Hormones, metabolites, and reproduction in Holsteins, Jerseys, and their crosses

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ABSTRACT

Cows in first (n = 163) and second (n = 101) lactation were studied to determine if reproduction, progesterone (P4), IGF-I, insulin, NEFA, and milk production differed between genetic groups. Thirty-five cows were Holstein-Jersey (HJ) crosses, 48 were Jersey-Holstein (JH) crosses, 51 were Holsteins (HH), and 29 were Jerseys (JJ). Days open (DO) was affected by genetic group. HH had 168.6 ± 9.6 DO which was different from HJ (142.9 ± 11.3 d), JJ (132.1 ± 12.1 d), and JH (127.2 ± 9.3 d). HH had 2.4 ± 0.1 services per conception which was different from JH (1.9 ± 0.1), but not different from HJ (2.1 ± 0.2) or JJ (2.1 ± 0.2). In HH P4 concentrations (1.6 ± 0.11 ng/mL) were not different from HJ (1.5 ± 0.12 ng/mL), but lower than JH (1.8 ± 0.10 ng/mL) and JJ (1.8 ± 0.13 ng/mL). NEFA concentrations were greater in lactation 2 (0.52 ± 0.02 mEq/L) than in lactation 1 (0.45 ± 0.02 mEq/L). Insulin in HH (0.83 ± 0.03 ng/mL) was not different from HJ (0.87 ± 0.04 ng/mL) or JH (0.76 ± 0.03 ng/mL), but was greater than JJ (0.66 ± 0.04 ng/mL). IGF-I gradually increased over the 10 wk period. The HH produced 10,348 ± 208 kg of milk, which was greater than the HJ (9,129 ± 230 kg), the JH (9,384 ± 192 kg), and the JJ (7,080.9 ± 261 kg). Reproductive measures, milk yield, hormones and metabolites were affected by genetic groups.

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INTRODUCTION

Crossbreeding is one solution to improve the reproductive efficiency within the dairy industry which has declined over the last four decades. Crossbreeding improves components of milk, fertility, and productive life (VanRaden and Sanders, 2003; Anderson et al., 2007; Heins et al., 2008a, b). Low reproductive efficiency in the purebred Holsteins has led to the interest in crossbreeding (Heins et al., 2008b). The decline in the reproductive ability in the Holsteins could be attributed to the state of negative energy balance (**NEB**) that they enter after parturition. Therefore, it is important to identify when a NEB state exists so that herd nutritional and management programs can be implemented to allow for adequate energy intake to optimize herd health and productivity (Stokol and Nydam, 2005).

Interaction between diet and reproduction can enhance or reduce fertility and may be an important combination for feed management (Lucy et al., 1992b). The most important period is during early and peak lactation when energy demand is the highest (Butler and Smith, 1989). Other factors that influence NEB are the diet that the animal received prior to calving and the diet provided postpartum. Non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHBA**), insulin, insulin-like growth factor-I (**IGF-I**), and glucose have been shown to describe the effects of NEB.

NEFA appear in the bloodstream when the animal breaks down adipose tissue to supply energy needed for milk production. Plasma NEFA are an important measure to assess energy balance of the cow because they are elevated when stored fat is mobilized (Holcomb

et al., 2001). One condition that increases NEFA concentrations reveals an energy deficiency (Tanaka et al., 2008).

Van Knegsel et al. (2007) found that low plasma insulin concentrations reduced glucose uptake by muscle and adipose tissue thus sparing glucose for uptake by the mammary gland, which is not responsive to insulin. During NEB or with low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of NEFA to the bloodstream (Nelson and Cox, 2000; Melendez et al., 2009). According to Staples et al. (1998), insulin concentrations usually reflect energy intake.

Decreased IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus, and cows with high milk production are in NEB and under nutritional stress. IGF-I concentrations in blood and follicular fluid in cattle usually exceeds 100 ng/mL (Echternkamp et al., 1990; Spicer and Enright, 1991; Spicer et al., 1992).

Fertility declines with age and older cows have higher milk yields and more clinical abnormalities than younger animals. Cows in first lactation have lower energy balance because they consume less feed and had energy requirements for growth in addition to lactation (Lucy, 2001). Lower energy balance in first lactation cows was associated with delayed intervals to first ovulation (Lucy et al., 1992b). Lucy (2001) stated that improved reproduction in high-producing herds probably reflected improved feeding, healthier cows, and better reproductive management.

Progesterone is known as the hormone of pregnancy; therefore, it is an indicator of ovarian activity. Ovulation is substantiated by collection of a single plasma sample in which progesterone increases above 1 ng/mL (Harrison et al., 1990; Senatore et al., 1996). Cows reached first estrus on the first of 3 consecutive samples that had a progesterone concentration greater than 1 ng/mL (Simmons et al., 1994). Butler and colleagues (1981) found that average time to first ovulation in multiparous cows was near 30 d.

Exploring these different factors and how they influence the cows' ability to return to estrus more quickly will allow producers to maximize production of their animals. Management, diseases, environmental events, and the quality of feed affect reproduction. Attempting to determine which factor hinders the cow's ability to maximize production and enhance reproduction may allow producers to control factors related to breed or lactation number.

CHAPTER 1: Review of the Literature

Crossbreeding

Crossbreeding is of current interest within the dairy industry, and the number of crossbreds has increased in the U. S. since 1993 (VanRaden and Sanders, 2003). Crossbreeding is a growing topic of interest because of the dairy producers' concerns regarding heifer and cow fertility, cow health, and calf survival (Funk, 2006; Heins et al., 2008b). Successful crossbreeding programs entail the use of high-ranking sires within breed based on certain indexes like Net Merit (McAllister, 2002). Crossbreeding provides a simplistic method to increase the health and efficiency of many plants and animals, by introducing favorable genes from other breeds, by eliminating inbreeding depression, and by maintaining gene interactions that cause heterosis. Crossbreeding improved components of milk, fertility, and productive life (VanRaden and Sanders, 2003; Anderson et al., 2007; Heins et al., 2008a, b). In a survey conducted by Weigel and Barlass (2003), dairy producers stated that crossbreeding enhanced fertility, survival, and profitability of dairy cows. Data obtained from large numbers of crossbred and purebred dairy cows in commercial herds may provide more current or more accurate estimates of heterosis for recorded traits and include more breeds than in previous studies (VanRaden and Sanders, 2003).

The decline in reproductive ability in Holsteins around the world has led to the interest in crossbreeding (Heins et al., 2008a). Some Holstein breeders have used crossbreeding to increase protein and fat percentages of milk or believe that the benefits of heterosis outweigh the breed differences. Crossbreeding often leads to an increased in health; however, higher milk yields of crossbreds may increase the stress on the udder and

could be the cause of the small net increase in somatic cell score (**SCS**). Matings between the Jerseys, Brown Swiss, and Holstein breeds can generate a crossbred progeny that will stay in the herd as long as or longer than the purebred Holsteins.

Olson and colleagues (2010) found that crossbred cows ate the same amount or less energy, required less energy for maintenance than the Holsteins, and required the same or less for growth when compared with the Holsteins, but gave the same energy in milk as the Holsteins. The Jerseys allocated a higher proportion of consumed energy to milk production when compared to the Holstein group. However, the Holsteins put more energy into growth than the Jersey-Holstein crosses and the Jerseys. Recent research suggested a 2 to 3 wk advantage of crossbreds vs. purebred Holsteins for days open, which should enhance the profitability of dairy production systems (Heins et al., 2006b; Dechow et al., 2007). A study conducted by Heins and colleagues (2008a) found that Jersey (**J**) x Holstein (**H**) crosses had a 23 d advantage for days open and that the smaller body size of J x H should provide benefits in lowering maintenance costs compared to the purebred Holsteins. Another study conducted by Olson and colleagues (2009) found that crossbred calves born from a Holstein dam were less likely born stillborn compared to crossbred calves born from a Jersey dam.

The Southern Regional Cooperative Research Project S-49 (McDowell, 1982) reported that crossbreds averaged 3-17% fewer days open than the purebreds at 3 locations, but differences between the crossbreds and purebreds did not favor the crossbreds consistently at the 4^{th} location. Holstein cows with clinical problems after calving had 16.6 ± 10.8 more days open than cows with no clinical issues. In Jerseys, the days open was affected by clinical abnormalities at parturition and postpartum, age at calving, and milk

yield during the first 70 DIM. Those Jersey cows that had clinical problems during the postpartum period had 15.1 ± 7.0 more days open than normal cows, whereas cows that had problems at calving had 20 ± 9.6 fewer days to conception compared to those cows with no issues (Fonseca et al., 1983). Jerseys that calved during December to May had higher conception rates at first insemination compared to those that calved during September to November. Butler and Smith (1989) found that the negative effects of high milk yield on conception rate most likely resulted through delays or failure of early resumption of ovulation in the postpartum period, thereby allowing fewer ovulatory cycles before insemination causing lower fertility.

Holstein cows were more likely to have a prolonged luteal phase when compared to Friesian cows (Lucy, 2001). Dairy producers responded that crossbreeding programs of Holstein cows mated with either Brown Swiss or Jersey bulls achieved higher conception rates than matings involving purebred Holsteins (Heins et al., 2006b). Crossbreeding of dairy cows is being investigated mostly for its potential to improve fertility, health, and survival of cows, and advantages for these traits might compensate for loss in production of crossbreds compared to the purebred Holsteins (Heins et al., 2006a).

Milk Yield

The level of milk production had the greatest negative effect on reproductive performance, suggesting that reduced fertility is caused by the inability of the cow to consume enough feed to meet nutritional requirements (Garmo et al., 2009). The total energy required by a cow to produce milk yield for one lactation can vary depending on the energy source to the mammary gland because the cost of metabolic processing of different

types of nutrients by cows is variable (Emmans, 1994). Genetic selection for yield enhances feed intake; however, milk production causes negative energy balance (**NEB**) and increased body tissue mobilization (Veerkamp et al., 2000). Typically cows that have a higher genetic merit for milk yield have greater milk energy output during early lactation, which is met by a combination of increased dry matter intake (**DMI**) and body tissue mobilization (Bauman, 2000). High yielding dairy cows typically are unable to consume adequate amounts of energy during early lactation to completely satisfy the energy requirements needed for milk production; therefore, they rely on the body reserves that were stored prepartum (Holcomb, 2001).

