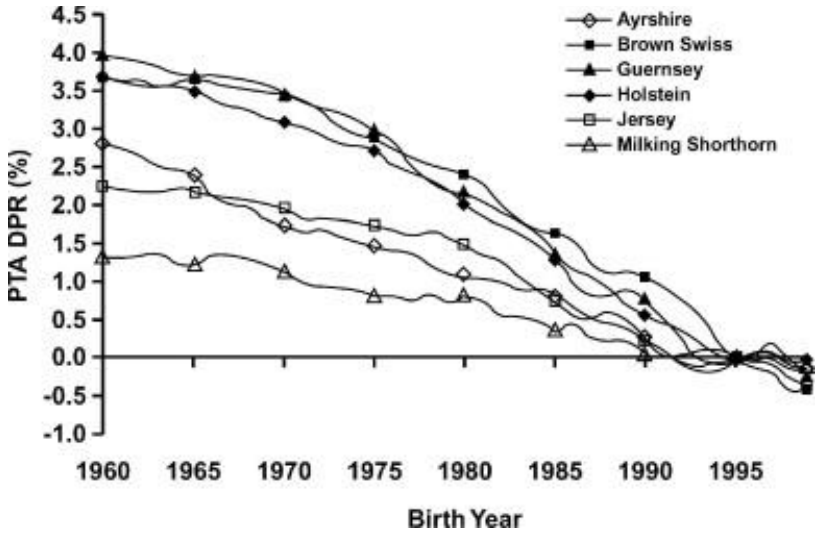

Figure 1.4. Trend in PTA DPR for bulls born from 1960 to 1999 by breed. Trends were estimated, but breed differences were not (VanRaden et al., 2004).

DPR is calculated with genetic evaluation expressed as deviation from a base pregnancy rate within each individual breed. An increase of 1 point in DPR is expected to

result in a reduction of the interval from calving to pregnancy of approximately 4 days (Norman et al., 2009). DPR is lowly heritable with a heritability of h^2=0.014 (Council on Dairy Cattle Breeding, 2019).

Heifer and Cow Conception Rate

Heifer conception rate (HCR) is defined as a virgin heifer's ability to conceive, calculated as the percentage of heifers that become pregnant at each service, while cow conception rate (CCR) is defined as a lactating cow's ability to conceive, calculated as the percentage of cows that become pregnant at each service (CCR) (Kiser et al., 2019). Both HCR and CCR have low heritability, similarly to DPR, with heritabilities of h^2=0.01 and h^2=0.016, respectively (Council on Dairy Cattle Breeding, 2019).

Environmental Factors that Influence Cyclicity

Heat Stress' Impact on Cyclicity

Cycling year round and without regard to changes in amount of daylight per day means that cattle are polyestrous. However, this does not mean they are not impacted by seasonality. Heat stress is when cattle generate and absorb more heat than they can easily diffuse leading to increased respiration rates and body temperatures. Heat stress in cattle is achieved when the temperature humidity index (THI) exceeds the comfort threshold (> 72), evoking a physiological response (Ravagnolo and Mistzal 2000). During summer months, heat stress can compromise fertility in both nulliparous heifers as well as primiparous and multiparous cows. However, lactating cows are more susceptible to the detrimental effects of heat stress due to the metabolic heat production associated with lactation, which makes bodily temperature regulation more difficult than in non-lactating heifers (Sartori et al, 2002). It has been previously estimated that fertility begins to

decline when uterine temperatures rise 0.5° C above normal temperatures in lactating cows (Gwazdauskas et al., 1973)

The level of stress induced by THI is not relegated to implications to the metabolic systems in an attempt to dissipate heat. Instead, THI can affect many other biological processes to include those of the reproductive system. The detrimental effects are found at both the uterine and ovarian level from direct insults as well as disrupted function through reduced hormone concentrations resulting from circulatory changes.

A previous study found lactating cows bred by artificial insemination had fertility rates of 88% in the winter which were lowered to 55% in the summer (Sartori et al., 2002). There is some evidence that there is higher pregnancy loss in heat stressed animals, as losses seen between days 34 to 45 of gestation and day 90 were 2% in cooler months and 12% in warmer months (García-Ispierto et al., 2006). Follicular dominance is reduced in cattle experiencing heat stress, meaning there is an increase in the overall number of large follicles present on the ovary, increasing FSH and decreasing E2 and inhibin in circulation (Roth et al., 2000). In a chronic heat stress study in mice, mice exposed to heat stress for 3 and 6 weeks had smaller ovaries smaller with less vasculature and higher incidence of atretic follicles were with more oocytes that were detached from the granular cell layer when compared to mice with no heat stress (Bei et al., 2020). Similarly, cows exposed to heat stress develop oocytes with reduced competence for fertilization and subsequent development (Roth 2008). This exposure has detrimental effects for a sustained period of time, and it takes a period of up to two or three estrous cycles in order to recover from heat damage and for competent oocytes to return (Roth et al., 2001).

Voluntary Milking Systems

Voluntary milking systems have the potential to increase milk production from cows while reducing labor input by utilizing robotic technology to milk cows. These systems allow cows to voluntarily milk themselves, meaning there is a behavioral component differing from conventional farms. The number of times a cow goes to milk at the robot and to consume feed at the feed bunk on these farms is heavily influenced by the design of cow traffic systems, with the series of gates guiding the cow to follow a set pattern within the barn (Ketelaar-de Lauwere et al., 1998). There are three types of systems: guided flow, free flow, and hybrid flow. Guided flow systems are designed to direct the cows to be milked in the robotic milking unit (RMU) before they can access the feed alley, while free flow systems allow the cow to move freely between freestalls, the RMU, and the feed alley. Hybrid flow systems have one-way gates around the robots but unrestricted access to feed bunks and stalls. Although these systems are voluntary, there is still a diurnal nature associated with them; less milking occurs between 2200h and 0700h (Wagner-Storch and Palmer, 2003).

In conventional systems cows who milk voluntarily and milk 3 to 4 times a day compared to the traditional 2 milkings a day were found to have a higher incidence of metabolic disorders and more days open (Ipema 1991). Cows who have a higher frequency of milkings have a higher milk yield without a proportional increase in feed intake, consequently leading to a deeper NEB, which could potentially be responsible for the faults to metabolic and reproductive success (Ipema 1991, Butler and Smith, 1989). This deeper NEB was found to be directly related to a longer postpartum interval and lower conception rates (Butler and Smith, 1989). Cows on dairies utilizing RMS may

experience NEB in the periparturient period and exhibit different patterns of metabolites compared to cows on conventional dairies resulting from different feeding behavior (Abeni et al., 2005). The opportunity for increased milking frequency and increased milk production on a robotic dairy is known to increase production efficiency in dairy cattle with potential to increase milk yield from 3 to 11% per cow (Baines 2002; de Konig et al., 2002; Wade et al., 2004). However, feeding behavior is altered on farms utilizing RMS, where feeding activity was seen to increase after human intervention of moving cows to the robots while on conventional farms, feeding activity was seen to increase after milking and feed delivery (Wagner-Storch and Palmer, 2003). Feed intake during the night is less frequent in farms utilizing RMS (Olofsson et al., 2000). Additionally, the social hierarchy seen in cows is exacerbated on farms utilizing RMS, and socially low-ranked animals spend less time in the feeding area and make less visits to the robots (Olofsson and Svennersten-Sjuanja, 2004). Consequently, increased milk production associated with RMS increases the risk for NEB and metabolic disorders, having negative implications for reproductive efficiency.

Working to Improve Reproductive Efficiency in Dairy Herds

The difficulties of estrous detection for artificial insemination (AI) in dairy herds can be exacerbated in modern herds due to large herd (Macmillan 2010). AI can be utilized more effectively in herds through implementation of estrous and/or ovulation synchronization protocols. However, the presence of hormones in dairy products is of concern to distrustful consumers, and it is necessary to explore other methods of improving reproductive efficiency beyond these synchronization protocols (Pieper et al., 2016). Therefore, understanding and identifying the behavioral aspects of estrus is the

first step to achieving optimal estrous detection rates without synchronization protocols and therefore leading to efficient reproduction.

Traditional methods of estrous detection consisted of visual observation of cattle, but with herd size increasing this is not as viable of a method. 90% of low estrous detection rates can be attributed to poor management observations, with only 10% of low detection rates resulting from the cow (Diskin and Sreenan, 2000). It has been reported that selecting for traits such as high milk production has a negative impact on estrous detection rates. The average duration of estrus in high producing Holstein cattle was found to be only 6.2 hours every estrous cycle (estimated at 21 days) (Lopez et al., 2004). Within those 6.2 hours, high producers (46.4kg/d ± 0.4) were only found to have 21.7 seconds (±1.3) of total standing time. While not seeing much improvement, low producers (33.5kg/d ± 0.3) were found to have estrus duration of 10.9 hours (±0.7) with total standing time of 28.2 seconds (±1.9) (Lopez et al., 2004).

Methods of Estrous Detection

Since there is such high variation in estrus durations and intensities, estrous detection in large herds poses as problematic. Automated sensor-based technologies offer producers a high efficacy, low input method of monitoring activity in cattle. These activity monitoring systems (AMS) have been evolved to continuously monitor and record data about each individual animal and have been heavily refined for accurate detection of estrus in cattle. There are several types of AMS used for reproductive management, including pressure-sensitive mount detectors, pedometers, and accelerometers.

Mount detectors such as HeatWatch® (DDx Inc., Boulder, CO, USA) are glued to the sacral region anterior to the trailhead and are triggered by the weight of a mounting animal for a minimum of 2 seconds to limit false-positive results, but up to 40% of mounts were found to be < 2 seconds (Walker et al., 1996). The information collected by the sensor is sent via radio signal to a receiver and are recorded for an algorithm to classify a standing event as three or more standing events in any 4 hour period (Diskin and Sreenan, 2000). This system was found to accurately detect 82.1% of ovulations and improved detection of estrus compared to traditional methods of visual observation (Lopez et al., 2004).

Pedometers can be attached to the leg of a cow and record the number of steps taken per specified unit of time. Data is stored in memory, transferred to receivers placed near the milking system, and transferred to the management software. Cows coming into estrus are identified by the pedometers as having an increased number of steps above the individual's average activity recorded during the same time period for within-cow comparison (Yániz et al., 2006).

Activity monitoring systems using accelerometers are attached to neck collars for individual cattle to wear. These devices on collars will continuously measure horizontal accelerations related to upward movement of an individual cow's head and neck throughout the day during both normal walking and mounting behavior (Reith et al, 2014). A baseline is established for each individual cow for within-cow comparisons to be made as data is collected from the devices. Algorithms are specially created to evaluate deviations from the previously stored individual cow's established baseline level of activity in order to identify and separate estrus behavior from normal daily activities.

The producer will receive an alert if this threshold level of baseline activity is surpassed, indicating a potential and probable estrus event. Information collected by these devices are received by an antenna and automatically transferred via IR signal to the herd management software providing the producer with lists and graphs to monitor and manage reproductive status of individual cows (Reith et al., 2014).

DeLaval's heat detection program, DelPro™, continuously records movement via a collar placed around the neck of the animal. Raw activity is recorded every 14 seconds and scored with a value of 0 (no activity) or 1 (activity), with a maximum number of activity records per hour for an individual animal being 255. An algorithm is generated for each individual cow and compares her history of activity against herself to determine standard deviations for determination of probable estrous events. Cows with more erratic behavior have increased activity, translating into a larger standard deviation from their normal activity. The larger the standard deviation, the more reliable the estrous event.

When these systems correctly identify an estrous event, the alert is classified as a true positive. If the system were to alert outside of an estrus event, it is classified as a false positive. Estrus events that occur without the system identifying them lead to false negative results. Algorithms and the sensitivity level of these systems must be refined in order to minimize the number of false negative and false positive alerts. The percentage of false alerts compared to the number of detected estrus events is considered the error rate to evaluate how the system is working for a specific farm (Firk et al., 2002).

All of this data culminates the idea that there are many influencing variables in the postpartum period that have implications for future reproductive success in the dairy cow. The knowledge of these implications can help producers change management

practices to better manage cows through the metabolically challenging transition period. These considerations for management changes can assist producers by increasing reproductive efficiency of cows in the early postpartum period by reducing days between calving and first insemination while also increasing herd longevity.

Objectives of the Study

The objectives of the upcoming study were to examine the relationship between variables impacting the time to resumption of cyclicity in the postpartum period. The first part of the study focuses on factors influencing cyclicity resumption on a robotic dairy farm. Dairies utilizing RMS have different behavioral and feeding aspects when compared to conventional dairies, which could have implications for cyclicity resumption. The second part of the study was used to investigate cyclicity resumption in Jersey cattle by utilizing genomics and production. Further analyzation was done in this piece to compare Jerseys and Holsteins. The third part of the study was to evaluate the uterine microbiome and its diversities and abundance in both Jersey and Holstein cattle and the implications on cyclicity resumption in the postpartum period.