Negative energy balance was associated with reduced expression of estrus and lessened responses to procedures for synchronization of estrus, which may be due to inconsistent growth and development of ovarian follicles (Lucy et al., 1992a). High milk production by dairy cows is associated with low reproductive performance (Staples et al., 1990). High milk-yielding underfed dairy cows have greater plasma concentrations of growth hormone and nonesterified fatty acid (**NEFA**) and lower insulin concentrations than lower yielding overfed dairy cows (Barnes et al., 1985). If milk production affects reproductive performance, then certain traits such as involution of the uterus and cervix, interval from parturition to first and subsequent ovulations, rate of detection of estrus, and the rate of conception should differ among cows with different milk yields. Also, Fonseca et al. (1983) found that conception rate increased for cows that had a deviation of less than 1,400 kg from herdmates and decreased for cows that had a deviation of more than 2,600 kg of milk from herdmates. Milk yield affected days to first observed estrus in Holsteins, days to

first insemination in both Holstein and Jerseys, and days open in Jerseys (Fonseca et al., 1983). Higher producing Jerseys had longer intervals to first insemination and conception, but these effects appeared independent of the effects of milk yield on physiological traits. Cows that had higher milk yield during the current or previous lactations had longer intervals to first service (Nebel and McGilliard, 1993), showing that higher milk yields were connected with lower conception rates or cyclicity of estrus expression, suggesting that metabolic demands of higher yield reduced fertility of high yielding cows. This suggests that parity may influence the resumption of ovarian activity.

Parity

Fertility declines with age and older cows have higher milk yields and more clinical abnormalities than younger cows. Older cows may become more susceptible to the possible effects of higher milk production on the involution of the cervix and uterus and time of first postpartum ovulation (Fonseca et al., 1983). A major subcomponent of infertility resulting in higher incidence of late embryonic mortality in dairy cattle was related to abnormal hormonal activity, causing effects ranging from prolonged luteal activity to delayed ovulation (Lamming and Darwash, 1998). Recent studies show that low fertilization rates and embryonic loss appear as the main factors contributing to infertility in dairy cattle (Santos et al., 2004; Morris and Diskin, 2008).

Clinical uterine diseases postpartum increase the risk of subclinical endometritis in lactating dairy cows, and suppress fertility (Rutigliano et al., 2008), partly because of the decreased fertilization rate (Cerri et al., 2009a). Lucy (2001) stated that improved

reproduction in high-producing herds probably reflected improved feeding, healthier cows, and better reproductive management.

First-lactation cows tend to have lower energy balance because they consume less feed and have energy requirements for growth in addition to lactation (Lucy, 2001). First-lactation cows usually represent a larger portion of the herd when cow turnover is increased due to infertility in older cows. Expanding herds or herds with fertility problems may have decreased herd reproductive efficiency because a larger portion of the herd consists of first-lactation cows whose reproduction may be compromised. Increased days open and extended lactation may lead to an increased dry period length. Friggens and colleagues (2007) found that first lactation cows tended to mobilize less energy from body tissue compared to multiparous cows.

Dry Period

The evaluation of the optimal dry period length was based upon milk production, but animal health and reproductive performance may need consideration in determining this decision (Watters et al., 2009). However, limited research has been done evaluating the effect of the dry period on reproduction. Studies have shown that cows with first ovulation occurring early in lactation may improve reproductive performance; therefore, reducing the dry period length.

Watters and colleagues (2009) stated that days open was lower in cows with a 56 d dry period than for cows with a 42 d dry period, but first-service conception rate were greater in multiparous cows with a 35 d dry period compared to a longer dry period. There was a reduction in days to first ovulation after cows had a shorter dry period than those with a

traditional 55 d dry period. An important observation was that cows tended to have a decrease in days to pregnancy when the dry period was shorter. A shorter dry period and earlier ovulation were associated with a decrease in NEB during the first 3 wk after calving. A more positive energy balance was mainly related to an increased DMI in cows that had a shorter dry period.

Short dry periods tend to reduce the milk production in the subsequent lactation in several species including cattle, rats, and humans (Annen et al., 2004); in cattle, this takes place because of decreased mammary epithelial cell turnover and secretory capacity (Annen et al., 2007). A decrease in the duration of the dry period reduced the inherent drive to produce milk in subsequent lactations, whereas increasing dietary energy density allowed dietary energy intake to meet energy requirements more closely (de Feu et al., 2009).

Uterine involution and resumption of cyclic ovarian activity play an important role in the subsequent fertility of dairy cows (Lewis, 1997). During the period from parturition to first ovulation, where progesterone concentration is minimal, the uterus is resistant to infections, and purulent uterine infections rarely develop (Sakaguchi et al., 2004). After ovulation, concentrations of progesterone increase, and the uterine immune system becomes down-regulated, creating an environment that is susceptible to infection (Lewis, 1997). Some studies have suggested that luteal concentrations of progesterone after early first ovulation increase the occurrence of uterine infections (Lewis, 1997) and reduce fertility (Smith and Wallace, 1998).

Transition Period

Dairy cows are vulnerable to NEB during the transition period (Stokol and Nydam, 2005). This is due to the high-energy demands of the growing fetus and subsequent milk production, coupled with DMI decreasing prior to calving (Drackley, 1999; Grummer et al., 2004). It is important to recognize when a state of excessive NEB exists in cows within the herd, so nutritional and management programs can be tailored to the increase transition energy intake and optimize herd health and productivity (Stokol and Nydam, 2005).

The transition period is recognized as the 3 wk prior to parturition until 3 wk after calving. This period is marked by nutritional, metabolic, hormonal, and immunological changes that influence the incidence of infections and metabolic diseases (Loiselle et al., 2009). The hallmark of the transition period of dairy cows is the dramatic increase in nutrient demands of metabolism to meet requirements for energy, glucose, amino acids, and calcium by the mammary gland following parturition (Overton and Waldron, 2004). It is characterized by a decline in feed intake around parturition (Bertics et al., 1992), and lipid mobilization that leads to increased plasma NEFA concentrations (Doepel et al., 2002). Holcomb et al. (2001) stated that the transition period is the most critical period in a cow's life. Cows are at high risk during this period for developing metabolic disorders like ketosis and diseases like mastitis (Chagunda et al., 2006). This period remains the most problematic for dairy farmers and metabolic disorders continue to occur at economically important rates on commercial dairy farms (Overton and Waldron, 2004). Most metabolic problems occur during the transition from the dry period into early lactation along with the health issues taking place during this period can result in a decrease in peak milk yields causing a loss of milk for the entire lactation (Holcomb, 2001). Peak milk yields decreased 5 to 10 kg/d,

which can amount to 1,000 to 2,000 kg of lost milk for the lactation. Cows having one or more reproductive, nutritional, or health related issue prior to 30 d postpartum had eaten 19 % less feed during the periparturient period (Zamet et al., 1979).

Early Postpartum

Lucy et al. (1992b) found that the shortest interval to first ovulation occurred in those cows that consumed the most DM throughout the experimental postpartum period. Cows with greater NEB had longer intervals to first ovulation and produced the least amount of milk; therefore, higher producing cows can have a less NEB and return to ovarian activity earlier. Patton and colleagues (2007) found that increased DMI during early lactation was associated with earlier resumption of cyclicity, greater conception rate to first insemination, and shorter calving to conception interval. Butler and Smith (1989) found the recovery period or improvement in energy balance from its most negative energy state at the onset of lactation towards a positive energy state may provide an important signal for initiation of ovarian activity. It is possible that the effects of lactation and energy status may influence fertility in stages after the period of early embryonic development (Cerri et al., 2009b). Energy status and lactation impact the occurrence of metabolic diseases and metabolic disorders.

Metabolic Factors

Metabolic changes can lead to diseases and can decrease reproductive performance (Goff et al., 2002). If metabolic and endocrine challenges are not compensated by the cow's homeostatic mechanisms, then the cow can encounter diseases like milk fever, hypocalcemia, ketosis, retained fetal membranes, metritis, mastitis, or displacement of the abomasum

(Melendez and Risco, 2005). Cows with metabolic disturbances or health problems during early lactation produced less milk than healthy cows, causing economic losses for dairy farmers (Drackley, 1999). Milking frequency is a factor that affects both milk yield and metabolic disturbances around calving (Rémond and Pomiès, 2005). Throughout the transition period, there are large variations in metabolites and hormones that are associated with several traits such as genetic line, breed, parity, diet, and days in milk (**DIM**) (Drackley, 1999; Ingvartsen and Anderson, 2000).

Metabolic Disorders

Dietary intake often fails to compensate for increasing energy demands associated with high milk yield during the early stages of lactation (Reksen et al., 2001). Proper nutritional management of cows throughout the dry period to uphold optimum body condition score (**BCS**) prior to calving and improving DMI throughout the early postpartum period is crucial to prevent severe and prolonged NEB during early lactation to support high milk production, and to prevent metabolic diseases (Shrestha, 2004). High-producing dairy cows are challenged postpartum with high metabolic demands initiated by the increase in energy requirements at the start of lactation, which are unable to be met with DMI alone (van Knegsel et al., 2007). Cows with metabolic disorders commonly associated with fat cow syndrome are at risk to develop reproductive disorders, which can result directly or indirectly reduce reproductive performance (Butler and Smith, 1989). The metabolic and endocrine effects of early lactation can be expected to affect follicular development and in turn have a negative impact on the dairy cow fertility through prolonged anovulatory periods (Beam and Butler, 1998). Characterization of follicular development prior to the first ovulation

postpartum and examination of the associated levels of energy balance and metabolic hormones were important steps towards understanding the metabolic restriction on post-partum ovarian activity.

The beginning of lactation imposes a considerable metabolic challenge to dairy cows because energy intake lags behind the energy expenditure required attaining and maintaining high levels of milk production (NRC, 2001). During early lactation, nutritional and metabolic conditions to support lactation favor increased plasma NEFA and ketones as a result of lipolysis and ketogenesis, which may lead to lower adipose tissue responsiveness to insulin (Holtenius, 1993). Observing energy balance on a regular basis could assist in the improvements of fertility and reproduction (Oikonomou et al., 2008). Management affects NEB that influence reproductive performance (Stockdale, 2001).

Reproduction

Reproductive ability has been decreasing within the dairy industry over the last 40 yr in high-yielding dairy cattle. The intensive genetic selection for increased milk production has led to the notable improvement in milk yield per cow, but has been associated with a worldwide decline in fertility of the dairy cow (de Feu et al., 2009). Lucy (2001) stated that the decline in reproductive efficiency in dairy cows could be connected to reduced expression of estrus.

Increasing requirements to maximize the economic efficiency of animal production has driven forward the intensification of farming systems (Law et al., 2009). One way to maximize the economic efficiency is to have cows calve once a year (Shrestha, 2004; Opsomer et al., 1998). In order to attain this goal, early onset of estrus postpartum, high

estrous detection rate, and high conception rates are necessary (Shrestha, 2004). Cows need to conceive by 85 d postpartum to achieve this goal (Opsomer et al., 1998). The ability of the dairy cow to partition energy for milk production at the cost of reproduction early in lactation has made energy balance a key factor for studying reproductive performance and milk production (Ospina et al., 2010).

Early reestablishment of ovarian activity was a prerequisite for superior fertility during later lactation (Darwash et al., 1997). Fertility should be included in breeding goals in order to reverse the trend of reproductive efficiency in dairy cows, because of the unfavorable genetic correlations that exist between milk yield and reproductive performance (Veerkamp et al., 2001; Royal et al., 2002; Pryce et al., 2004). Conception rates are influenced by the number of estrous cycles prior to AI (Beam and Butler, 1999; Thatcher and Wilcox, 1973).

Reproductive performance is frequently measured by calving interval or some component of calving interval like days open (days nonpregnant). Calving interval combines days open and length of gestation (Fonseca et al., 1983). Days open is defined as the time between calving and conception. Days open are used as the major determinant of lifetime production and herd replacement rates (Lee et al., 1989). Days open are the primary economic measure of reproductive performance in dairy cattle (Lee et al., 1989). Kuhn et al. (2004) defined days open as a function of days to first breeding, number of services to conception, and intervals between services.

The variation of calving interval results from the changes of days open; a function of interval from parturition to first insemination, rates of conception at first and subsequent

inseminations, and intervals between successive inseminations. Lucy (2001) found that first-service conception rates in American herds declined from approximately 65% in 1951 to 40% in 1996.

Higher producing dairy cows have a greater number of days to first ovulation, estrus, and first breeding, lower conception rates, and more days open than those cows that do not produce as much milk (Staples et al., 1990). The decline in conception rates for lactating cows reflects the effects of higher milk production, not genetic selection for lower fertility (Butler and Smith, 1989).

The USDA/Animal Improvement Programs Laboratory uses an upper limit of 250 d for days open (VanRaden et al., 2003). For most states, estimates of heritability at thresholds ≥ 250 d were identical and differed from heritability estimates at the 200 d or less threshold (Oseni et al., 2004). This may imply that there is a greater genetic variability at days open thresholds ≥ 250 d. The heritability estimates for days open were higher or identical at days open thresholds ≥ 250 d when compared with lower thresholds, indicating greater genetic variability for long days open records (Oseni et al., 2004).

Disease exposure can increase days open and ultimately decrease both productivity and profitability (Lee et al., 1989). Animals that had retained placenta, nonsystemic metritis, systemic metritis, ovarian cysts, or lameness tended to have lower conception rates and higher days open than healthy animals.

Diet &Reproduction

Interaction between diet and reproduction can either enhance or reduce fertility and may be an important factor when making decisions for future feed management (Lucy et al.,

1992b). Certain fatty acids have specific effects on different tissues, with potential benefits to the fertility of dairy cows (Cerri et al., 2009b). These benefits tend to be independent of the stipulation of calories and changes in energy balance of the cow (Staples et al., 1998; Santos et al., 2008). Considering the relationship between dietary energy and reproduction in dairy cattle, the most important period is during early and peak lactation when energy demand is the highest in the cow (Butler and Smith, 1989). Other factors that influence the effects of NEB are the diet that the animal is receiving prior to calving along with the diet provided postpartum. NEFA, β-hydroxybutyrate (**BHBA**), insulin, insulin-like growth factor-I (**IGF-I**) and glucose describe the effects of NEB. The regulatory hormones such as growth hormone, insulin, and IGF-I affected reproduction such that during NEB their altered concentrations restricted follicle growth and steriodogenesis (Roche et al., 2000; Webb et al., 2004). Mills et al. (1986) stated that cows in early postpartum that eventually became ketotic had larger changes in concentrations of plasma glucose, free fatty acids, BHBA, and insulin than those cows that did not become ketotic.

Progesterone

Progesterone is known as the hormone of pregnancy, since it is produced by the corpus luteum to maintain pregnancy. Ovulation is substantiated by collection of single plasma progesterone above 1 ng/mL (Harrison et al., 1990; Senatore et al., 1996). Cows reached first estrus on the first of 3 consecutive samples that had a progesterone concentration greater than 1 ng/mL (Simmons et al., 1994). Butler and colleagues (1981) found that average time to first ovulation in older cows was near 30 d. Other studies reported ovulation taking place when progesterone concentrations remained elevated when samples

were collected over a period of several days (Lucy et al. 1992b; Spicer et al., 1990; Zurek et al., 1995).

Negative energy balance may influence reproduction through its direct or indirect influence on progesterone secretion by the corpus luteum (Nebel and McGilliard, 1993). Reestablishment of ovulatory cycles early after parturition guarantees multiple estrous cycles prior to the breeding period, which influences conception rate (Butler and Smith, 1989). Increased concentrations of plasma progesterone were associated with improved conception rates of lactating ruminants (Staples et al., 1998). Conception rates increased 1.44% for every 1 ng/mL increase in plasma progesterone.

Analyzing progesterone profiles allows for an objective way of characterizing postpartum ovarian activity in dairy cows. A normal progesterone profile usually contains a period of low progesterone after calving followed by increasing concentrations of progesterone, which indicate first postpartum ovulation (Figure 1.1; Petersson et al., 2006). Shrestha et al. (2004) found that cows with successful pregnancies had a higher progesterone concentration in the cycle prior to breeding. However, deviations can occur from the normal which can be associated with decreased fertility in dairy cattle. They found that all fertility disorders were associated with an atypical progesterone profile, whether that profile be

delayed, prolonged, or indicative of cessation of cyclicity.

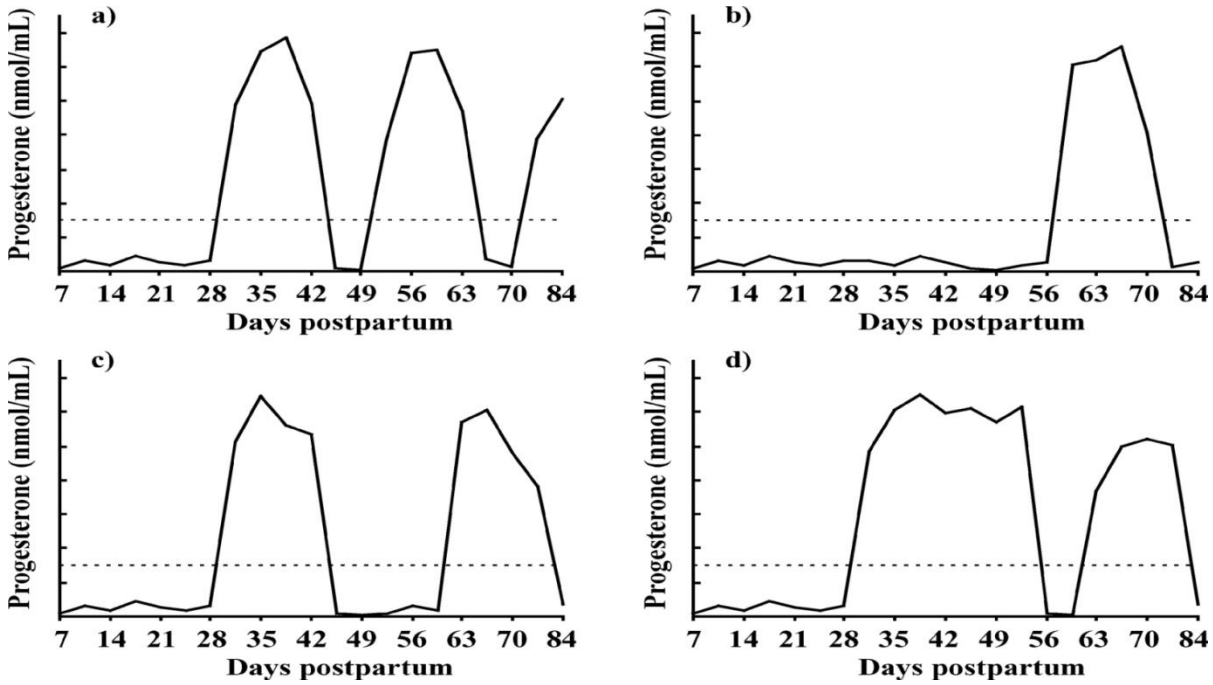

Figure 1.1. Schematic illustration of the different progesterone profiles for Swedish Holstein and Swedish Red and White dairy cows: a) normal profile, b) delayed cyclicity, c) cessation of cyclicity, and d) prolonged luteal phase (Petersson et al., 2006).

A period of postpartum anestrus is normal in the dairy cow; however, if it is too long it can extend into the breeding season, particularly of seasonally bred cows causing a delay in timing of pregnancy and increases the risk of reproductive failure (Chagas et al., 2007). McCoy and colleagues (2006) found that cows that had any abnormal profile had a significantly longer interval to first service, lower conception rates to first and all services, and longer interval to conception. Reduced fertility may be related to lower progesterone concentration, due to the lack of cycles before insemination (Shrestha, 2004).

Fonseca et al. (1983) found that progesterone was affected by season of calving and change in BW for Jerseys. They found that cows that calved between September and

November had higher progesterone concentrations than cows that calved during the rest of the year. Progesterone concentrations found in the blood after AI were higher for the Holsteins that conceived compared to those that did not; however, no difference was seen in the Jerseys.

Shrestha et al. (2004) found that postpartum cows that had abnormal ovarian cycles, including prolonged luteal phase and anovulation, during the pre-service period had a reduced reproductive performance than those cows that experienced normal ovarian cycles. Those cows with prolonged luteal phases had lower AI rates, conception, and pregnancy within 100 d postpartum compared to those having normal cycles. Cows experiencing anovulation had lower conception rates and pregnancy within 100 d postpartum, and required longer intervals from calving to AI, and to conception, and more inseminations per conception than cows with normal cycles. The increasing severity of NEB in early lactation was associated with impaired ovarian function and delayed resumption of estrous cycles (Jolly et al., 1995).

Energy Balance

NEB is associated with body energy loss and positive energy balance with body energy gain (Coffey et al., 2002). Dairy cows usually enter a NEB state postpartum when the combined energy requirements for maintenance and milk production exceed dietary intake (Patton et al., 2008). Heavier reliance on body fat reserves during early lactation has a negative impact on fertility and health (Hansen, 2000; Banos et al., 2006). An extended period of NEB may lead to health problems, reduced reproductive performance, decreased production, and can lead to early culling or death (Coffey et al., 2004; Collard et al., 2000;

De Vries and Veerkamp, 2000). The magnitude and length of NEB state is dependent on the direct and interactive effects of numerous factors including genotype, plane of nutrition, and body condition score (**BCS**) at calving (Patton et al., 2008).