CHAPTER 2

EVALUATING THE INFLUENCE OF HERITABLE, METABOLIC, AND PRODUCTION PARAMETERS ON CYCLICITY RESUMPTION IN A DAIRY WITH A ROBOTIC MILKING SYSTEM

Johnson, Sarah. To be submitted to *Journal of Dairy Science*.

Abstract

The postpartum period is a challenging time in the dairy cow's life with production demands often overshadowing the need to resume cyclicity. Therefore, this study aimed to explore the metabolic markers, non-esterified fatty acids (NEFA) and glucose (GLU), alongside the heritable markers of insulin (IN), insulin resistance (INR), and anti-Müllerian hormone (AMH) and their influence on resumption of cyclicity postpartum in a robotic milking system (RMS). Holstein cows (n=95) were enrolled at 14 ± 3 days in milk (DIM) with blood samples taken weekly. All samples were analyzed for evaluation of metabolic profiles of NEFA, GLU, and IN until 49 ± 3 DIM with a surrogate index of RQUICKI used to estimate INR. RMS visits and milk production data were collected on day of sampling and averaged with data on the day preceding and the day following. Animals were fitted with a DeLaval activity meter and monitored from day of calving until 100 DIM using DelPro™ Farm Manager. Both (++) and (+++) reproductive attentions were used to identify an estrous event with estrous intensity (EI) recorded. Animals were considered to have normal (N) resumption of cyclicity if an estrous event was identified on or before 45 DIM or delayed (D) resumption beyond 45 DIM. Data were analyzed using the MIXED procedure of SAS with resumption of cyclicity as treatment and Pearson's correlation coefficients. GLU was higher in N cows on d 35 ($P = 0.020$) and d 42 ($P = 0.018$). NEFA concentrations decreased in all animals over time ($P < 0.05$). NEFA was greater in delayed animals on d 21 ($P = 0.044$) and d 28 ($P = 0.061$) but greater in normal animals on d 49 ($P = 0.050$). IN increased over time in all animals ($P < 0.05$) but did not differ between treatments ($P > 0.05$). Higher milk yield corresponded with increased RMS visits ($P < 0.001$, $r = 0.39$). RQUICKI did not change

over time for either treatment ($P > 0.05$). EI was not different in animals that had normal (191% ± 6.6) versus delayed (179.1% ± 5.97) resumption of cyclicity ($P > 0.05$). AMH was not different between N (223.4 ± 28.62 pg/ml) and D animals (217.6 ± 25.22 pg/ml) ($P > 0.05$) but tended to increase EI at first estrus (P = 0.052). Milk production and its association with glucose and RMS visits are key drivers to cyclicity resumption in a RMS dairy.

Introduction

Reproductive inefficiency is one of the most common causes for cull decisions made by producers. The single-trait selection pressure for milk production has historically been one of the strongest influencers for genetic selection of animals, which led to a concurrent decline in general dairy cattle fertility (Crowe, 2018). Although not directly causing poor reproduction, other factors such as the increased metabolic demand and negative energy balance associated with higher milk production have a negative impact on resumption of cyclicity postpartum and eventual successful reproduction (Butler and Smith, 1989). To maximize reproductive efficiency postpartum, cows must resume normalized ovarian cyclicity, have complete uterine involution, and have estrous intensity strong enough for estrus detection (Crowe, 2008).

The transition period is a critical time in dairy cattle health, as they are undergoing major physiological and metabolic changes. Rising milk production in early lactation coupled with inadequacies in meeting this metabolic demand causes cows to enter a state of negative energy balance (NEB) whereby they begin to mobilize body reserves to meet energetic needs (Butler and Smith, 1989). During NEB, there is increased lipolysis of adipose tissue to supply the body with appropriate levels of energy

(Bauman and Currie, 1980). These mechanisms have implications for concentrations of metabolites including glucose, NEFA, and insulin in circulation, which all potentially impact fertility in the postpartum cow.

Cows on dairies utilizing robotic milking systems (RMS) may have different transitional needs in the periparturient period due to different patterns of metabolites compared to cows on conventional dairies (Abeni et al., 2005; Solano et al., 2022). The opportunity for increased milking frequency and increased milk production on a robotic dairy is known to increase production efficiency with improvements in milk yield from 3 to 11% per cow (Baines 2002; de Konig et al., 2002; Wade et al., 2004). To support the increased milk production, feeding behavior can be altered on farms utilizing RMS depending on flow system (free, hybrid, or guided) with some flow systems encouraging cows to milk before accessing food, along with cows receiving concentrate in the form of pellets while being milked. Previous studies find feeding increases after human intervention of fetching the cow to the robots while on conventional farms, feeding increases are seen after milking and feed delivery (Wagner-Storch and Palmer, 2003). Additionally, the social hierarchy seen in cows is exacerbated on farms utilizing RMS, and socially low-ranked animals spend less time in the feeding area and make less visits to the robots (Olofsson and Svennersten-Sjuanja, 2004). The tendency for increased milk production associated with RMS could increase the risk for NEB and metabolic disorders, potentially having negative implications for reproductive efficiency.

It is well documented that the metabolic demand stemming from milk production can lead to a reduction in estrous expression with a representative study reporting high milk producers (46.4 ± 0.4 kg/d) were found to be in estrus an average of 6.2 hours every

estrous cycle and within that time frame, were only found to have 21.7 ± 1.3 seconds of total standing time (Lopez 2004). Comparatively, low milk producers (33.5 ± 0.3 kg/d) were found to have extended estrus duration (10.9 hours) with 28.2 ± 1.9 seconds of total standing time. High variability in both duration and intensity of estrous events and influential variables on cow behavior make visual detection of estrus difficult (Reith and Hoy, 2018). Activity monitoring systems (AMS) offer the opportunity to capture estrous events with lower intensities than traditional methods of observation, identifying animals that may have been considered sub-fertile before. Research indicates that multiple different commercial activity monitoring systems correctly identified and alerted for estrus 15 to 35% more cows in heat than traditional visual observation occurring four times a day (Mayo 2019).

While AMS can identify estrus events, there are few predictive tools to promote reproductive success in dairy cattle beyond genomics. In the evaluation of biological methods to identify more fertile animals, Anti-Müllerian hormone (AMH) is promising as a biological marker for fertility in dairy cows. AMH is highly associated with the number of antral follicles on the ovaries and has been historically used as a marker for the ovarian reserve (Riberio et al., 2014). AMH has been found to be moderately heritable (h^2 =0.36±0.03) and with positive correlation with ovarian follicular reserve, AMH's potential relationship with estrous intensity is of high interest in this study (Nawaz 2018). AFC was found to be extremely variable between animals, but highly repeatable within animals over time, allowing AMH to be used reliably for phenotyping (Burns et al., 2005). If a correlation between AMH concentrations and estrous intensity is discovered, AMH could be a useful tool for future reproductive improvement in dairy cattle selection.

The objectives of this study were 1) to evaluate how production and metabolites impact postpartum resumption of estrous cyclicity and intensity of estrus, and 2) to explore the relationship between AMH and time to the first estrous event and estrous intensity at that event. It was hypothesized that high production cows and/or cows with metabolic imbalance in the transition period would have an extended days in milk at first estrus and lowered estrous intensity as compared to cows who are lower in production and/or without metabolic imbalance.

Materials and Methods

Animals

Holstein cows (n= 95) of varying lactations (1^{st}-7^{th}) without clinical signs of disease were enrolled in the study at 14 (± 3) DIM and housed on a commercial dairy operation with a robotic milking system (RMS) in Northeast Georgia. Animals were housed in two separate groups allocated by farm management to distribute lactation number and milk production. All animals were housed with free stall housing and received the same pellet allocated at levels to match production in addition to the same free choice partially mixed ration (PMR). Each group had access to two DeLaval VMS™ V300 robotic milkers and all animals were equipped with DeLaval collars with activity monitored for estrous events utilizing the DeLaval DelPro™ system. Days in milk at first estrus and intensity of that estrous event were recorded for each animal. Milk production and visits to the RMS were recorded on day of sampling and averaged with data on the day preceding and the day following. All sampling and data collection concluded after an estrous event was identified for each animal. If no estrous event was recorded at 100

DIM, the animal was ultrasounded and sampling concluded. No exogenous hormones were used throughout the duration of the study.

Estrous Event Identification

DeLaval's activity monitoring program, DelPro™, continuously records movement via a collar placed around the neck of the animal and was used to determine estrous events for this study. Estrous events are determined by 3 levels of estrus alarms in DelPro™: (+) = activity 3.8 standard deviations above normal, (++) = activity 5 standard deviations above normal, (+++) = activity 6 standard deviations above normal. A (+++) alert is the most reliable and accurate estrous alert. Estrous intensity is reported on a scale of 0% - 255%, with 100% being average activity and 255% being the maximum amount of activity. To be considered an estrous event in the current study, the alert must have been (++) or (+++). Days in milk at first estrus and estrous intensity were recorded for all animals with intensity reported on a scale of 0% - 255%

Ultrasonography

Animals not having an estrous event as identified by DelPro™ by 100 DIM were transrectally ultrasounded to evaluate the uterus and ovaries (n=9). The uterus was observed for any abnormalities or anomalies. The ovaries were scanned for ovarian abnormalities as well as to confirm cyclicity status, with the presence of a corpus luteum (CL) indicating resumption of cyclicity was achieved. Of the nine animals ultrasounded, three were truly non cycling while one of the noncycling animals had a uterine infection. These observations were recorded for later analysis.

Blood Collection

Blood samples for progesterone analysis were collected weekly beginning at enrollment (14 ± 3 DIM) and continuing until animals experienced a first estrous event or reached 100 DIM with no estrous event. An additional blood sample was collected between 45-60 DIM for Anti-Müllerian hormone (AMH) analysis. All samples were collected via coccygeal venipuncture into vacutainer tubes using no additives.

Blood Processing, Storage, and Analysis

Following collection, tubes were immediately placed on ice and transported to the lab. Samples were centrifuged at 3000 rpm for 25 minutes to separate serum. Serum was extracted using a pipette and aliquoted to microcentrifuge tubes in triplicate labeled with animal number and sampling date. Microcentrifuge tubes were stored in a -20° C freezer until future analyses. Serum was analyzed for progesterone at Clemson University (Clemson, SC, USA) using a radioimmunoassay (RIA) to detect the amount of antigen in the sample using antibodies. Frozen serum was shipped on dry ice overnight to Motive Biosciences Inc. (Webster, TX) for AMH analysis using an enzyme-linked immuno-absorbent assay (ELISA). The assay uses unique monoclonal antibodies and is calibrated using recombinant bovine AMH.

Metabolic Analysis

Blood samples taken weekly from 14± 3 DIM until 49 ± 3 DIM were analyzed for glucose, non-esterified fatty acids (NEFA), and insulin. Glucose was analyzed using the Autoki Glucose by FUJIFILM WAKO for the quantitative determination of glucose in serum using a 10% CV threshold. This kit uses an enzymatic colorimetric method that combines the traditional glucose oxidase method with the enzyme mutarotase to facilitate the conversion of a-D-Glucose to b-D- Glucose. NEFA was analyzed using the NEFA-

HR(2) kit (FUJIFILM WAKO, Minato City, Tokyo, Japan) for the quantitative determination of NEFA in serum. Average assay CV for NEFA was 9.1%. The FUJIFILM Wako kit uses enzymatic methodology and relies upon the acylation of coenzyme A (CoA) and ultimately results in a purple-colored sample that is measured colorimetrically at 550 nm. Insulin was analyzed using the Mercodia Insulin ELISA for specific measurement of insulin in serum. Average assay CV for insulin was 2.3%. Insulin resistance was calculated using the Revised Quantitative Insulin Sensitivity Check Index, RQUICKI = 1/ [log (Glucose) + log (Insulin) + log (NEFA)].

Statistical Analysis

Days to first estrus, AMH, and intensity at first estrus were analyzed using PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). Normal cyclicity was defined as the animal experiencing first estrus before 45 DIM, with delayed cyclicity occurring after 45 DIM. The statistical models included treatment (normal or delayed cyclicity) as the fixed variable, with cow nested within treatment as the random variable. Repeated measures data collected (metabolic parameters, milk production, robot visits) were analyzed by PROC MIXED procedure. The statistical models included treatment, time, and treatment by time interaction as fixed variables, with cow nested within treatment as the random variable. When the interaction between treatment and time was significant or tended to be significant, the SLICE function was used to detect treatment difference at each time point. Correlation coefficients were determined for all associated variables. T-tests were used to evaluate differences in the averages of each treatment.

Results

The average DIM at 1st estrus was 33.3 ± 1.85 days in N cows (n=43) and was earlier compared to D cows (n=52) who experienced 1st estrus on average at 66.6 ± 1.85 days ($P < 0.010$) (Table 2.1). Estrous intensity at first estrus was numerically higher in N cows (191 ± 6.0) compared to D cows (179 ± 6.0) but was not statistically different ($P > 0.05$).

Table 2.1: Average days in milk at 1st estrus and average intensity at 1st estrus.