A study by Reksen et al., (2001) found that energy balance was lower during the first 5 wk postpartum in first lactation cows that had a late resumption of luteal activity (> 30 d) postpartum. Energy balance was low for cows with a midluteal response (luteal response between 19-30 d postpartum) for the first 3 wk postpartum when compared with cows in first lactation that had an early resumption of luteal activity (<19d). For second lactation cows, those that had late resumption of luteal activity had low energy balance during wk 3 to 10 postpartum and energy balance was lower for those cows with a midluteal response during the 4^{th} wk compared to those with an early resumption of luteal activity. They found that first lactation cows that resumed luteal activity late produced more milk than herd-mates with an early luteal response during the entire 14 wk period. First lactation cows that had a midluteal response produced more milk than cows with an early luteal response except during wk 5 and 6 postpartum. Milk yield was higher during the first 6 wk postpartum for second lactation cows with late resumption of luteal activity, and milk yield was higher for those cows with a midluteal response during the 3^{rd} and 4^{th} wk postpartum compared to those cows in second lactation having an early resumption to luteal activity. In addition, first lactation cows that experienced an early resumption of luteal activity reached the greatest NEB status during the second week postpartum compared to the first lactation cows that had mid or late resumption of luteal activity that reached the lowest energy balance level during wk 1. In summary, moderate yielding cows, which compensated for energy deficits by

decreasing milk yield experienced an early reoccurrence of ovarian activity. Therefore, days to first luteal activity and first regular cycle can be used to assess the impact of energy balance on reproduction.

The shift from NEB to positive energy balance during the course of early lactation may affect reproduction performance (Butler and Smith, 1989). Cows which lose body tissue and energy in early lactation usually return to positive energy balance around 40 to 80 d postpartum (Sutter et al., 2000; Coffey et al., 2001; Veerkamp et al., 2000). Potential factors such as increased milk production which is associated with NEB, larger herd sizes, and higher inbreeding percentages were suggested as reasons for infertility in dairy cows (Lucy, 2001). Higher producing cows usually do not consume enough feed during early lactation to maintain a positive energy balance (Olson et al., 2010). Increased milk production, increases the animal's demand for energy and she ultimately enters into a NEB state. This is because the animal does not have enough energy to meet all of the demands the body is trying to overcome.

Olson et al. (2010) found that purebred Holsteins (**HH**) required more energy for growth when compared with the Jersey-Holstein (**JH**) or purebred Jerseys (**JJ**); the Holstein-Jersey (**HJ**) required more than the JJ, but the crosses were not different from each other. The HH, HJ, JH required more energy for milk production than the JJ. This study reported a trend that the HH and HJ carried more body condition than the JH genetic group.

Nutritional factors and their effect on energy balance in the early lactation period and periparturient diseases appear as the main factors influencing the onset of cyclicity or luteal activity (McCoy et al., 2006). DMI was reduced severely, the dependence on lipid stores

increased, with a later increase in the probability of hepatic lipidosis and metabolic disorders developing (Goff and Horst, 1997). Feeding diets that were more energy dense than recommended by the NRC prepartum led to reductions in both plasma NEFA concentrations and lipid infiltration of the liver (VandeHaar et al., 1999). After parturition, high-producing dairy cows usually experience a variable period of NEB because the DMI failed to meet the increasing energetic requirements of milk production. The severity and length of the NEB state during early lactation affected the postpartum interval to first ovulation (de Feu et al., 2009). Negative energy balance involved a decrease in circulating concentrations of insulin, glucose, and IGF-I, and increased concentrations of BHBA and NEFA (Grummer, 1995).

NEFA

Successful entry into lactation requires maintaining feed intake throughout the transition period, because prepartum DMI was inversely associated with concentrations of NEFA and BHBA (DeFrain et al., 2006). During periods of high energy deficit, key hormone expression and tissue responsiveness are altered to increase lipolysis and reduced lipogenesis, optimizing NEFA mobilization to uphold physiological equilibrium (Bauman and Currie, 1989; Bell, 1995; Bauman, 2000). This is known as homeostasis and the net result of mobilization of adipose tissue reserves in response to NEB (Roche et al., 2009). Cows in a NEB state mobilize more body fat reserves and produce glycerol for an energy resource, which leads to increased NEFA concentrations in blood (De Vries and Veerkamp, 2000). Homeostatic control means that when the nutritional environment is sufficient the lactating dairy cow can meet its energy demands from DMI and tissue mobilization will be reduced (Roche et al., 2009). According to Grummer (1995), feeding high-concentrate

rations prepartum may supply more glucogenic metabolites (propionate), increase ruminal papillae development and capacity to absorb volatile fatty acids (**VFA**), and decrease NEFA accumulation and associated clinical ketosis near parturition.

NEFA appear in the bloodstream when the animal is breaking down adipose tissue to supply the body with the energy it needs to complete the task of milk production. NEFA are taken up by the liver and can be oxidized for extra energy supply or esterified into triglycerides, which can lead to ketosis or fatty liver disorder (De Vries and Veerkamp, 2000). NEFA are associated with liver triglyceride accumulation and ketone production, which are related to delayed ovulation, estrus, and pregnancy (Jorritsma et al., 2000). Plasma NEFA are an important measure to help assess energy balance of the cow because they are evaluated when stored fat is mobilized (Holcomb et al., 2001). An increase in NEFA concentrations reveals an energy deficiency (Tanaka et al., 2008). Prior to calving, the concentrations of serum NEFA increase; however, occasionally they reach concentrations that are harmful to the health status of postpartum dairy cows (Melendez et al., 2009). Increased plasma NEFA concentrations is a common characteristic in dairy cows during the periparturient period and reflects the increased reliance on adipose tissue reserves to support energy requirements and milk fat synthesis (Pires et al., 2007). Doepel and colleagues (2002) found that NEFA concentrations declined as DMI increased after calving.

NEFA is utilized to make upwards of 40% of milk fat during the first days of lactation (Overton and Waldron, 2004). Skeletal muscle used some NEFA for fuel, particularly as it decreases its reliance on glucose as a fuel during early lactation. They found that plasma NEFA concentrations increased in response to increased energy needs

associated with inadequate feed intake; DMI and plasma NEFA concentrations were usually inversely related. The liver takes up NEFA in proportion to their supply, but the liver typically does not have enough capacity to completely dispose of NEFA through export into the blood or catabolism for energy (Overton and Waldron, 2004).

Westwood et al. (2002) found that cows with delayed calving to first ovulation interval had serum NEFA concentrations ≥ 313.3 μmol/L throughout the first 10 wk of lactation and were 20 times less likely to conceive by d 150 of lactation. In addition, they found that cows with a shorter interval to ovulation and less metabolic stress had lower milk production and serum NEFA concentrations along with a higher DMI and improved reproductive performance. Greater NEFA concentrations during the first 10 wk of lactation tended to be associated with a lower probability of conception by d 150 of lactation. Studies in the US found that higher NEFA concentrations at calving (1.1 to 1.2 mEq/L) may be indicative of excessive fat mobilization (Melendez et al., 2009).

There was a relationship between the NEFA concentrations at calving and the incidence of certain periparturient diseases. Moyes and colleagues (2009) found that increased NEFA concentrations were an indicator of increased lipolysis and cows that developed clinical mastitis and subclinical mastitis during the first week of lactation had mobilized more body tissue reserves than cows that did not develop mastitis. Therefore, NEFA concentrations may be a useful potential marker for risk of mastitis occurring in early lactation. When NEFA concentrations were ≥ 0.72 mEq/L postpartum, there was a decreased risk of pregnancy within the 70 d post-voluntary waiting period. Milk production decreased in animals with prepartum NEFA concentrations ≥ 0.33 mEq/L. In heifers sampled

postpartum, there was an increase in milk production when postpartum NEFA was ≥ 0.57 mEq/L; however, milk production was decreased when NEFA were ≥ 0.72 mEq/L postpartum (Ospina et al., 2010).

Oikonomou et al. (2008) suggested that genetic predisposition for high NEFA concentrations may be a part of decreased reproductive performance. There was a positive genetic correlation of NEFA concentrations with calving interval, metritis, and reproductive problems, and a negative genetic correlation involving NEFA concentrations with conception rate following first AI. Out of all the blood metabolites, NEFA concentration were assumed the best sign of energy balance because elevated NEFA concentration is one of the first indications of lipolysis (Reist et al., 2002). Concentrations of NEFA had the highest correlation (r = -0.685) with energy balance, followed by concentrations of glucose (r = 0.457) and BHBA (r = -0.451). Moyes and colleagues (2009) found that age had a significant effect on circulating concentrations of metabolites, where primiparous cows calving > 27 mo of age had higher NEFA and BHBA and lower glucose and insulin levels than cows < 24 mo of age at calving. Increased NEFA concentrations were associated with insulin resistance in Holstein cows (Pires et al., 2007). Plasma NEFA was a causal factor in insulin resistance in dairy cows during periods of NEB. The effect of NEFA-induced insulin resistance in the periparturient dairy cow will depend on the magnitude and on which tissues become insulin resistant.

Hormones

IGF-I and insulin enhanced both the proliferation and steroidogenesis of bovine granulosa cells in dose-dependent fashions (Gong, 2002). IGF and insulin control follicular

development within the bovine ovary. Lower plasma insulin and IGF-I concentrations appear linked to differences in reproductive factors such as: basal LH concentrations, LH pulse frequency, and subsequent peripheral progesterone concentrations that follow ovulation induced by GnRH. Gong (2002) showed that reproductive function was compromised in dairy cows that were selected for higher milk yield, which was linked with the changes in metabolic hormone profiles throughout early lactation. Diets that promote increases in insulin and IGF-1 may improve follicular development and endorse fertility; although, there is a limit to the quantity of grain that may be included to a particular ration for lactating dairy cow without hindering rumen fermentation (Ferguson, 2005). Insulin and IGF-I act synergistically with LH to promote follicular development in postpartum dairy cows (Lucy, 2000).

Insulin

Insulin, a peptide hormone containing acidic and basic chains connected by disulfide bridges, is secreted from β-cells of the islets of Langerhans in the pancreas (Hayirli et al., 2002). Van Knegsel et al. (2007b) found that low plasma insulin concentration reduced glucose uptake by muscle and adipose tissue and allowed glucose availability for uptake by the mammary gland, which is not responsive to insulin. During the NEB state or with low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of fatty acids in its nonesterified form to the bloodstream (Melendez et al., 2009). According to Staples et al. (1998), insulin concentrations usually reflect energy intake. They found that the low plasma insulin concentration might result from a greater clearance of insulin from the blood by tissues,

including those of the reproductive tract, which would stimulate the growth of ovarian follicles. Insulin was a powerful stimulator of ovarian follicle cell function. Doepel and colleagues (2002) found that plasma insulin concentrations declined steadily from wk -3 to calving and remained low during the postpartum period.

Hypo-insulinemia and reduced insulin responsiveness of skeletal muscle and adipose tissue occur concurrently in early lactation (Bell and Bauman, 1997; Vernon and Pond, 1997), causing an increase in glucose availability for insulin-independent uptake by the mammary gland and greater mobilization of tissue reserves (Roche et al., 2009). Dairy cows that are selected for increased milk yield have greater insulin resistance (Chagas et al., 2009), which is coupled with increased body lipid mobilization and a lower BCS nadir (Smith and McNamara, 1990; Roche et al., 2006; Kay et al., 2009). Reduced insulin concentrations during prolonged NEB postpartum may impair ovarian function and inhibit conception (Nebel and McGilliard, 1993).

A diet which resulted in a high insulin concentration tended to decrease the intervals from calving to first service and conception, but did not affect conception rate to first service and the number of services required for conception. Diets that were either isonitrogenous or isoenergetic would allow for acetate or propionate release from the rumen. Gong et al. (2002) reported that acetate would result in normal concentrations of circulating insulin; however, propionate would cause a greater insulin release in response to feeding. The conclusion on insulin was that feeding a diet that increase the amount of insulin circulating throughout early lactation can enhance reproductive performance. The most important aspect that came out of feeding a diet that increased circulating insulin concentrations was that the diet did not have a

negative influence on milk yield or on the energy balance status, meaning that it may be feasible to improve fertility in the modern high yielding dairy cows by using appropriate nutritional management practices. The average insulin concentrations in the blood of beef and dairy cattle are usually < 10 ng/mL (Elsasser et al., 1989; Richards et al., 1989). Feeding increases insulin concentrations in ruminants and the increase is greater after feeding concentrates than after feeding hay (Barnes et al., 1985). Ferguson (2005) showed that in cattle, IGF-I may interact with insulin to induce cell proliferation and estradiol production, influence LH receptor induction, and be a factor in the selection of the dominant follicle destined to ovulate.

IGF

IGF (IGF-I and IGF-II) are essential hormones that link nutrition with growth (Ferguson, 2005). IGF are single polypeptides structurally related to insulin (Froesch et al., 1985). They are important regulators of follicular and luteal function; however, it remains uncertain whether there is significant local production within the bovine ovary. Perks and colleagues (1999) found that both IGF-I and IGF-II influence ovarian activity. A potential hormonal mediator of ovarian function is IGF-I (Spicer et al., 1990). IGF-I activity in the blood decreased in lactating cows with high milk production. Plasma IGF-I decreases with nutrient restriction, and is connected with interference with normal ovarian cycling and inhibition of folliculogenesis and ovulation (Ferguson, 2005).

IGF-I is a potent stimulator of progesterone production by bovine luteal cells and IGF-I secretion is decreased during a NEB state, IGF-I holds potential as a hormonal mediator of the effects of energy balance on luteal function (Spicer et al., 1990). Decreased

IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus. IGF-I concentrations in the blood appear influenced by the nutritional status of the animal, and cows with higher milk production are under greater nutritional stress. IGF-I concentrations in blood and follicular fluid in cattle usually exceeds 100 ng/mL (Echternkamp et al., 1990; Spicer and Enright, 1991; Spicer et al., 1992). Results from Stewart et al. (1995) and previous studies show that IGF-I concentrations in follicular fluid increase as follicles enlarge, indicating that IGF-I may be an important regulator of follicular growth (Spicer and Echternkamp, 1995).

Plasma IGF-I concentration was directly related to energy balance, and IGF-I was cited as a potential hormonal mediator or nutritional control of fertility (Zulu et al., 2002). However, Patton and colleagues (2007) found that increased plasma IGF-I in the first 2 wk of lactation was associated with an increased likelihood of earlier ovulation. The plasma IGF-I concentration during the early postpartum period was connected with conception rate to first service.

Beam and Butler (1998) stated energy balance was positively correlated with IGF-I concentrations in serum which supported previous reports. Lactating dairy cows that had a positive energy balance had greater circulating concentrations of IGF-I than those cows in NEB.

Mastitis

The immunosuppression that is normally seen during the transition period as a result of the change in the physiological state is one of the main factors connected with the increased incidence of mastitis during early lactation (Mehrzad et al., 2001). Mastitis

remains the costliest disease afflicting the dairy cow. Unfavorable body energy status may lead to elevated somatic cell count (**SCC**) and mastitis incidence, thus compromising udder health (Banos et al., 2006). Mastitis can be either clinical or subclinical (Moyes et al., 2009). Signs of subclinical mastitis are elevated SCC in milk, decreased milk production, presence of inflammation, and no visible abnormalities in the milk or gland (Sordillo et al., 1997). Clinical mastitis is distinguished by elevated SCC in the milk and visual signs of inflammation including clumpy, watery, bloody, or yellowish milk, and isolation of an intramammary pathogen (Moyes et al., 2009). Clinical mastitis may cause a decrease in DMI, swelling of the udder, and in the extreme cases, septicemia or endotoxemia, which can lead to death (Bradley, 2002).

The degree of severity of NEB during the transition period is characterized by the amount of NEFA and BHBA circulating and the degree of decrease in glucose, which may contribute to the suppression of the immune system (Moyes et al., 2009). Cows that exhibit a severe NEB during the transition period are more susceptible to mastitis than those cows experiencing a moderate state of NEB.

Environmental Factors

Environmental factors such as temperature and photoperiod influence the health and productivity of dairy cows during lactation, possibly via similar physiological effects (do Amaral et al., 2009). Reproduction in dairy cows is extremely sensitive to heat stress because of the high metabolic rate that comes with lactation (Lucy, 2001). Higher ambient temperatures will add to reproductive loss by causing heat stress in the summer and by expanding the regions within the United States where heat stress occurs. Heat stress that

occurs during lactation accounts for a 10 to 25% milk production loss (Collier et al., 2006). However, exposing cows to cooler air during the dry period increased milk production relative to animals exposed to heat stress (Avendaño-Reyes et al., 2006). Long-day photoperiod during lactation improves milk yield, and short photoperiod during the dry period improves health and subsequent lactation performance (Dahl, 2008). Summer infertility was greatest in high milk producing dairy cattle. There was an additive effect of heat stress and greater milk production leading to a decrease in first-service conception rate in dairy cows (Lucy, 2001). Wolfenson and colleagues (2000) found cows with low conception in the summer did not achieve normal fertility until the late fall, long after heat stress had subsided.

Conclusion

There are multiple factors that influence the reproductive performance of dairy cows after parturition. NEB is one of the larger issues regardless of breed and parity. NEFA is the best indicator of energy balance and certain metabolic diseases. IGF-I and insulin both respond to nutritional status and affect reproductive status. Progesterone is the main hormone of the estrous cycle and of pregnancy. Analyzing progesterone and how it and other factors are influenced by NEB may identify ways to manage the NEB state. There are other non-management factors that influence the return to estrous cycling including genetic group, temperature, season, and parity. It is difficult to control all factors that may influence the reproductive performance, but determining the main problem may indicate ways to alleviate it. That will then allow the full reproductive potential of the dairy cows to be manifest without losing the milk production since that is what drives the dairy industry.

Hypothesis and Objectives

We hypothesize that the Holsteins will come into estrus later in lactation because they produce more milk than Jerseys. Therefore, the progesterone concentration should be lower during early lactation for the Holsteins when compared to the Jerseys. Research indicates that first lactation cows have greater NEB than later lactations because they tend to eat less and have energy requirements both for growth and lactation. NEB status in first lactation cows was associated with delayed intervals to first ovulation. Thus, NEFA concentrations are expected to be higher during 2nd lactation because cows will be producing more milk causing a higher demand for energy. Based on previous literature, we assume that IGF-I concentrations will be lower when the cow is in a NEB state. Decreased concentrations of IGF-I could be the cause of failure to ovulate. Previous literature has shown that insulin concentrations decrease during the first 2 wk postpartum. After classifying progesterone changes during the first 70 d of lactation into 4 profiles, we assume that those cows having a delayed progesterone profile will be associated with lower concentrations of insulin. Those cows with higher concentrations of insulin will have one of the other 3 profiles: short, early, or normal. Clinical issues such as mastitis, ketosis, and other health events may play a role in determining when a genetic group returns to estrus.

The overall objectives of this study will be to evaluate the physiological changes within genetic groups to determine the potential effects on reproductive performance of purebred and crossbred dairy cattle from a designed experiment and where cattle are managed the same.

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CHAPTER 2: Hormones, metabolites, and reproduction in Holsteins, Jerseys, and their crosses

ABSTRACT

One hundred sixty-three cows in first and second (n = 101) lactation were sampled to determine if reproduction, progesterone (P4), insulin-like growth factor I (IGF-I), insulin, nonesterified fatty acids (NEFA), and milk production differed between genetic groups. Thirty-five cows were Holstein-Jersey (HJ) crosses, 48 were Jersey-Holstein (JH) crosses, 51 were Holsteins (HH), and 29 were Jerseys (JJ). Blood samples were collected weekly for the first 10 wk postpartum. Statistical models included genetic group, parity, 'year-season', maximum daily temperature, week of lactation, and interactions. Analyses were conducted using the MIXED, chi-square and Glimmix procedures of SAS. Seasons of calving were cold (November to May) and hot (June to October) and were combined with year to form eight 'year-seasons'. Days open was affected by genetic group. The HH were open 168.6 ± 9.6 d which was different from HJ (142.9 ± 11.3 d), JJ (132.1 ± 12.1 d), and JH (127.2 ± 9.3 d). Service number was affected by genetic group. The HH had 2.4 ± 0.1 services per conception which was different from JH (1.9 ± 0.1), but not different from HJ (2.1 ± 0.2) or JJ (2.1 ± 0.2). The progesterone concentrations (P4) were affected by 'year-season', week of lactation, maximum daily temperature, and lactation number by week interaction. P4 levels increased over the 10 wk period. The P4 levels were greater in the hot seasons in yr 1 and 2 compared with cold seasons. NEFA were affected by lactation number, week of lactation, 'year-season', lactation number by week, and breed group by week interactions. NEFA were greater in lactation 2 (0.52 ± 0.02 mEq/L) than in lactation 1 (0.45 ± 0.02 mEq/L). NEFA

concentrations decreased over the 10 wk period. NEFA were greater in the cold season except in yr 3. Insulin was affected by genetic group, lactation number, week of lactation, 'year-season', and maximum daily temperature. NEFA in HH (0.83 ± 0.03 ng/mL) were not different from HJ (0.87 ± 0.04 ng/mL) or JH (0.76 ± 0.03 ng/mL), but greater than in JJ (0.66 ± 0.04 ng/mL). Insulin in lactation 1 (0.81 ± 0.02 ng/mL) was greater than in lactation 2 (0.75 ± 0.02 ng/mL). Insulin decreased at wk 2 then gradually increased. IGF-I were affected by week of lactation, 'year-season', maximum daily temperature, and genetic group by lactation interaction. IGF-I gradually increased over the 10 wk period. In yr 1, 3 and 4, IGF-I were greater in hot season than in cold. Milk production (actual yield in the first 305 d) was affected by genetic group, lactation number, 'year-season', and first week insulin. The HH produced 10,348 ± 208 kg of milk, which was greater than the HJ (9,129 ± 230 kg), the JH (9,384 ± 192 kg), and the JJ (7,080 ± 261 kg). Lactation 2 (9,676 ± 163 kg) milk production was greater than lactation 1 (8,295 ± 160 kg). Genetic group comparisons are "across lactation" while lactation number comparisons are across "genetic groups." They are very different measurements and possibly affect the interpretation of the results. Reproductive measures and milk yield appear to be more effected by genetic group. P4, NEFA, IGF-I, and insulin was affected by lactation number, 'year-season', maximum daily temperature, and week postpartum

INTRODUCTION

The decline in the reproductive ability in the purebred Holsteins has led to the interest of crossbreeding (Heins et al., 2008b). Crossbreeding improves components of milk, fertility, and productive life (VanRaden and Sanders, 2003; Anderson et al., 2007; Heins et al., 2008a, b). The lower reproductive ability in the Holsteins could be attributed to the state of negative energy balance (**NEB**) that occurs when they enter lactation after parturition. Thus, it is important to identify when a NEB state exists so that herd nutritional and management programs can be implemented to allow for adequate energy intake and optimize herd health and productivity (Stokol and Nydam, 2005).