		Normal (N)		Delayed (D)	
Parameter	n	Mean ± SD	n	Mean ± SD	*P-value*
DIM at 1st Estrus	43	33.3 ± 1.85	52	66.6 ± 1.85	< 0.010
Estrous Intensity at 1st Estrus	43	191 ± 6.0	52	179 ± 6.0	0.180

The average AMH for N cows was numerically higher at 223.4 $\pm$ 28.62 pg/mL but was not different from D cows with an average AMH of 217.6 $\pm$ 25.22 pg/mL as observed in Figure 2.1 ($P > 0.05$). Estrous intensity and AMH had a low degree of correlation (r=0.21) but tended to be higher in animals with greater estrous intensity ($P = 0.052$).

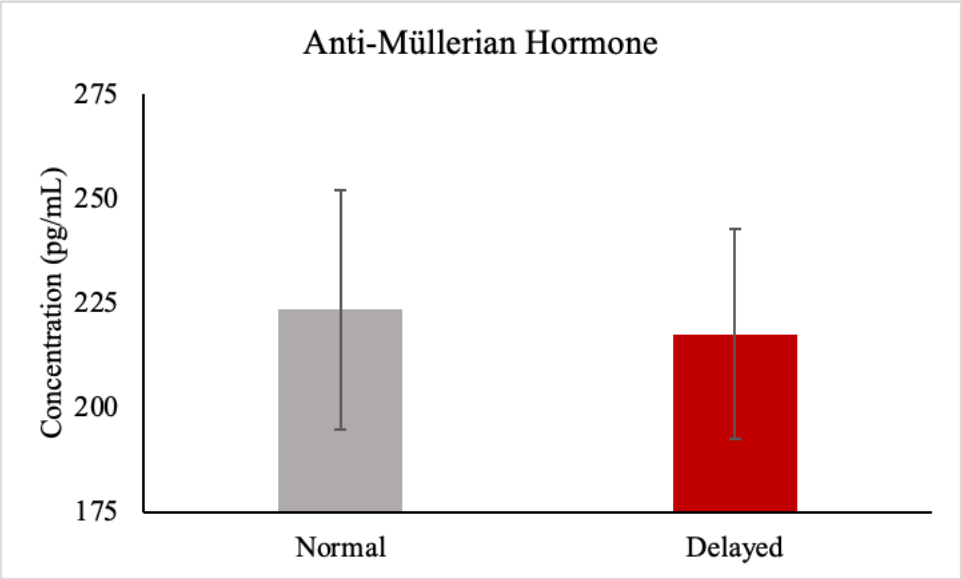

Figure 2.1: Average AMH concentrations.

Production Parameters

Milk production increased from d 14 to 49 in both N cows and D cows ($P <$ 0.001) (Figure 2.2). On d 42, milk production tended to be higher in D cows ($P = 0.082$) and on d 49, milk production was greater in D cows ($P = 0.010$).

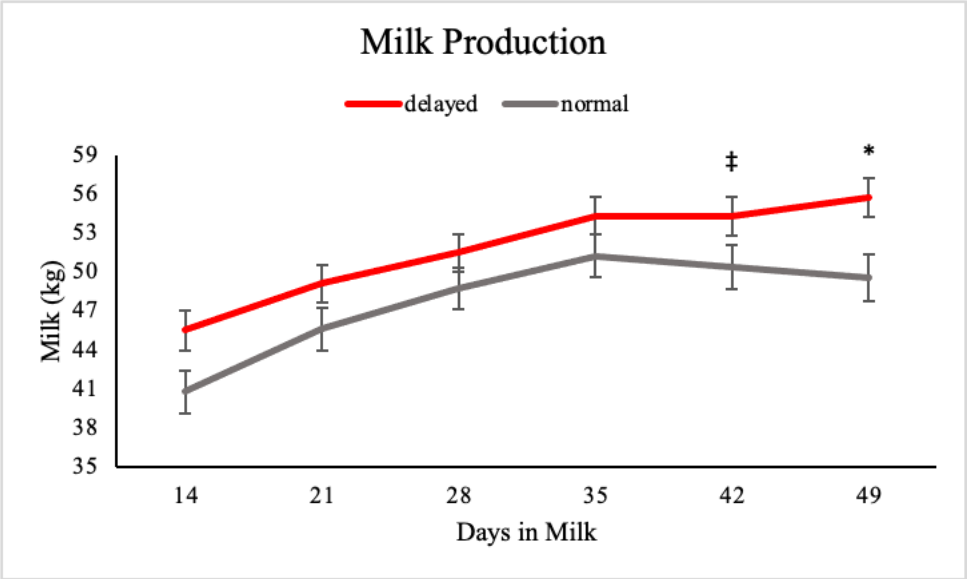

Figure 2.2: Milk production across the experimental period. * indicates $P < 0.05$. ‡ indicates $0.05 < P < 0.10$.

Visits to the RMS increased in both N cows and D cows from d 14 to d 49 ($P < 0.001$) as observed in Figure 2.3 but there were no differences in number of visits to the RMS across the entire experimental period between treatments ($P > 0.05$). An increase in visits to the RMS led to an increase in milk production ($P < 0.001$; r = 0.39).

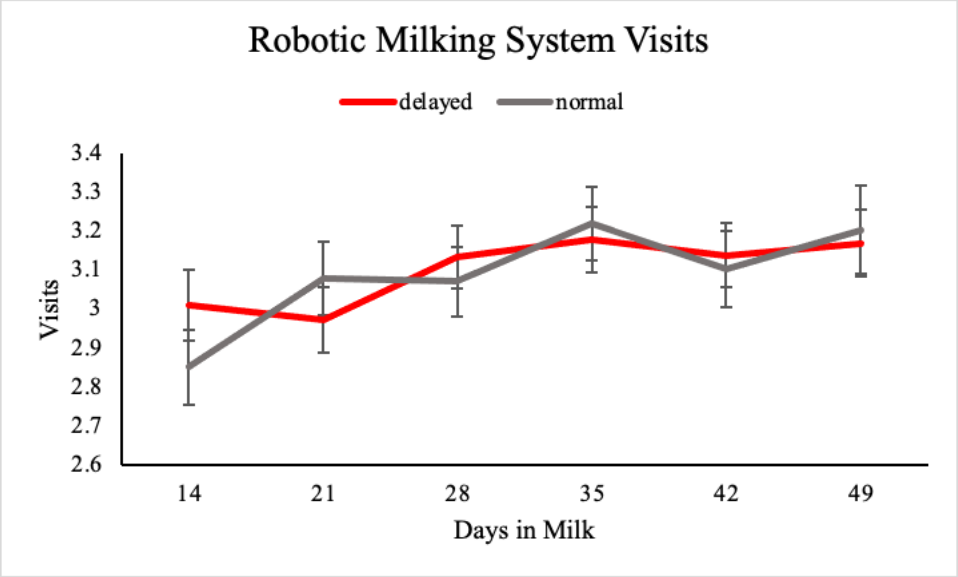

Figure 2.3: Visits to the volunteer milking system for the experimental period.

Metabolite Profiles

There was no overall treatment effect on glucose ($P > 0.05$) but there were treatment*time interactions (Figure 2.4). On d 28, N cows tended to have increased glucose in circulation when compared to D cows ($P = 0.096$) with those differences becoming significantly distinct at d 35 ($P = 0.020$) and d 42 ($P = 0.018$) before resuming no difference on d 49 ($P > 0.05$). Glucose had a significant negative correlation with milk production ($P < 0.001$; r = -0.253).

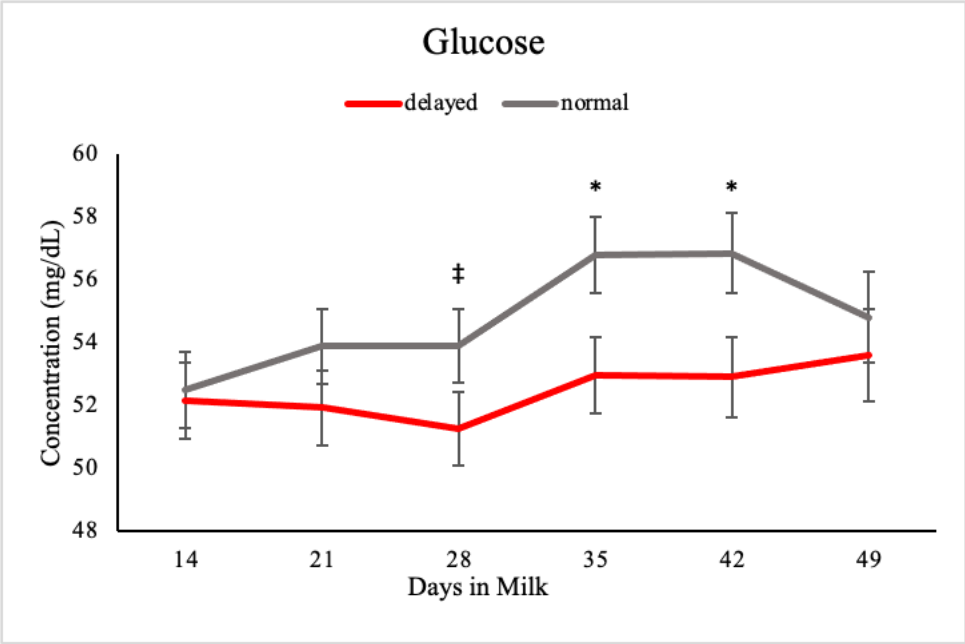

Figure 2.4: Serum glucose concentration for the experimental period.

Concentrations of NEFAs declined in all cows from d 14 to d 49 ($P < 0.001$) (Figure 2.5). Greater NEFA concentrations were seen in D cows on d 21 when compared to N cows ($P = 0.044$) and D cows tended to maintain greater concentrations on d 28 ($P = 0.061$). On d 35, there was no difference between N and D cows ($P > 0.05$). However, on d 42 N cows had numerically greater NEFA ($P > 0.05$) which was further increased on d 49 ($P = 0.05$).

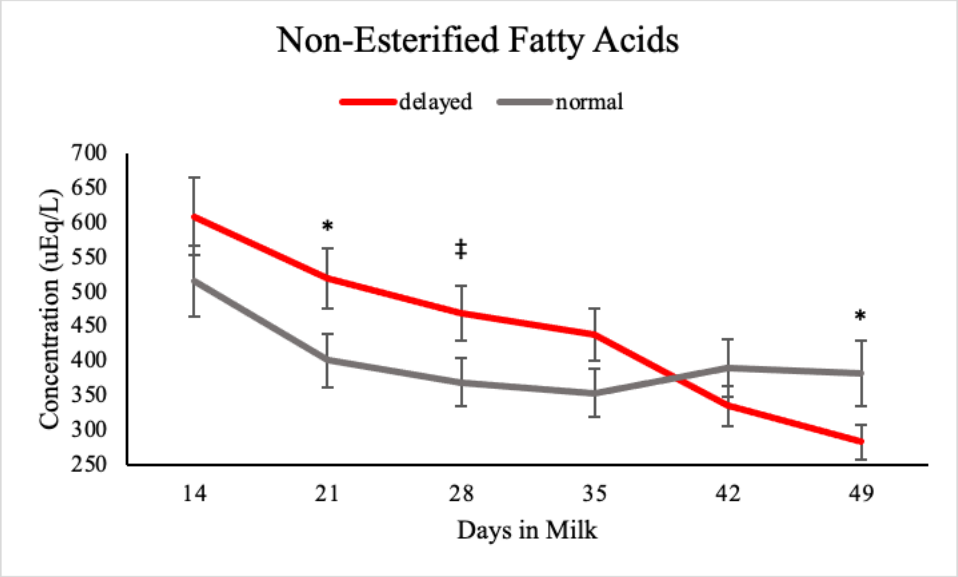

Figure 2.5: Serum NEFA concentrations for the experimental period. * indicates $P < 0.05$. ‡ indicates $0.05 < P < 0.10$.

There were no differences in insulin between N cows and D cows at any time point ($P > 0.05$) as observed in Figure 2.6. Insulin concentration increased from d 14 to d 49 in both N cows and D cows ($P < 0.001$). Concentrations of insulin in circulation were lower in cows with high milk production on d 14 ($P = 0.008$, r = -0.329), d 21 ($P = 0.009$, r = -0.296) and d 28 ($P = 0.04$, r = -0.200) and became insignificant beginning on d 35.