Interaction between diet and reproduction can enhance or reduce fertility and may be an important combination for feed management (Lucy et al., 1992b). The most important period is during early and peak lactation when energy demand is the highest (Butler and Smith, 1989). Other factors that influence NEB are the diet that the animal received prior to calving and the diet provided postpartum. Non-esterified fatty acids (**NEFA**), β-hydroxybutyrate (**BHBA**), insulin, insulin-like growth factor-I (**IGF-I**), and glucose can be used to describe the state of NEB.

NEFA appear in the bloodstream when the animal breaks down adipose tissue to supply energy needed for milk production. NEFA are taken up by the liver and can be oxidized for extra energy supply or esterified into triglycerides, which can lead to ketosis or fatty liver disorder (De Vries and Veerkamp, 2000). NEFA are associated with liver triglyceride accumulation and ketone production, which in high concentrations are related to

delayed ovulation, estrus, and pregnancy (Jorritsma et al., 2000). Plasma NEFA are an important measure to assess energy balance of the cow because they are elevated when stored fat is mobilized (Holcomb et al., 2001). Increases in NEFA concentrations were associated with an energy deficiency (Tanaka et al., 2008). Prior to calving, the concentrations of serum NEFA normally increase; however, occasionally they reach concentrations that are harmful to the health status of postpartum dairy cows (Melendez et al., 2009). Increased plasma NEFA concentration is a common characteristic in dairy cows during the periparturient period and reflects the increased reliance on adipose tissue reserves to support energy requirements and milk fat synthesis (Pires et al., 2007). Doepel and colleagues (2002) found that NEFA concentrations declined as dry matter intake (**DMI**) increased after calving.

Insulin, a peptide hormone containing acidic and basic chains connected by disulfide bridges, is secreted from β-cells of the islets of Langerhans in the pancreas (Hayirli et al., 2002). Van Knegsel et al. (2007) found that low plasma insulin concentration reduced glucose uptake by muscle and adipose tissue thus sparing glucose for uptake by the mammary gland, which is not responsive to insulin. During NEB or with low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of NEFA to the bloodstream (Nelson and Cox, 2000; Melendez et al., 2009). According to Staples et al. (1998), insulin concentrations usually reflect energy intake. They found that the low plasma insulin concentration may result from a greater clearance of insulin from the blood by tissues, including those of the reproductive tract, which would stimulate the growth of ovarian follicles. Doepel and colleagues (2002) found

that plasma insulin concentrations declined steadily from wk -3 to calving and remained low during the postpartum period.

Insulin like-growth factor (**IGF-I**) and insulin enhanced both the proliferation and steroidogenesis of bovine granulosa cells in dose-dependent fashions (Gong, 2002). IGF-I and insulin control ovarian follicular development. Low plasma insulin and IGF-I concentrations appear linked to differences in reproductive factors such as: basal luteinizing hormone (**LH**) concentrations, LH pulse frequency, and subsequent peripheral progesterone concentrations that follow ovulation induced by GnRH. Gong (2002) showed that reproductive function was compromised in dairy cows that were selected for a high milk yield, which was linked with the changes in metabolic hormone profiles throughout early lactation. Insulin and IGF-I act synergistically with LH to promote follicular development in postpartum dairy cows (Lucy, 2000).

IGF-I is a potent stimulator of progesterone production by bovine luteal cells and IGF-I secretion is decreased during NEB. IGF-I holds potential as a hormonal mediator of the effects of energy balance on luteal function (Spicer et al., 1990). Decreased IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus and cows with high milk production are in NEB and under nutritional stress. IGF-I concentrations in blood and follicular fluid in cattle usually exceeds 100 ng/mL (Echternkamp et al., 1990; Spicer and Enright, 1991; Spicer et al., 1992).

Fertility declines with age and older cows have higher milk yields and more clinical abnormalities than younger animals. Cows in first lactation have lower energy balance because they consumed less feed and had energy requirements for growth in addition to

lactation (Lucy, 2001). Lower energy balance in first lactation cows was associated with delayed intervals to first ovulation (Lucy et al., 1992b). Lucy (2001) stated that improved reproduction in high-producing herds probably reflected improved feeding, healthier cows, and better reproductive management.

Progesterone is the hormone of pregnancy; therefore, it is an indicator of ovarian activity. Ovulation is substantiated by collection of a single plasma sample in which progesterone increases above 1 ng/mL (Harrison et al., 1990; Senatore et al., 1996). Cows reached first estrus on the first of 3 consecutive samples taken twice weekly that had a progesterone concentration greater than 1 ng/mL (Simmons et al., 1994). Butler and colleagues (1981) found that average time to first ovulation in multiparous cows was near 30 d. Other studies have classified ovulation occurring after the progesterone concentrations remained elevated over a period of days (Lucy et al. 1992b; Spicer et al., 1990; Zurek et al., 1995).

Negative energy balance may influence reproduction through its direct or indirect influence on progesterone secretion by the corpus luteum (Nebel and McGilliard, 1993). Reestablishment of ovulatory cycles early after parturition guarantees multiple estrous cycles prior to the breeding period, which influences conception rate (Butler and Smith, 1989). Increased concentrations of plasma progesterone were associated with improved conception rates of lactating ruminants (Staples et al., 1998). Conception rates increased 1.44% for every 1 ng/mL increase in plasma progesterone. The decline in conception rates for lactating cows reflects the effects of higher milk production, not genetic selection for lower fertility (Butler and Smith, 1989).

The USDA/Animal Improvement Programs Laboratory uses an upper limit of 250 d for days open (VanRaden et al., 2003). For most states, estimates of heritability at thresholds ≥ 250 d were identical and differed from heritability estimates at the 200 d or less threshold (Oseni et al., 2004). This may imply that there is a higher genetic variability at days open thresholds ≥ 250 d. The heritability estimates for days open were higher or identical at days open thresholds ≥ 250 d when compared with lower thresholds, indicating greater genetic variability for long days open records (Oseni et al., 2004).

Disease exposure can increase days open and ultimately decrease both productivity and profitability (Lee et al., 1989). Animals that had retained placenta, nonsystemic metritis, systemic metritis, ovarian cysts, or lameness tended to have lower conception rates and higher days open than healthy animals. Clinical uterine diseases postpartum increase the risk of subclinical endometritis in lactating dairy cows, and suppress fertility (Rutigliano et al., 2008), partly because of the decreased fertilization rate (Cerri et al., 2009a).

Mastitis remains the costliest disease afflicting the dairy cow. Unfavorable body energy status may lead to elevated somatic cell count (**SCC**) and mastitis incidence, thus compromising udder health (Banos et al., 2006). Mastitis can be either clinical or subclinical (Moyes et al., 2009). Signs of subclinical mastitis are elevated SCC in milk, decreased milk production, presence of inflammation, and no visible abnormalities in the milk or gland (Sordillo et al., 1997), whereas the symptoms of clinical mastitis are elevated SCC in the milk and visual signs of inflammation including clumpy, watery, bloody, or yellowish milk, and isolation of an intramammary pathogen (Moyes et al., 2009). Clinical mastitis may

cause a decrease in DMI, swelling of the udder, and in the extreme cases, septicemia or endotoxemia, which can lead to death (Bradley, 2002).

Environmental factors such as temperature and photoperiod influence the health and productivity of dairy cows during lactation, possibly via similar physiological effects (do Amaral et al., 2009). Reproduction in dairy cows is extremely sensitive to heat stress because of the high metabolic rate that comes with lactation (Lucy, 2001). High ambient temperatures will add to reproductive loss by causing heat stress in the summer and by expanding the regions within the United States where heat stress occurs. Heat stress that occurs during lactation accounts for milk production loss of a 10 to 25 % (Collier et al., 2006). However, exposing cows to cooler air during the dry period increased milk production relative to animals exposed to heat stress (Avendaño-Reyes et al., 2006). Long-day photoperiod during lactation improves milk yield, and short photoperiod during the dry period improves health and subsequent lactation performance (Dahl, 2008). Summer infertility was greatest in high milk producing dairy cattle. There was an additive effect of heat stress and greater milk production leading to a decrease in first-service conception rate in dairy cows (Lucy, 2001). Wolfenson and colleagues (2000) found cows with low conception in the summer did not achieve normal fertility until the late fall, long after heat stress had subsided.

There are multiple factors that influence reproductive performance of dairy cows after parturition. NEB is one of the larger issues regardless of breed and parity. NEFA is the best indicator of energy balance and certain metabolic diseases. IGF-I and insulin affect both nutritional and reproductive status. Progesterone is the main hormone of pregnancy and

analyzing that and how the other factors influence it may show how to alleviate some of the effects of NEB. There are other non-management factors that influence the return to estrous cycling. Some of these include genetic group, temperature, season, and parity. It is difficult to control all factors that may influence the reproductive performance, but determining where the main problem is may alleviate it. Research will hopefully allow producers to obtain the full potential of the dairy cows without losing the milk production since that is what drives the dairy industry.

The overall objectives of this study was to evaluate the physiological changes within genetic groups to determine the potential effects on reproductive performance of purebred and crossbred dairy cattle from a designed experiment and where cattle are managed the same.

MATERIALS AND METHODS

Experimental design

Researchers from 3 universities, Virginia Tech, the University of Kentucky, and North Carolina State University, collaborated to conduct a crossbreeding experiment involving the university research herds and Holstein (H) and Jersey (J) breeds, details of the design are in Olson et al., 2009. The measures of "return to luteal activity" were only conducted at Virginia Tech. The crossbreeding study began at Virginia Tech in the fall of 2002. The design of the study was a diallel model with H and J dams as the foundation. These dams were then mated with 4 H bulls and 4 J bulls allowing for the creation of HH, HJ, JH, and JJ genetic groups with the sire listed first and the breed of the dam second. There were 163 cows with 51 HH, 35 HJ, 48 JH, and 29 JJ. The group of cows consisted of both first and second lactation (n = 101). Cows were given prostaglandin and GnRH for synchronization.