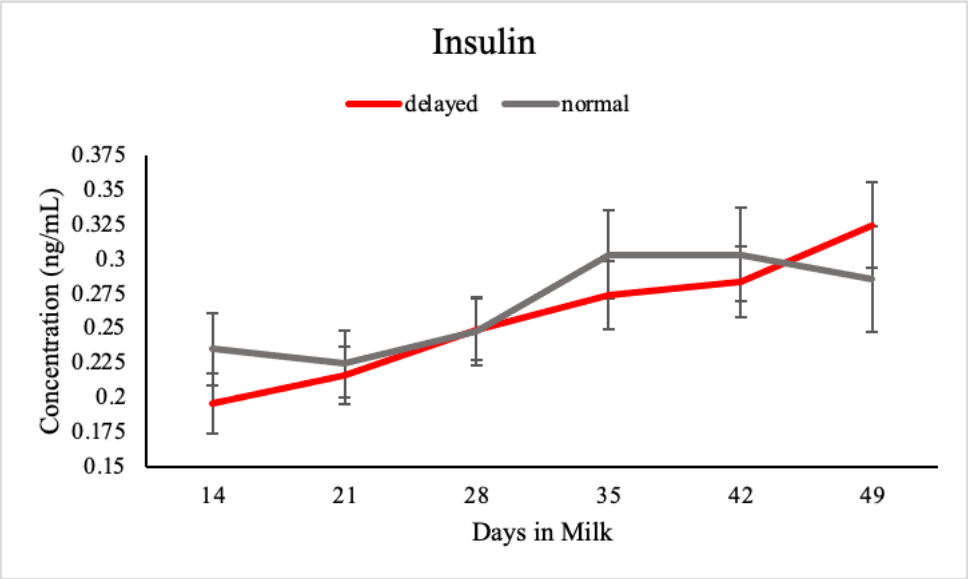

Figure 2.6: Serum insulin concentrations for the experimental period.

There was no difference in RQUCKI between N cows and D cows between d 14 and 35 or on d 49 ($P > 0.05$) (Figure 2.7). There was a tendency for D cows to have increased RQUICKI on d 42 ($P = 0.099$). There was a moderate correlation between RQUCKI and insulin ($P < 0.001$; r = -0.31).

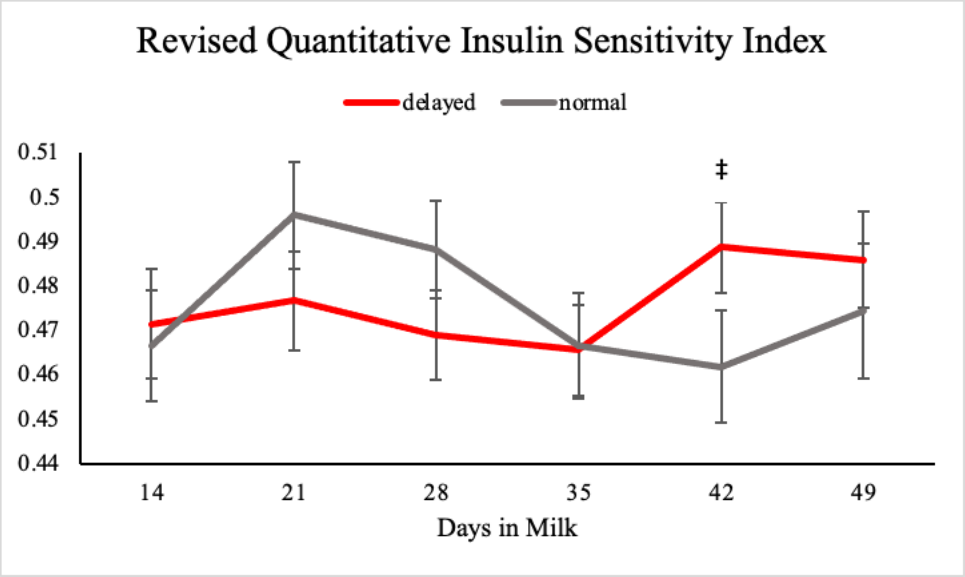

Figure 2.7: Revised quantitative insulin sensitivity index (RQUICKI) for the experimental period.

Metabolic Ratios

Although the glucose to insulin ratio decreased from d 14 to 49 in all cows ($P < 0.001$) there were no differences in this ratio between N cows and D cows across the entire experimental period ($P > 0.05$) (Figure 2.8). As concentrations of glucose in circulation increased, insulin also increased ($P < 0.001$; $r = 0.35$).

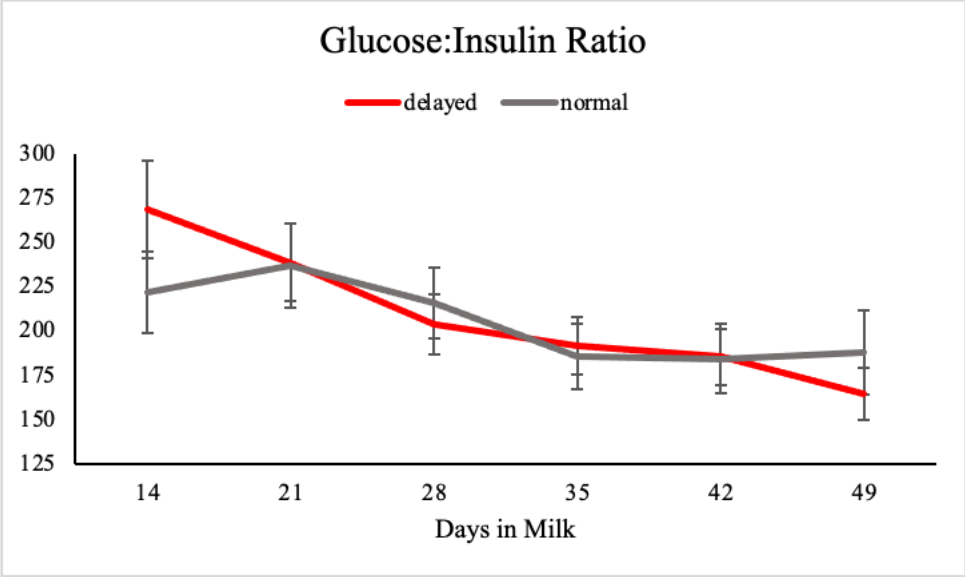

Figure 2.8: Glucose:Insulin ratio for the experimental period.

The NEFA:Insulin ratio decreased from d 14 to d 49 in both N cows and D cows as seen in Figure 2.9 ($P < 0.001$). The NEFA:Insulin ratio was numerically greater in D cows from d 14 to d 35 ($P > 0.05$) but numerically greater in N cows on d 42 and d 49 ($P > 0.05$). There was a treatment by time interaction for the NEFA:Insulin ratio ($P = 0.037$). As NEFA decreased, concentrations of insulin increased ($P < 0.001$; r = -0.28).

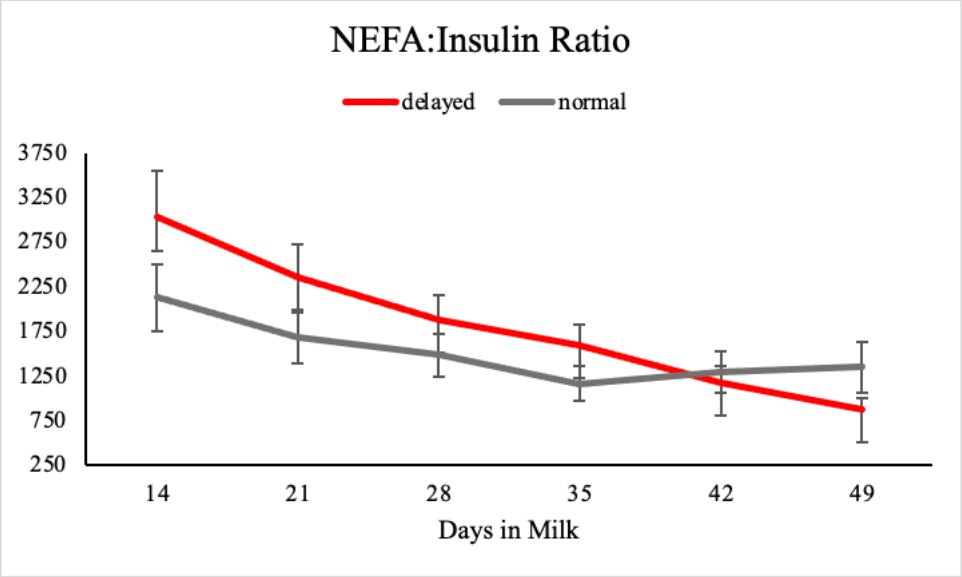

Figure 2.9: NEFA:Insulin ratio for the experimental period.

Discussion

It is well documented that an increased frequency of milking on robotic dairy farms can correspond with increased milk production in dairy cattle (Tse et al., 2018; De Koning and Rodenburg, 2004; Bernier-Dodier et al., 2010). Milk production and visits to the RMS increased from day 14 to day 49 in both animals with normal and delayed resumption of cyclicity. Animals with increased milk production were more likely to have delayed resumption of cyclicity. These high production animals experience greater steroid hormone clearance, leading to a decrease in reproductive efficiency in the periparturient period (Wiltbank et al., 2006).

It was hypothesized that concentrations of glucose would be increased in animals who had a normal resumption of cyclicity postpartum compared to delayed animals (Wathes et al., 2011; Gaverick et al., 2013). This relationship was to be expected because early postpartum animals will increase gluconeogenesis to provide glucose to the

mammary gland for milk production. In prior studies, the need for gluconeogenesis was seen to increase from 1,200 g/d three weeks prior to calving to approximately 3kg/d at three weeks postpartum as milk production reached 36kg/d (Reynolds et al., 2003; Ascenbach et al., 2010). This leaves decreased availability of glucose for bodily tissues other than the mammary gland, including reproductive organs, decreasing their potential for reproductive efficiency (Green et al., 2012). Blood glucose concentration was not different on day 14 between animals with normal versus delayed resumption of cyclicity. Because milk production is still increasing at this point, metabolic demand has yet to be exacerbated (Gaverick et. al, 2013). The tendency for greater blood concentrations of glucose in animals with normal resumption of cyclicity seen on day 28 and the increase seen on days 35 and 42 may have been responsible for the normal resumption of cyclicity observed. Animals with increased glucose in circulation will have increased energy availability to support reproduction rather than just support lactation.

In the present study, the ratio between glucose and insulin statistically decreased from day 14 to 49 in both cows with normal and delayed resumption of cyclicity, meaning insulin release is more responsive to glucose stimulation. The increase in insulin concentrations was associated with an increase in blood glucose concentration from day 14 to day 49. The increase in insulin concentrations in the periparturient period seen in both animals with normal and delayed resumption of cyclicity was similar to results in previous work (Weber et al., 2016). Interestingly, insulin concentrations did not have an impact on resumption of cyclicity postpartum, which was similarly observed in a study by Garnsworthy et al (2009) in which cows were fed rations formulated to induce high or low levels of insulin and there was no impact on reproduction.

The concentration of NEFA decreased over time in both normal and delayed animals, which was expected as they are high prepartum and begin to decrease at parturition. Both groups were able to rely more on glucose for energy over time allowing less mobilization of body fats and decreasing the NEFA in circulation. Elevated blood concentrations of NEFA are used as an indicator for negative energy balance, which is known to be a cause of reduced reproductive success in the postpartum dairy cow (Butler and Smith, 1989; Patton et al., 2007). In the postpartum period, animals with NEFA > 0.57 mEq/L are considered to be at an increased risk for developing metabolic disease such as ketosis (Ospina et al., 2010). Animals with delayed resumption of cyclicity were found to have elevated NEFA from day 14 to 35. On day 14, the NEFA concentration in delayed animals surpassed the threshold of 0.57 mEq/L, but this concentration declined below the risk threshold by day 21. Interestingly, on day 49 animals with normal resumption of cyclicity had significantly greater NEFA. On this same day, normal animals saw a decrease in glucose in circulation, meaning the body was not able to utilize glucose for energy and was instead partitioning glucose toward lactation. In response to a decrease in glucose, the body would initiate lipolysis to produce more NEFA for energy consumption. Animals with delayed resumption of cyclicity exhibit a deeper negative energy balance in the early periparturient period until day 35, which could negatively impact time to first estrus postpartum.

The RQUICKI method can be utilized as an index to estimate changes in insulin sensitivity in dairy cattle (Holtenius and Holtenius, 2007). Insulin sensitivity is decreased in adipose tissue and muscles in the postpartum period so glucose can be reserved for the mammary gland and production of milk (Bell et al., 1995). Previous work shows cows

with a higher body condition score were found to be less insulin sensitive than thinner cows, exemplifying how animals with lower RQUICKI are able to deposit glucose in adipose tissue (Holtenius et al., 2003). Although the current study did not collect BCS data, RQUICKI was still utilized to estimate insulin sensitivity. Delayed animals had greater RQUICKI on day 42 showing higher levels of insulin sensitivity so the body could deposit glucose in adipose tissue and muscle. This increase was unexpected because as animals approach peak milk production they are known to become less sensitive to insulin in order to partition glucose toward the mammary gland (Oliveira et al., 2016). This unexpected difference could be due to RQUICKI being an estimate of insulin sensitivity rather than the true value.