Blood Collection

Blood sampling began in 2005 and finished in late 2009. Samples were collected weekly for the first 10 wk after parturition via a coccygeal vessel into two 6 mL K_2-EDTA tubes (Fisher, Suwanee, GA). Samples were immediately placed on ice until they were centrifuged for 30 min at 3,000 x g at 4°C. Plasma was harvested and stored at -20°C until the analysis of progesterone, NEFA, IGF-I, and insulin.

Data

Milk yield, reproductive, and clinical data were collected daily and stored in PCDART. PCDART is a DHI-supported, herd management system with extensive data on

individual cows including breedings, confirmed pregnant, calved, given synchronization protocols, individual milk production, and records of health status This program allows personnel to see the events that took place during a particular lactation. PCDART information contained pregnancy dates, number of times cows were bred, types of clinical issues, and when immunization or synchronization injections were given.

Radioimmunoassays

Progesterone. Progesterone concentrations were analyzed by Coat-a-Count kits (TKPG2, Siemens, Los Angeles, CA). These RIA kits contained antibody coated tubes and ^{125}I-labeled progesterone. All samples were assayed in duplicate. Residual pellets were counted using the Cobra II auto-gamma counter (Packard Company, Virginia Beach, VA). The intra and inter assay CV were 7.4 and 14.5 %, respectively.

IGF-I. Concentrations of IGF-I were quantified using a double antibody RIA. All samples were assayed in duplicate. Prior to the IGF-I assay, the binding proteins were acid-ethanol extracted as previously described by Sharma et al. (1994). Plasma concentrations of IGF-I were measured as described by Berry et al. (2003). Briefly recombinant human IGF-I (Grow Prep, Adelaide, Australia) was used for iodination and standards. Iodination was carried out as described for α-lactalbumin (Akers et al., 1986). Mouse anti-human IGF-I primary antibody (1:70,000) was a gift from Dr. Bernard Laarveld (University of Saskatchewan, Saskatchewan, Canada). Goat anti-mouse secondary antibody (1:20) was purchased from Sigma Aldrich Chemical Company (St. Louis, MO). A quality control of pooled bovine plasma extract was evaluated in all assays. Samples were counted by the

Cobra II auto-gamma counter. The intra and inter assay CV were 11.3 and 18.3 %, respectively.

Insulin. Insulin concentrations were determined by a double antibody RIA. Samples were assayed in duplicate. The insulin assay was previously described by McFadden et al., 1990 and Daniels et al., 2008. For the insulin assay, purified bovine insulin (Lot 615-70N-80; Eli Lily and Co., Indianapolis, IN) was used for iodination and standards. Guinea pig anti-bovine insulin (lot GP20) was the primary antibody (Miles Laboratory, Elkhart, IN) and used at a final dilution of 1:12,000. Ovine anti-guinea pig gamma globulin antiserum was used at a final dilution of 1:15. A quality control of pooled bovine plasma was evaluated in all assays. Samples were counted on the Cobra II auto-gamma counter (Packard Company). The intra and inter CV were 8.1 and 14.5 %, respectively.

NEFA. Non-esterified fatty acids (NEFA) were analyzed by a colorimetric procedure using reagents and solvents from Wako (Wako Diagnostics/Chemicals Richmond, VA). Reagents A and B were prepared as described in the protocol. Samples were analyzed in duplicate via the Beckman Clinical CX5 Analyzer. The intra and inter CV were both less than 1%.

SAS and Data Management

All data recorded from blood samples were stored into Microsoft Excel program, version 2007 (Microsoft Corporation, Redmond, WA). Graphs and tables were created in Excel from the data output from the statistical analysis software SAS version 9.2 (SAS Institute Inc., Cary, NC). Statistical analyses were done with SAS using a MIXED procedure with repeated measures with cow within breed as the repeated variable for each model, Chi-

Square, and Glimmix. Chi-square showed the frequency distribution among genetic groups within the four P4 profiles. A correlation procedure was run on those variables that had only one observation per cow per lactation. There was an additional correlation procedure for analysis of those variables that had repeated measures per cow per lactation.

Dependent variables were days open, service number, progesterone, NEFA, insulin, IGF-I, clinical condition, and milk yield. Independent variables included profile, slope, P4 > 1 ng/mL and P4 > 3 ng/mL (described below), genetic group (HH, HJ, JH, and JJ), year-season, lactation number, week, genetic group by week, genetic group by lactation number and lactation number by week interactions. The profile variable classified the way the progesterone concentration increased during the early portion of lactation. Profile consisted of 4 different categories based on Petersson et al., 2006: normal, early, short, and delayed. 'Normal' was defined as an increase in plasma progesterone above 1 ng/mL between d 28 to 30 of lactation that remained elevated above the threshold of 1 ng/mL for 2 to 3 wk. 'Early' meant the same as normal except progesterone rose above 1 ng/mL before 21 DIM. 'Short' was where there was an increase in progesterone concentrations above 1 ng/mL around 28 to 30 DIM, but then it decreased below the threshold level at the next sampling. 'Delayed' profile was assigned to cows that had progesterone concentrations that never met threshold levels of 1 ng/mL before 30 DIM. The slope variable indicated the change in progesterone concentrations over the first 30 d of lactation. The variable P4 > 1ng/mL was either a 'yes' or a 'no' to whether or not the cow increased above 1 ng/mL before 30 DIM. The variable P4 > 3ng/mL was also a 'yes' or 'no' response to whether or not the cow increased above 3 ng/mL of P4. The variable 'year-season' combined the year and the season of calving. The

'year-season' variable takes into effect management practices, environmental temperature, and feed quality. There were 4 yr, since the first year and second were combined because relatively few calvings occurred and few samples were collected in 2005, the initial year of the study. Season was divided into 'Hot' for the months June to October and 'cold' for November to May. The maximum daily temperature was recorded from the Blacksburg National Oceanic and Atmospheric Administration weather station located in close proximity to the Dairy Cattle Center. Maximum daily temperature was placed into the model to see how it affected P4, NEFA, insulin, and IGF-1 concentrations. Maximum daily temperature is different from the year-season as it only accounts for the environment effects, whereas year-season includes environmental as well as management and feed quality effects, but maximum daily temperature does not consider June and October to be the same as season does. Milk production was actual yield through 305 d of lactation. For cows not milked a full 305 d, a 305 d yield was calculated by multiplying the last milk recorded from DHI and the days remaining to 305 This approach assumes a flat lactation curve at the end of lactation which is likely more appropriate for first then second lactation. Milk yield was not calculated if the lactation was less than 280 d. A MIXED procedure with repeated measures was used for analysis with cow within breed as the repeated variable using the following model:

$Y_{ijklmn} = \mu + B_i + C_{(i)j} + L_k + R_l + MT_m + W_n + BL_{ik} + BW_{in} + LW_{kn} + e_{ijklmn}$

where:

Y_{ijklmn} = the dependent variable

μ = the mean of Y;

B_i = effect of genetic group,

$C_{(i)j}$ = effect of cow within genetic group,

L_k = effect of lactation number,

R_l = effect of year-season,

MT_m = effect of maximum daily temperature

W_n = effect of week of lactation (n = 1 to 10),

BL_{ik} = interaction of genetic group and lactation number,

BW_{in} = interaction of genetic group and week of lactation,

LW_{kn} = interaction of lactation number and the week of lactation, and

e_{ijklmn} = error (genetic group, lactation number, week interaction)

Independent variables describing P4 were genetic group, lactation number, week of lactation, maximum daily temperature, 'year-season', and lactation number by week of lactation interaction. The independent variables describing NEFA were genetic group, lactation number, week of lactation, maximum daily temperature, 'year-season', genetic group by lactation number, and lactation number by week of lactation interactions. IGF-1 was described by the independent variables of genetic group, lactation number, week of lactation, maximum daily temperature, 'year-season', and genetic group by lactation number interaction. The insulin model was described by the independent variables of genetic group, lactation number, week of lactation, maximum daily temperature, and 'year-season.' The independent variables describing actual milk yield were genetic group, lactation number, 'year-season,' first week insulin concentrations, and the genetic group by lactation number interaction.

The GLIMMIX model was used to analyze whether or not the genetic group or lactation number affected the P4 increasing above 1 ng/mL or above 3 ng/mL prior to 30 DIM. It was also used to determine if the different clinical conditions were affected genetic group or lactation number. The dependent variables were P4 > 1 ng/mL, P4 > 3 ng/mL, mastitis, metritis, ketosis, and displaced abomasum. The independent variables describing P4 > 1 ng/mL and P4 > 3 ng/mL and, mastitis, metritis, ketosis, and displaced abomasum were genetic group and lactation number.

Tukey's adjustments were performed on the main effects of genetic group and lactation number to account for multiple two-way comparisons in the MIXED procedure. Additionally, contrasts were conducted between breed group and lactation number and lactation number and week. The Chi-square analysis was conducted on the genetic group by profile frequency.

Dunnett's adjustments were performed on the main effects of genetic group and lactation number to account for the multiple two-way comparisons in the GLIMMIX procedure. Significance was considered to be at $P < 0.05$.

RESULTS

Reproductive results

There was a significant genetic group effect ($P < 0.02$) on profile of progesterone (Table 2.2) based on the chi-square analysis. It appears that HH and HJ had higher percentages of delayed cycles (~ 61%) and fewer early cycles (~ 14%) than JH and JJ cows. The JH and JJ cows were more common in later year-season groups. Overall almost 50% of

the cows in the study had a delayed progesterone profile. Over the 4 yr of sampling, days open ranged from 127.2 to 168.6 in the model with P4 > 1 ng/mL and 129 to 170.3 in the model with P4 > 3 ng/mL. Genetic groups significantly affected days open when P4 was > 1 ng/mL and when P4 was > 3 ng/mL. (Table 2.3 and 2.5; Appendix A.1 and A.2). The HH had the greatest days open and the JH had the least days open ($P < 0.01$). There were no differences in days open with other genetic group combinations. Days open was positively correlated with service number (r = 0.76; $P < 0.0001$) as expected. As days open increases so did the number of services required for a cow to become pregnant. Days open was positively correlated with milk production (r = 0.14; $P < 0.03$) suggesting that higher producing cows had greater days open. Service number had a significant genetic group effect when P4 > 1 ng/mL and P4 > 3 ng/mL were used in the model. (Table 2.4 and 2.6; Appendix A.3 and A.4). Services per conception ranged from 2.4 to 1.9 in the model including P4 > 1 ng/mL and 2.3 to 1.8 in the model including P4 > 3 ng/mL

Progesterone

Progesterone was positively correlated with days postpartum (r = 0.30; $P < 0.0001$). The progesterone concentrations increased with week of lactation. The initial increase above 1 ng/mL occurred on at 3 wk postpartum, suggesting that the first ovulation occurred before this time. After ovulation, P4 can be expected to be above 1 ng/mL for approximately 14 d. Genetic group had a significant effect on whether the P4 concentrations > 1 ng/mL (Appendix A.10). The HH had 42.2 ± 5.4 % of cows with progesterone concentrations increasing above 1 ng/mL in the first 30 DIM compared to 43.9 ± 6.6 % of HJ, 61.7 ± 5.4 %

of JH, and 67.4 ± 7.2 % of JJ (Table 2.7). The chance of progesterone increasing above 3 ng/mL during the first 30 DIM was lower than the chance of 1 ng/mL increase. There was a trend for differences in the percentages of cows with progesterone above 3 ng/mL plasma by 30 DIM ($P < 0.066$). The HH had a 25.6 ± 4.8 % of cows, the HJ had a 32.1 ± 6.2 % of cows, the JJ had a 40.1± 5.5 % of cows, and the JH had a 42.4± 5.5 % of cows with progesterone increases above 3 ng/mL before 30 DIM ($P < 0.10$).