There was no difference in AMH concentration between cows with normal and delayed resumption of cyclicity which is not consistent with previous results linking AMH with increased fertility in cattle (Mossa et al., 2017; Alward and Bohlen, 2019). While the data show no difference in AMH between normal and delayed animals, AMH had a tendency to be higher in animals with more intense estrus. Animals with greater AMH are known to be more reproductively successful due to its positive relationship with antral follicle count and decreased pregnancy loss, which could be associated with increased estrous expression. In a previous study by Ribeiro et al (2014), average concentrations of AMH in lactating cows were 320.3 ± 251.1 pg/mL, in which there was an increased average concentration of AMH and much greater range of AMH than seen in the current study. Differences in the herd composition in the current study could account for the discrepancy in range of AMH values.

Although some of the data from this study matches previous works, it also demonstrates that not one single parameter determines the time to resumption of cyclicity postpartum. Many of the previous studies conducted were on conventional dairies, while this study was performed on a robotic dairy. Differences in the data could be due to qualities of a robotic farm, like voluntary milking, receiving pellets and a PMR versus receiving a TMR, and overall management style differences. The interplay between all parameters previously described must be considered in concert when evaluating the periparturient period and resumption of cyclicity. Still, for the threshold set by the current study to classify cows as N or D (i.e., 45 DIM), we observed that D cows were producing more milk than N cows around that time, which further corroborates the negative association between milk production and fertility.

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CHAPTER 3

CHARACTERIZING POSTPARTUM RESUMPTION OF CYCLICITY ON A BIOLOGICAL AND GENETIC BASIS IN JERSEY CATTLE

Johnson, Sarah. To be submitted to *Journal of Dairy Science*.

Abstract

Although often masked by management and breeding programs, selection intensity for production traits has led to a decline in many aspects of reproductive efficiency. Genetic markers for reproductive recovery are available, and heritable and biological markers for fertility such as anti-Müllerian hormone (AMH) are well documented. However, other variables are yet to be fully described. Therefore, the objective of this study was to characterize the genetic and biological factors influencing postpartum resumption of cyclicity in Jersey cattle. An additional objective of this study was to evaluate the breed differences in the early postpartum period and the potential impact on resumption of cyclicity. Jersey cows (n=25) and Holstein cows (n=25) of varying lactations were enrolled in the study at 14 ± 3 days in milk (DIM) with weekly blood samples collected for progesterone analysis. Blood collected at d 45 was analyzed for AMH determination in all animals. Ear tissue samples from Jerseys (n=24) were genetically analyzed for daughter pregnancy rate (DPR), heifer conception rate (HCR), and cow conception rate (CCR). All animals were fitted with a DeLaval activity collar and monitored via DelPro^{TM}. Estrous events and estrous intensities (EI) were recorded if activity surpassed a threshold of ++, +++, and/or a threshold of 120% EI. Animals were removed from the study after an estrous event occurred or at 100 DIM if no estrous event was recorded. Animals were considered to have normal (N) resumption of cyclicity if 1st estrus occurred on or before 45 DIM or delayed (D) resumption beyond 45 DIM. There was no difference in AMH or EI between Jerseys with D resumption (305.8 ± 61.85 pg/ml and 128.9% ± 2.89, respectively) and N resumption (319.3±91.69 pg/ml and

139.0% ± 4.92, respectively). AMH increased with lactation number in Jerseys (P=0.001; R^2=0.6) while 1st estrous EI increased with DPR in Jerseys (P<0.05; R^2=0.45). Jerseys had greater AMH (304.3 pg/mL ± 49.21) than Holsteins (182.4 pg/mL ± 20.22) (*P* = 0.026) while Holsteins had greater first estrous intensity compared to Jerseys (*P* = 0.024). Tendencies in the data additionally indicate that as DPR increases in Jerseys, DIM at first estrous decreases (P=0.094). As a preliminary trial, these conclusions provide avenues of further investigation into the influence of genetic and biological data on postpartum resumption of cyclicity in Jersey cattle and the comparison of fertility in the postpartum period with Holstein cattle.

Introduction

The single-trait selection pressure for milk production has historically been one of the strongest influencers for genetic selection of animals while leading to a concurrent decline in general dairy cattle fertility (Crowe 2018). Factors such as the increased metabolic demand and negative energy balance associated with higher milk production have a negative impact on resumption of cyclicity postpartum and eventual successful reproduction (Butler and Smith, 1989). A method of overcoming poor reproductive performance is utilization of timed artificial insemination programs; however, consumer drive for minimal exogenous hormone use may leave producers looking to alternative methods of improving reproductive efficiency in the coming years. There are many tools to evaluate animal fertility that include biological markers, genomic markers, and technology. However, the utilization of these tools in combination is not well published.

In the evaluation of biological methods to identify more fertile animals in the postpartum period, anti-Müllerian hormone (AMH) is promising as a biological marker

for fertility in dairy cows. AMH is directly correlated with antral follicle count and has been found to be moderately heritable (h^2 =0.36±0.03) (Nawaz 2018). AFC was found to be extremely variable between animals, but highly repeatable within animals over time, allowing AMH to be used reliably for phenotyping (Burns et al., 2005). AMH has a positive association with pregnancy maintenance and is known to be increased in Jersey cattle in comparison with Holsteins (Riberio et al., 2014). If a positive correlation between AMH concentrations and estrous intensity is discovered, AMH could be a useful tool for future reproductive improvement in Jersey cattle selection.

Currently, genomic markers for reproductive efficiency are selected for in Jersey breeding programs. The three genomic markers for utilization concerning reproductive efficiency are Daughter Pregnancy Rate (DPR), Cow Conception Rate (CCR), and Heifer Conception Rate (HCR). Fertility traits account for 11% of the Jersey Performance Index, with DPR accounting for 7% and CCR and HCR accounting for 2% each, showing the economic importance of evaluating these specific traits (American Jersey Cattle Association, 2018). While AMH is moderately heritable, the heritability for other genomic markers is much lower at 0.014 (DPR), 0.016 (CCR), and 0.01 (HCR). When coupling genomic markers with AMH, their merit for utilization may be increased when making breeding selections and/or culling decisions.

In the same realm of genomic markers for fertility, reproductive numbers are generally lowly heritable, including estrus expression with a h^2 of ≤ 0.05 (Council on Dairy Cattle Breeding, 2019). Jerseys were found to have significantly shorter duration of episodes of estrous events and less intense episodes of estrous when compared to

Holsteins (Løvendahl and Chagunda, 2010). This makes Jersey cows even more difficult to identify estrus on and can potentially lead to extended days open.

With consumer demand increasing for hormone-free products and potential for reduced estrous expression in Jerseys compared to Holsteins, alternative methods for accurate estrous detection are necessary to ensure reproductive efficiency is achieved. Activity monitoring systems (AMS) have been evolved to continuously monitor and record data about each individual animal and have been heavily refined for accurate detection of estrus in cattle. Research has shown that multiple different commercial activity monitoring systems correctly identified and alerted for estrus 15 to 35% more cows in heat than traditional visual observation occurring four times a day (Mayo 2019).

The current study aims to investigate basic influences on the resumption of cyclicity postpartum between Holstein and Jersey cattle and further explore genetic impact on cyclicity resumption within the Jersey breed. With a moderate heritability and positive correlation with ovarian follicular reserve, AMH's relationship with estrous intensity is of high interest in this study. The objectives of this study were 1) to determine the relationship between heritable, biological, and genomic markers for fertility on intensity of estrus in Jersey and Holstein cows, 2) evaluate the relationship between heritable, biological, and genomic factors on time to resumption of cyclicity postpartum in Jersey and Holstein cows, and 3) compare cyclicity information in the early postpartum period between Jersey and Holstein cows.

Materials and Methods

Animal Management

Jersey cows (n=25) of varying lactations (1^{st} – 5^{th}) and Holstein cows (n=25) of varying lactations (1^{st} – 8^{th}) were selected for enrollment in the study at 14 ± 3 days in milk (DIM) and housed in confinement housing at the University of Georgia Teaching Dairy in Northeast Georgia. All animals were milked twice daily at 3:00 AM and 2:00 PM and were all fed the same TMR twice daily which was formulated to meet their specific nutrient requirements. The animals were equipped with DeLaval collars and activity was monitored for estrous events utilizing the DeLaval DelPro™ system. Days in milk at first estrus and intensity of that estrous event were recorded for each animal. Milk production data was collected on day of sampling and averaged with production on the day preceding and day following. All sampling and data collection concluded after an estrous event was identified for each animal, and if an animal had no estrous event, collections concluded when 100 DIM was reached. No exogenous hormones were used throughout the duration of the study.

Estrous Event Identification

DeLaval's activity monitoring program, DelPro™, continuously records movement via a collar placed around the neck of the animal and was used to determine estrous events for this study. Estrous events are determined by 3 levels of estrus alarms in DelPro™: (+) = activity 3.8 standard deviations above normal, (++) = activity 5 standard deviations above normal, (+++) = activity 6 standard deviations above normal. A (+++) alert is the most reliable and accurate estrous alert. Estrous intensity is reported on a scale of 0 - 255% of relative activity, with 100% being average activity and 255% being the maximum amount of activity.

An additional report was created to capture animals with more subtle estrous events. To be entered on that report, cows had to either elicit a (+), (++), or (+++) response, or cross a threshold of 120% relative activity, and the report recorded the maximum relative activity the cow experienced that day. The maximum relative activity achieved during the first estrous event and days in milk at occurrence were recorded. To be considered an estrous event for the current study, (++) or (+++) alerts or entry onto the report with relative activity > 120% were all identified as estrous events. Days in milk at first estrus and estrous intensity were recorded for all animals.

Ultrasonography

Animals not having an estrous event as identified by DelPro^{TM} by 100 DIM were transrectally ultrasounded to evaluate the uterus and ovaries (n=20). The uterus was observed for any abnormalities or anomalies. The ovaries were scanned for ovarian abnormalities as well as to confirm cyclicity status, with the presence of a corpus luteum (CL) indicating resumption of cyclicity was achieved. Of the 20 animals ultrasounded, 3 were truly noncycling. These observations were recorded for later analysis.

Blood Collection

Blood samples for progesterone analysis were collected weekly beginning at enrollment and continuing until animals experienced a first estrous event or reached 100 DIM with no estrous event. An additional blood sample was collected between 45-60 DIM to be analyzed for Anti-Müllerian hormone (AMH). All samples were collected via coccygeal venipuncture into vacutainer tubes using no additives.

Blood Processing, Storage, and Analysis

Following collection, tubes were immediately placed on ice and transported to the lab. Samples were centrifuged at 3000 rpm for 25 minutes to separate serum from red blood cells. Serum was extracted using a pipette and aliquoted to microcentrifuge tubes in triplicate labeled with animal number and sampling date. Microcentrifuge tubes were stored in a -20° C freezer until future analyses. Serum was analyzed for progesterone at Clemson University (Clemson, SC, USA) using a radioimmunoassay (RIA) to detect the amount of antigen in the sample using antibodies. Frozen serum was shipped on dry ice overnight to Motive Biosciences Inc. (Webster, TX) for AMH analysis using an enzyme-linked immuno-absorbent assay (ELISA). The assay uses unique monoclonal antibodies and is calibrated using recombinant bovine AMH.

Genomic Sampling and Analysis

Genomic analysis was performed using a tissue sample from the ear enrolled Jerseys (n=24). Samples were analyzed for production and wellness traits by Zoetis using Clarifide® Plus, the first commercially available genetic test for the Jersey breed. Reproduction traits evaluated included the genetic predictions of Daughter Pregnancy Rate (DPR), Cow Conception Rate (CCR), and Heifer Conception Rate (HCR).

Statistical Analysis

Days to first estrus, AMH, and intensity at first estrus were analyzed using PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). Normal cyclicity was defined as the animal experiencing first estrus before 45 DIM, with delayed cyclicity occurring after 45 DIM. The statistical models included treatment (normal or delayed cyclicity) as the fixed variable, with cow nested within treatment as the random variable. Repeated measures data collected (milk production) were analyzed by PROC MIXED procedure. The

statistical models included treatment, time, and treatment by time interaction as fixed variables, with cow nested within treatment as the random variable. An additional statistical model included breed, treatment, time, breed by treatment, breed by time, treatment by time, and breed by treatment by time interactions as fixed variables, with cow nested within treatment as the random variable. When the interaction between treatment and time was significant or tended to be significant, the SLICE function was used to detect treatment difference at each time point. Correlation coefficients were determined for all associated variables.

Results

Jersey vs Holstein Parameters

Holsteins had greater milk production when compared to Jerseys across the entire experimental period ($P < 0.001$) as observed in Figure 3.1. For both breeds, milk production increased over time ($P < 0.001$).

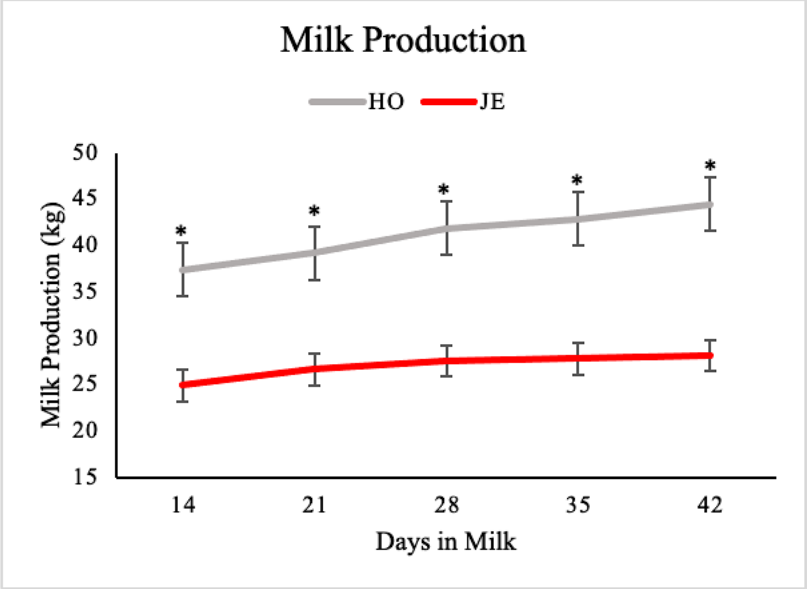

Figure 3.1: Average milk production for Holsteins (n=25) and Jerseys (n=25) across the experimental period. * indicates $P < 0.05$.

There were no differences for days in milk at first estrus when comparing N Holsteins and Jerseys as well as when comparing D Holsteins and Jerseys ($P > 0.05$) (Figure 3.1). Holsteins with normal resumption of cyclicity had greater estrous intensity compared to N Jerseys ($P = 0.001$), but there was no difference between delayed animals ($P = 0.933$). Overall, Jerseys had greater AMH (304.3 pg/mL ± 49.21) than Holsteins (182.4 pg/mL ± 20.22) ($P = 0.026$) but there was no treatment impact on AMH ($P > 0.05$). Holsteins overall had greater first estrous intensity compared to Jerseys ($P = 0.024$) with an average estrous intensity of 152.3% ± 5.75 while Jerseys had an average estrous intensity of 136.3 % ± 3.80.

Table 3.1: Reproductive parameters of Holsteins and Jerseys.

	N Holstein	N Jersey	*P*-value	D Holstein	D Jersey	*P*-value
DIM at 1st estrus	29.4 ± 3.1	25.7 ± 3.3	0.415	69.9 ± 4.8	63.4 ± 4.0	0.305
Estrous Intensity	154.7 ± 5.7	135.1 ± 4.6	0.001	133.9 ± 6.6	132.9 ± 5.4	0.933
AMH	168.1 ± 34.5	248.5 ± 44.6	0.157	171.8 ± 50.8	282.6 ± 58.3	0.161

Jersey Parameters

There were no differences in milk production between normal and delayed Jerseys ($P > 0.05$). (Figure 3.2).

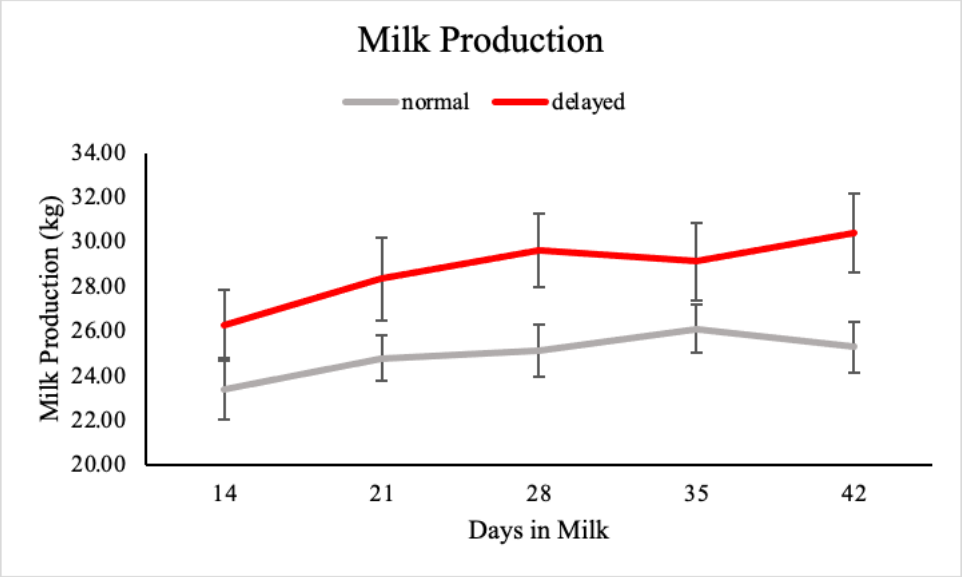

Figure 3.2: Average milk production during the experimental period for Jersey cattle.

Average AMH for N cows was 305.77 ± 61.85 pg/mL while average AMH for D cows was 301.70 ± 86.17 pg/mL and were not different ($P > 0.05$) (Figure 3.3). AMH was unrelated to estrous intensity at 1st estrus ($P = 0.298$) but had a significant positive correlation with lactation number ($P = 0.002$; r = 0.599). AMH was unrelated to all fertility traits ($P > 0.05$).

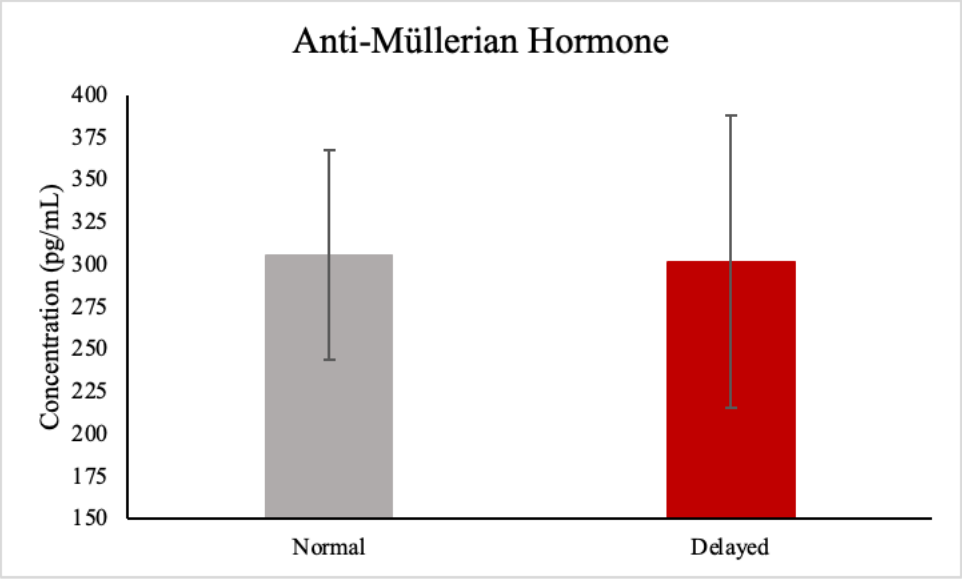

Figure 3.3: Average anti-Müllerian hormone concentrations in Jersey cattle.

There was a difference in the DPR of N cows (-0.26) and D cows (-0.78) ($P < 0.05$) (Figure 3.4). As DPR increased, estrous intensity at 1st estrus increased ($P = 0.049$; r= 0.405). Animals with a higher lactation number had higher DPR ($P = 0.046$; r=0.411). There was no difference in the CCR or HCR of N cows (0.23, 1.04 respectively) and D cows (-0.30, 0.86 respectively) There was a tendency for CCR to be higher in higher lactation animals ($P = 0.058$; r=0.393). HCR had no relationship with lactation number ($P > 0.05$).

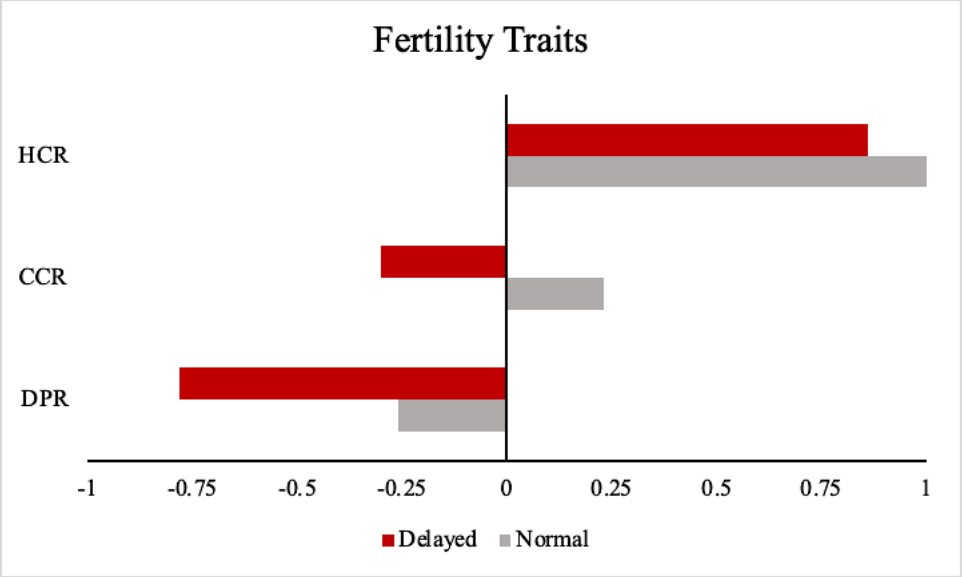

Figure 3.4: Average fertility traits of HCR, CCR, and DPR for D cows (n=8) and N cows (n=16).

Discussion

It is well documented that animals with high milk production in the postpartum period experience negative energy balance, which negatively impacts reproductive efficiency (Shrestha et al., 2004; Nakao et al., 1992). While Holsteins have higher milk production, Jerseys are known to produce more pounds of milk per pound of bodyweight

with higher components. Jerseys have increased calcium demands postpartum along with decreased vitamin D receptors in the intestine, leading to less active Ca absorption capabilities compared to Holsteins (Cerbulis and Farrell, 1976; Goff et al., 1995). As a result, Jerseys are at higher risk of developing metabolic disorders postpartum which are associated with impaired reproductive efficiency (Chiwome et al., 2017; Valldecabres et al., 2018; Chapinal et al., 2012). The results of the current study are inconsistent with this as Jerseys and Holsteins did not have different time to resumption of cyclicity between normal or delayed groups. While this study evaluated milk production between Jerseys and Holsteins in kilograms, evaluating milk production instead as energy corrected milk may be more informative for exploring metabolic stressors in Jerseys. This would further explore the impact high component milk has during the postpartum period rather than evaluating on a quantity basis alone.

Concentrations of AMH were greater for Jerseys than Holsteins but were not different amongst Jerseys with delayed and normal resumption of cyclicity, and these findings are consistent with previous work. A study by Ribeiro et al (2014) evaluating AMH concentration in Jerseys, Holsteins, and Jersey x Holstein crossbreds found that Jerseys had the highest AMH in circulation with no impact on days in milk at first service. Jerseys are known to be a more fertile breed, and coupled with the positive implications AMH has on fertility, it was hypothesized there would be a difference in AMH between cows with normal and delayed resumption of cyclicity. It is possible that low sample size in the present study led to insignificance in AMH differences between normal and delayed Jerseys.

Jerseys with normal resumption of cyclicity had greater DPR than those with delayed resumption of cyclicity, aligning with previous research. An estimated increase of 1 point in DPR is expected to result in a 4-day reduction of the time between calving and first service (Norman et al., 2009). Jerseys with increased DPR were also found be higher in lactation number, showing increased herd longevity, and were found to have increased estrous intensity. Cows with higher estrous intensity are more likely to be identified in estrus by visual or technological means, making them more reproductively efficient and increasing herd longevity. The results of the current study affirm promotion of DPR in genetic selection could potentially allow more reproductively efficient animals in the postpartum period and increased retention of animals over time.

Holsteins were found to have a higher intensity of estrous than Jerseys. In a previous study by Fonseca et al (1983) Jerseys were found to have a higher rate of estrous detection (73%) when compared to Holsteins (43%). However, in an additional study Jerseys were found to have less intense episodes of estrous events (0.951ln ± 0.022) when compared to Holsteins (1.029ln ± 0.017) (Løvendahl and Chagunda, 2010). These differences in studies could be explained by herd composition and/or differences in the early postpartum period, such as disease incidence, nutrition, and lactation number. Additionally, environment and management style play a significant role in estrous expression and detection and could be responsible for differences observed. Increasing animal numbers in the current study would be beneficial to further explore the dynamics of estrous intensity between Holsteins and Jerseys.

Although some of the data from this study matches previous works, differences in the data could be due to management style or size of the study population. This work

demonstrates that not one single parameter determines the time to resumption of cyclicity postpartum. When used in concert, criteria like AMH and DPR can be utilized for selection of animals for potential increased reproductive efficiency in the postpartum period.

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CHAPTER FOUR

CHARACTERIZATION OF THE UTERINE MICROBIOME IN POSTPARTUM HOLSTEIN AND JERSEY COWS

Johnson, Sarah. To be submitted to *Frontiers in Animal Science.*

Abstract

Over half of dairy cattle experience uterine microbial disease in the early postpartum period, which have negative implications for reproductive performance. This study aimed to explore the uterine microbiome in both Holstein and Jersey cattle and implications it has for resumption of cyclicity in the postpartum period. Holstein cows (n=21) and Jersey cows (n=20) of various lactations were sampled in duplicate using sterilized double culture swabs between 45-60 days in milk (DIM) for uterine microbiome analyses. Samples were extracted using Qiagen QIAamp BiOstic Bacteremia DNA Kit and stored in a -80° C for future analyzation. Samples were shipped to Element Biosciences for Element LoopSeqTM Long-Read 16S ribosomal ribonucleic acid (rRNA) Gene Sequencing.

Introduction

Uterine diseases are some of the most significant factors responsible for poor reproductive performance in dairy cattle, with over half of dairy cows experiencing uterine microbial disease postpartum (Sheldon et al., 2009). These diseases include retained placenta, metritis, endometritis, pyometra, and other non-specific infections of the uterus (Noakes et al., 2001). Typically considered a "sterile" environment, the uterus is protected by the barrier of a closed cervix, which prevents bacterial ascension (Dadarwal et al., 2017). During parturition however, the cervix dilates and thus the protection of the "sterile" environment of the uterus is compromised. This dilation, which may last for up to several week postpartum, allows for exposure of the uterus to pathogens. The bacteria that may infiltrate the uterine environment during this time are considered to originate from feces and the environment (Nguyen et al., 2019). Eventually

the cervical diameter will regress and the uterine body undergoes full involution, once again providing a protective barrier by controlling and tolerating any pathogenic bacteria that entered while the uterus was compromised (Karstrup et al., 2017; Moore et al., 2017; Machado et al., 2012). However, the immune system is overwhelmed by reduced tolerance and a significant bacterial challenge in high-producing animals and/or health compromised animals, allowing uterine disease occurrence in the early postpartum period (Galvão et al., 2019). Between 80-100% of cattle were found to have bacteria in their uterine lumen within the first 2 weeks postpartum (Sheldon et al., 2008).

Prior works relied on culture information for determination of bacterial clearing in the postpartum cow. Unfortunately, culture data may not provide enough information to make that determination. Traditional culture methods were only able to study 0.1-15% of naturally occurring microbes as most uterine bacteria cannot be cultured (Lamont et al., 2011). The development of technologies such as 16S rRNA gene sequencing allowed researchers to discover bacterial communities of commensal microbes in low density environments, like the uterine environment of a healthy animal (Santos et al., 2011). Using these methods, healthy animals were found to have greater bacteria richness and diversity than animals with disease (Galvão et al., 2019).

The uterine microbiome of healthy cows has been explored with varying results showing inconsistencies in the most abundant phylum between herds. However, in multiple studies, *Firmicutes* was found to be the most abundant phylum of the uterine microbiome, showing dominating abundances of 31.3%, 52.3%, and 76.7% in the uterine microbiota of healthy cows early postpartum (Clemmons et al., 2017; Machado et al., 2012; Wang et al., 2018). Two differing studies reported that *Proteobacteria* was the

most abundant phylum in the uterine microbiota of healthy cows (Sicsic et al., 2018; Santos et al., 2011). Other phyla composing the majority of the uterine microbiome are *Bacteroidetes*, *Fusobacteria*, *and Tenericutes* (Luecke et al., 2022). Some of the microbes that healthy animals harbor are also found in animals with uterine disease, and combined with decreased microbial diversity in diseased animals, the immune system plays a role in the extent of overgrowth of pathogenic bacteria (Moore et al., 2017).

There are disease-related alterations in these phyla as pathogenic bacteria can suppress existing communities by reducing overall bacterial diversity and colonize new bacterial populations (Fredericks et al., 2005; Manichanh et al., 2006; Ott et al., 2004). The abundance of *Bacteroidetes*, particularly with regards to the families *Bacteroidaceae* and *Porphyromonadaceae* was found to be increased in cows suffering from metritis (Machado et al., 2012; Bicalho et al., 2011). *Escherichia coli* and *Truperella pyogenes* are commonly associated with inflammation of the uterus and poor reproductive performance (Bicalho et al., 2011). When the cow cannot tolerate pathogenic bacteria and overgrowth occurs, they are known to have reduced feed intake, further inducing negative energy balance in an already challenging transition period, inhibiting the HPG axis (Moore et al., 2019). Along with HPG axis inhibition, bacterial pathogens can also act directly on developing follicles and downregulate response to gonadotropins, preventing ovulation from occurring (Bromfield and Sheldon, 2013; Lüttgenau et al., 2016; Oguejiofor et al., 2015). Anti-Müllerian hormone is a biomarker for fertility in cattle and is known to be directly correlated with antral follicle count on the ovaries. Because pathogens are known to impact developing follicles, a relationship between AMH and the microbial community of the uterus is of interest in this study.

The uterine microbial density and diversity in the postpartum period and its impact on reproductive health is not well documented. Therefore, the objectives of this study were to 1) characterize the uterine microbial diversity and abundance in Holstein cattle, 2) characterize the uterine microbial diversity and abundance in Jersey cattle as the uterine microbiome has never been studied in Jerseys, 3) compare uterine microbiome density and diversity between Holstein and Jersey cattle, 4) evaluate the relationship between the uterine microbial population and time to resumption of cyclicity postpartum, and 5) explore the relationship between the uterine microbial population, genomic, and heritable parameters. It was theorized that cows with a delayed resumption of cyclicity postpartum would have reduced bacterial diversity but higher abundance of known pathogenic genera. It was also theorized that cows with higher AMH concentrations and stronger genomic profiles for reproduction would have increased bacterial diversity.

Materials and Methods

Animal Management

Holstein cows (n=21) and Jersey cows (n=20) of varying lactations were selected for enrollment at 14 ± 3 days in milk (DIM). The cows were housed in confinement at the University of Georgia Teaching Dairy in Northeast Georgia and were milked twice daily at 3:00 AM and 2:00 PM. Animals were fed a TMR twice daily which was formulated to meet their specific nutrient requirements. All enrolled cows were equipped with DeLaval DelPro^{TM} activity monitoring collar and monitored for estrous activity with DIM at first estrus and intensity of first estrus recorded. Animals were monitored for uterine health from day of calving by farm management with both diagnoses and treatments recorded. Metritis (n=2) and retained placenta (n=4) were diagnosed and treated with antibiotics.

Additionally, mastitis (n=2) and a displaced abomasum (n=1) were diagnosed and treated with antibiotics.

Blood Collection for Anti-Müllerian Hormone

At 45-60 DIM a blood sample was collected via coccygeal venipuncture into vacutainer tubes containing no additive for anti-Müllerian hormone (AMH) analysis. Following collection, blood samples were immediately placed on ice and transported to the laboratory where they were centrifuged at 3,000 rpm for 25 minutes to separate the serum from red blood cells. The serum was aliquoted into microcentrifuge tubes in duplicate and labeled with animal number and date of sampling then stored at -20° C. Frozen serum was shipped on dry ice overnight to Motive Biosciences Inc. (Webster, TX) for AMH analysis using an enzyme-linked immuno-absorbent assay (ELISA). The assay uses unique monoclonal antibodies and is calibrated using recombinant bovine AMH.

Uterine Microbiome Sample Collection

Uterine microbiome samples were collected for analysis on all animals between 45-60 DIM. Prior to collection, the vulva was wiped clean with diluted chlorhexidine, dried with a clean paper towel, then cleaned again with a 70% isopropyl alcohol-soaked paper towel. Samples were collected using a sterilized double guarded culture swab with an outer tube, inner sheath, and sterile swab to minimize contamination as the swab has to enter through the vagina and pass trans-cervically into the uterus (Figure 4.1).

The instrument was gently passed through the cervix and positioned in the uterine body where the internal sheath and swab were exposed, and the swab was carefully rolled against the wall of the uterine body. After the sample was collected, the swab was

withdrawn into the inner sheath then withdrawn into the outer tube and removed from the cow.

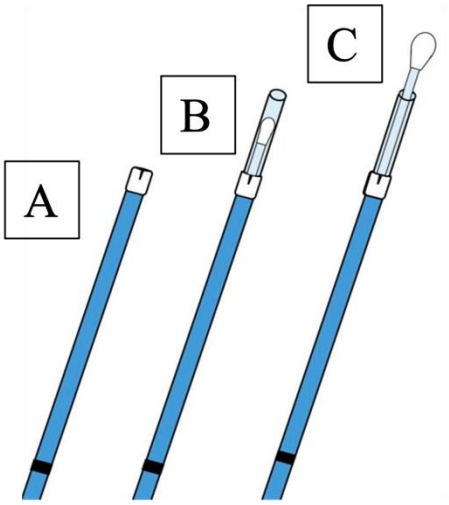

Figure 4.1: Illustration of the sterile double guarded culture swab used for uterine microbiome sampling. Image depicts A) outer tubing through vagina and cervix, B) inner tubing rupturing at opening to uterine body, and C) exposed sterile swab in uterine body. Adapted from Reproductive Provisions, Walworth, Wisconsin, USA.

The samples were collected in duplicate. After collection, both swabs were placed into a single, sterile, 13 mL conical tube, immediately placed on dry ice, and transported to the lab for storage in a -80° C freezer until being used for further analysis.

To confirm a sample was taken in the correct location, ultrasonography was used to evaluate the uterine endometrium. As seen in Figure 4.2, hyperechoic white line embedded within the issue is representative of irritation of the uterine endometrium, confirming the swab collection occurred against the wall of the uterine body.

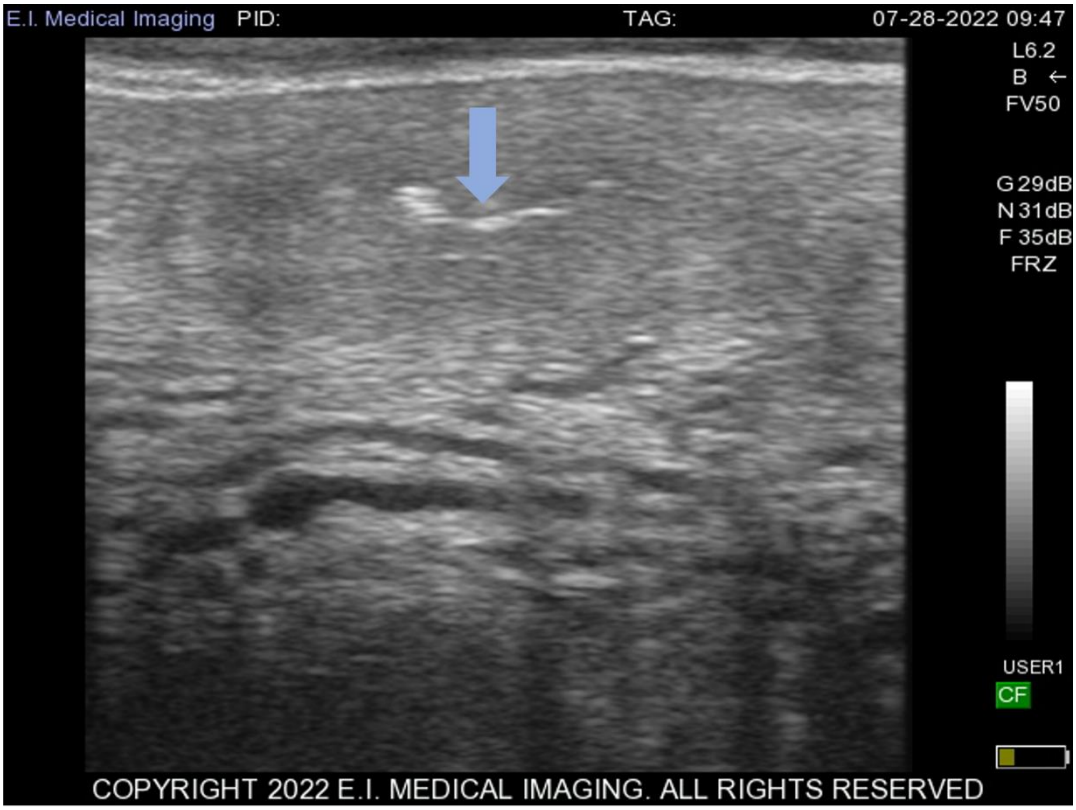

Figure 4.2: Ultrasound image of the uterine endometrium after sample collection has occurred. The blue arrow points to the hyperechoic portion of the uterine endometrium where the sample was collected.

Uterine Microbiome Sample Extraction

Uterine microbiome samples were removed from the -80° C and thawed in a cold water bath before extraction. Extractions were performed using the QIAamp BiOstic Bacteremia DNA Kit (QIAGEN, Venlo, Netherlands) and following the manufacturer's specifications with slight modifications for the uterine microbial population as described below. To prepare for extraction, 2 mL of sterile phosphate-buffered saline (PBS) solution was added to each 13 mL conical tube containing both uterine swabs for each cow from the study. The conical tubes containing PBS solution and uterine swabs were

vortexed in a multi tube-vortexer for 10 minutes and 1 mL of the resulting solution was transferred into sterile 2 mL PowerBead tubes (QIAGEN, Venlo, Netherlands). This was combined with 450 µL of proprietary solution MBL, vortexed at maximum speed for 20 seconds, then heated in a 70° C water bath for 15 minutes. The PowerBead tubes were vortexed again at maximum speed for 10 minutes using a QIAGEN vortex adapter (QIAGEN, Venlo, Netherlands), completing the lysis of the bacteria in the samples. The PowerBead tubes were then centrifuged at 10,000 g for 1 minute, allowing debris and PowerBeads to be removed from the supernatants, and 1 mL of the supernatants were transferred to new, sterile 2 mL tubes. The supernatants had 100 µL of proprietary solution IRS added and were vortexed at maximum speed for 20 seconds before incubating at room temperature for 5 minutes. Following incubation, the supernatants were centrifuged at 10,000 g for 1 minute to pellet the debris, then 0.8 mL of the sample was transferred to new, sterile 2 mL tubes. Following transfer, 1 mL of proprietary solution BB was added to the supernatants then pulse vortexed for 5 seconds 3 times to mix and again centrifuged at 10,000 g for 1 minute. Following, 600 µL of the lysate was added to the MB Spin Column (QIAGEN, Venlo, Netherlands) and centrifuged at 10,000 g for 1 minute with the flow-through being discarded. This step was repeated 2 additional times to ensure all of the lysate had been processed, allowing all the purified genomic DNA to bind to the MB Spin Column filter membrane. The MB Spin Columns were transferred to new, sterile 2mL tubes and are washed with 500 µL of proprietary solution Solution CB and centrifuged at 10,000 g for 1 minute with flow-through being discarded. Solution CB washes impurities and salts off the MB Spin Column filter membrane. This step was repeated and the flow-through was discarded again. The 2mL tubes containing

the MB Spin Columns were then centrifuged at 13,000 g for 2 minutes to dry the MB Spin Column membrane, ensuring that all ethanol was removed so DNA could be released from the membrane. The MB Spin Columns were transferred to new, sterile 2 mL tubes and were eluted with 50 µL of Solution EB directly in the center of the membrane. The MB Spin Columns were incubated for a final time at room temperature for 5 minutes to maximize elution before being centrifuged at 10,000 g for 1 minute. After this process, the DNA was ready for downstream applications.

Following extraction, the determination of DNA concentration and purity in the resulting eluate was performed spectrophotometrically using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA). Samples with a volume of 30 µL consisting of a minimum volume of DNA yield of 3 ng/µL of DNA were stored at -80° C for further analysis.

16S rNA Gene Sequencing

Following DNA extraction, samples were shipped overnight on dry ice to Element Biosciences (San Diego, California, USA) for Element LoopSeqTM Long-Read 16S ribosomal ribonucleic acid (rRNA) Gene Sequencing. The entire 16S rRNA gene libraries were prepared from genomic DNA, and synthetic long reads were assembled from the short-read sequencing reads (Callahan et al., 2021). Sequences were analyzed utilizing the Quantitative Insights Into Microbial Ecology (QIIME) bioinformatics pipeline, version 2-2021.11.

Results

Population dynamics for Holstein (n=21) and Jersey (n=20) cows sampled in the present study for analysis of the uterine microbiome can be seen in Table 4.1.

Table 4.1: Population dynamics for Holstein (n=21) and Jersey (n=20) cows sampled for uterine microbiome analysis.

	n	Yield (ng/mL)	n	Lactation	n	DIM at 1st Estrus	n	EI at 1st Estrus	n	AMH (pg/mL)
HO	21	93.21	21	3.52	19	41.78	19	147%	20	180.54
JE	20	69.94	20	2.90	20	39.95	20	137%	21	269.70

DNA yield following extraction tended to be higher in cows with delayed resumption of cyclicity 109.28 ± 24.67 ng/mL while normal cows had average yield of 65.16 ± 14.57 ng/mL ($P = 0.075$) (Figure 4.3). Although there was a tendency for difference between treatments, DNA yield was not different between Holsteins (93.21 ± 16.52ng/mL) and Jerseys (69.94 ± 16.30 ng/mL) ($P = 0.323$) (Figure 4.4). Throughout the entire population, as lactation number increased, DNA yield increased ($P = 0.020$; r= 0.362). There was no relationship between DNA yield and DIM at first estrus, intensity at first estrus, or AMH ($P > 0.05$).

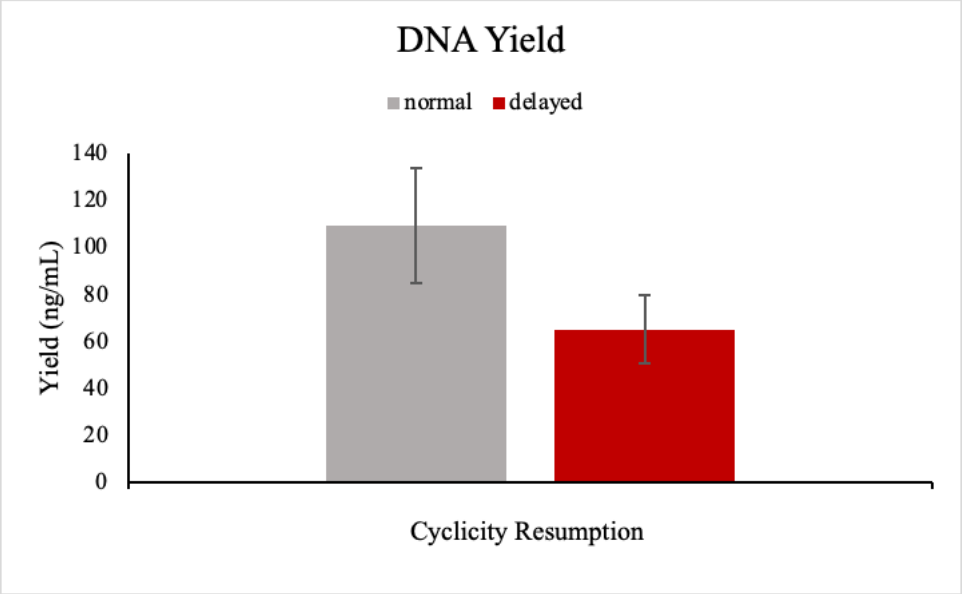

Figure 4.3: DNA yield in normal and delayed animals after extraction. Quantified using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA).

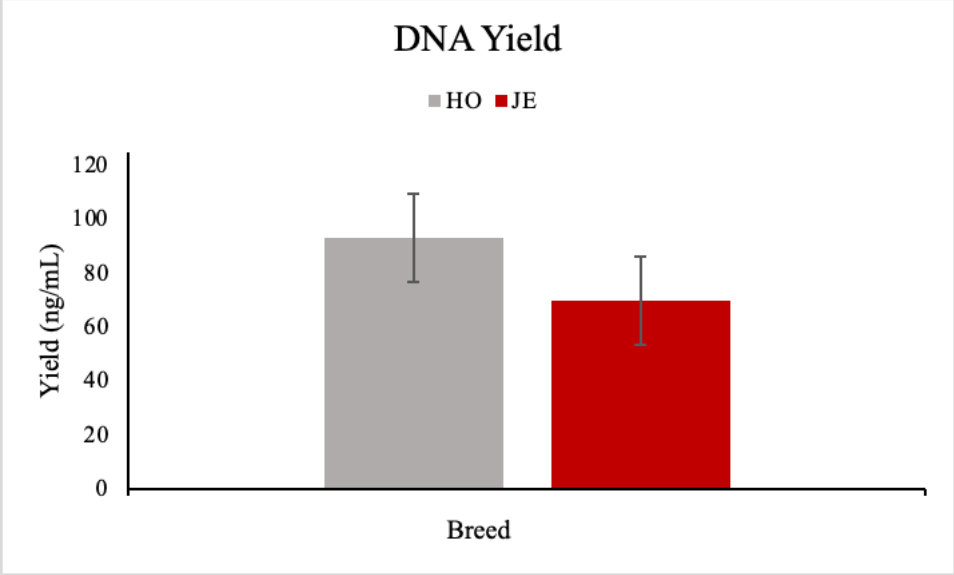

Figure 4.4: DNA yield in Holstein and Jersey cows after extraction. Quantified using the Synergy LX Multi-Mode Microplate Reader in conjunction with the Take3 Micro-Volume Plate (BioTek Instruments Inc; Winooski, VT, USA).

Discussion

Although the data have not been analyzed yet, a tendency for decreased DNA yield was seen in cows with delayed resumption of cyclicity. Animals with delayed resumption have difficulty regaining normal control of the HPG axis in the postpartum period for various reasons, and this could be due to incidence of pathogenic bacteria that entered the cervix in the calving process. It is expected that animals with delayed resumption of cyclicity will have an increased relative abundance of pathogenic bacteria which inhibit the HPG axis effectively disrupting cyclicity resumption. Animals with normal resumption of cyclicity are expected to have increased microbial diversity and abundance of commensals, promoting reproductive efficiency. However, the DNA yield is a summary of how much microbial DNA was present after the extraction process. This is not indicative of whether the microbes are pathogenic or commensal and the sample must be normalized for further sequencing before reporting information on abundance and diversity of bacteria within the uterus.

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CHAPTER FIVE

CONCLUSION

After evaluating groups of dairy cows on both a conventional and robotic farm with varying breed composition, results indicate time to resumption of cyclicity postpartum is influenced by metabolites, milk production, and/or heritable traits. However, despite the many variables with potential to impact the animal's reproductive status, milk production and its associated negative energy balance was consistently found to be important in determination of a cow having normal or delayed resumption of cyclicity.

The results from the first study indicate that cows on a robotic farm with voluntary milking access and pellet allotments in addition to feed still have potential to be negatively impacted by negative energy balance in the early postpartum period. Although milk production had no direct impact on resumption of cyclicity postpartum, metabolites associated with increased production impacted the timing of cyclicity resumption. Cows with delayed resumption of cyclicity experienced increased NEFA in circulation while normal cows experienced increased glucose in circulation. The cows who were able to regain cyclicity in a normal time frame were potentially able to utilize glucose more efficiently for reproduction instead of prioritizing glucose for milk production. Conversely, the cows who had delayed resumption of cyclicity did not have enough glucose in circulation and had to utilize lipolysis to increase NEFA in circulation to prioritize lactation during metabolic demand. Although some differences in reproductive

parameters were seen in delayed and normal cows, they were not exacerbated to the level hypothesized. Additional samples during the pre-partum period would allow us to expand our knowledge on the changes that occur metabolically during the transition period and the implications those changes have on reproduction. To expand on this study, measuring feed and/or pellet intake along with body condition score would allow us to further understand the metabolic imbalance in the transition period and its impact on resumption of cyclicity postpartum.

In the second study, our results indicate Jersey cows had increased AMH but lower estrous intensity at 1st estrus postpartum compared with Holsteins. It must be considered Jerseys will potentially have less estrous expression and producers may have to rely on alternative methods beyond traditional visual observation for reproductive efficiency. DPR was the only fertility trait found to be impactful and was found to be positively associated with increased estrous intensity and normalized time to resumption of cyclicity. Milk production was seen to have no impact on Jerseys or Holsteins time to resumption of cyclicity postpartum. However, it is known that Jerseys are more efficient milk producers by producing more pounds of milk per pound of bodyweight. Comparing breed differences using energy corrected milk would allow more direct comparison by evaluating any influence on cyclicity associated with the increased components in Jersey cow milk. To further explore the differences between Holstein and Jersey reproductive efficiency during the transition period, metabolic testing will be performed. Additional samples taken during the pre-partum period will allow us to investigate the depth of impact the transition period has on two breeds with vastly different milk production and

composition. This will help us better understand how to manage the breeds appropriately to achieve successful resumption of cyclicity.

The third study was designed to explore the uterine microbiome in early postpartum Holsteins and Jerseys in an effort to identify relationships with microbial community and fertility, within and between breeds. These results will additionally indicate whether cows had subclinical infections at the time of sampling and the implication that could have on their future fertility that would otherwise be unknown. While the results have not yet been analyzed, this data could provide invaluable information about the impact microbial colonization during the calving process has on reproductive success in the current lactation and future reproductive success through herd longevity. To expand on this study in the future, samples taken concurrently from swabs of the vaginal wall could allow for comparison of the uterine and vaginal environments to ensure no contamination occurred during sample collection.

Collectively, our results indicate that regardless of environmental factors such as parlor or management style, there are many variables influencing the time to resumption of cyclicity postpartum. Moreover, some of these variables can be controlled and immediately changed, but other variables like genetics can only be changed through selection for desirable traits over time. It is necessary for producers to mitigate the risks of a delayed resumption of cyclicity by minimizing negative energy balance, managing disease in the postpartum period, and promoting change through long term selection of reproductively heritable genetic traits in order to promote reproductive efficiency in dairy herds.

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