Progesterone concentrations were negatively correlated with slope (r = -0.11; $P < 0.0001$) and NEFA concentrations (r = -0.12; $P < 0.0001$). As NEFA concentration decreased, the progesterone concentrations increased. Progesterone concentrations were significantly affected by year-season ($P < 0.0005$), week of lactation ($P < 0.0001$), maximum daily temperature ($P < 0.0020$) and lactation number by week of lactation interaction ($P < 0.0003$) (Appendix A.5). Progesterone concentrations increased above 1 ng/mL at wk 3 postpartum and were maximum at wk 6 followed by a decline and an increase to 10 wk of lactation; however, wk 5 to 10 were affected by the timed AI programs that were used (Figure 2.1). The overall progesterone concentration was 1.58 ± 2.45 ng/mL. Progesterone concentrations were greater ($P < 0.0005$) in the hot season (season 1) in the year 2005-2006 and 2007. However, in 2008 and 2009 the progesterone concentrations were slightly higher in the cold season (season 2) than in the hot season (Figure 2.2). Lactation number by week interaction was significant with the initial progesterone maximum at wk 6 for lactation 2 compared with wk 7 for lactation 1. This was followed by greater progesterone in wk 9 and 10 for first lactation cows compared with second lactation (Figures 2.3).

NEFA

NEFA concentrations at wk 1 were positively correlated with milk production (r = 0.17; $P < 0.01$). When NEFA concentrations were low, days open and milk production was lower. However, NEFA concentrations were negatively correlated with days postpartum (r = -0.40; $P < .0001$), progesterone (r = -0.12; $P < 0.0001$), IGF-I (r = -0.10; $P < 0.0001$), and insulin concentrations (r = -0.31; $P < 0.0001$; Table 2.1). When NEFA concentrations are high days postpartum, progesterone, IGF-I, and insulin concentrations tended to be lower. Higher NEFA concentrations occurred early in lactation, as suggested by the negative correlation between NEFA and week of lactation.

Lactation number, week of lactation, year-season, genetic group by lactation number and lactation number by week of lactation interaction significantly affected the NEFA concentrations (Appendix A.6). The NEFA concentrations were higher ($P < 0.0002$) in second lactation (0.52 ± 0.02 mEq/L) when compared with first lactation (0.45 ± 0.02 mEq/L). NEFA was highest at wk 1 and gradually decreased over the first 10 wk of lactation (Figure 2.4). NEFA concentrations were highest ($P < 0.0001$) in the cold season (season 2) compared to the hot season (season 1) in 2005-2006, 2007, and 2009. In 2008, the NEFA concentrations were highest in the hot season compared to the cold season (Figure 2.5). The lactation number by week effect ($P < 0.0058$) showed that NEFA in both lactations gradually declined over the first 10 wk, but second lactation NEFA concentration starts out greater than first lactation (Figure 2.6). The slice analysis revealed that there were significant differences between first and second lactation NEFA in cows occurring at wk 1, 2, 4, and 5. The interaction between genetic group and lactation number showed that all 4 genetic groups had

higher NEFA concentrations in second lactation ($P < 0.0114$) compared to first. The HH in second lactation were significantly different than the JH in second lactation ($P < 0.0039$) (Figure 2.7).

Insulin

Insulin was positively correlated with days postpartum (r = 0.08; $P < 0.0001$), week of lactation (r = 0.09; $P < 0.0001$), and IGF-I concentrations (r = 0.12; $P < 0.0001$). Insulin concentrations increased when days postpartum, week of lactation, and IGF-I concentrations increased. Insulin was negatively correlated with milk production (r = -0.28; $P < 0.0001$). When milk production increases, the insulin concentration is declines.

Genetic group, lactation number, week of lactation, maximum daily temperature, and year-season affected insulin concentrations (Appendix A.7). The insulin concentrations ranged from 0.66 to 0.86 ng/mL. The HJ had the greatest insulin concentrations and the JJ had the lowest, but insulin in HH and HJ was different from insulin in JJ (Table 2.8). Insulin concentrations were greater ($P < 0.0066$) in first lactation (0.81 ± 0.03 ng/mL) compared with second lactation (0.72 ± 0.03 ng/mL). Over the first 10 wk of lactation, the insulin concentrations started out high and then decreased for the next 3 wk and gradually increased (Figure 2.8). Insulin concentrations were highest in the hot season (season 1) ($P < 0.0001$) compared with the cold season (season 2) during 2005-2006 and 2007, but in 2008 and 2009 the concentrations were higher in the cold season compared to the hot season (Figure 2.9).

IGF-I

IGF-I concentrations were positively correlated with days postpartum (r = 0.11; $P < 0.0001$), week of lactation (r = 0.11; $P < 0.0001$), and insulin concentrations (r = 0.12; $P < 0.0001$). As days postpartum, week of lactation, and insulin concentrations increased, IGF-I concentrations increased.

Week of lactation, year-season, maximum daily temperature, and genetic group by lactation number interaction affected the IGF-I concentrations (Appendix A.8). The average IGF-I concentration was 80.04 ± 66.75 ng/mL. IGF-I concentrations gradually increased ($P < 0.0001$) over the first 10 wk of lactation (Figure 2.10). IGF-I concentrations were similar in both the hot season (season 1) and cold season (season 2) in 2005-2006. In 2007, the IGF-I concentrations were greater in the cold season compared with the hot season. In 2008 and 2009, the IGF-I concentrations were greater in the hot season compared with the cold season (Figure 2.11). The HH had higher IGF-I concentrations in first lactation compared with second lactation ($P < 0.0018$). The HJ had higher IGF-I concentrations in second lactation compared with first lactation. The JH were like the HH in that they had higher IGF-I concentrations in first lactation compared to second lactation. The JJ had the same IGF-I concentrations in both first and second lactation (Figure 2.12).

Milk

Milk production was positively correlated with lactation number (r = 0.44; $P < 0.0001$) and days open (r = 0.14; $P < 0.03$). The higher the lactation number the cow is in means she is expected to produce more milk than she did in the previous lactation. Increased

milk production leads to increased days open. Milk production was negatively correlated with insulin concentrations (r = -0.28; $P < 0.0001$).

Actual 305 d milk production was affected by genetic group, lactation number, year-season, and first week insulin concentrations (Appendix A.9). The genetic group by lactation number interaction was not significant ($P < 0.3813$). All four genetic groups had higher actual milk yield in second lactation compared to first. The HH cows produce the greatest amount of milk yield in both first and second lactation and the JJ had the lowest in both lactations. The JH came close in actual 305 d milk yield to the HH. The HH had 11,171 kg and the JH had 10,195 kg. Milk production ranged from 7,080 to10,348 kg. The HH were different from HJ, JH, and JJ. The HJ and JH were different from the JJ (Table 2.9). Milk production was greater ($P < 0.0001$) in second lactation (9,676 ± 163 kg) compared with first lactation (8,294 ± 160 kg). Milk production was greater in cold season (season 2) in 2005-2006, 2007, and 2008 than in the hot season (season 1). In 2009, milk production was greater in the hot season compared with the cold season (Figure 2.13).

Clinical Conditions

Clinical conditions of mastitis, metritis, ketosis, and displaced abomasum were affected the genetic groups and lactation number differently. Clinical conditions were only recorded if they took place within the first 70 d of lactation. Mastitis ($P < 0.006$) was significantly different by genetic group ($P < 0.0344$) and lactation number ($P < 0.0006$) (Table 2.10). The JJ (10.3 ± 4.7 %) had the greatest chance of getting mastitis. The HH (1.1 ± 0.9 %) chance of getting mastitis differed from the HJ (9.4 ± 4.1%), the JH (8.1 ± 3.4 %), and the JJ. There was a greater incidence of mastitis ($P < 0.0006$) in first lactation cows

(17.6 ± 3.6 %) compared with second lactation cows (1.6 ± 1.1 %). The HH (16.9 ± 4.2 %) had a higher incidence of metritis. . The incidences of ketosis or displaced abomasum did not differ among genetic groups or lactation number. However, the HH had 11 ± 3.5 % incidence of ketosis. The HJ had 10.8 ± 4.2 %, the JH had 8.7 ± 3.1 %, and the JJ had 9.9 ± 4.7 % chance of getting ketosis. The HH had 14.5 ± 3.9 % occurrence of displaced abomasum. The HJ had 7.1 ± 3.4 %, the JH had 7.4 ± 2.9 %, and the JJ had 2.4 ± 2.3 % chance of getting a displaced abomasum.

An overall means for all dependent variables for each genetic group and lactation number are shown in Table 2.11.

DISCUSSION

Throughout this study at Virginia Tech, it was apparent that the particular AI sires of the cows may contribute more to fertility than their dams. The HH and HJ cows appear to follow the same pattern in having a delayed P4 profile and increase in P4 above 1 ng/mL plasma as the JJ and JH follow each other. For services per conception, the HH had the highest services (2.3 to 2.4) followed by the HJ (2.1), JJ (2.1), and finally the JH (1.8 to 1.9). The days open analysis followed the same pattern with the HH (169) having the highest days open, then the HJ (143), then JJ (132), and last the JH (127). The general pattern looks like the HJ are better reproductively than the HH and the JH are reproductively better than the JJ. The correlations regarding these two variables agree. Service number was correlated with days open (r = 0.76). As days open increases it is going to take the producer more attempts

at successfully getting the cows pregnant. Recent research has suggested that there is a 2 to 3 wk advantage of days open for crossbreds vs. purebred Holsteins, which would enhance the profitability of dairy production systems (Heins et al., 2006b; Dechow et al., 2007). It was shown in the 1982 study by McDowell that the crossbreds averaged 3 to 17% fewer days open compared to the purebreds at three locations. The current data at Virginia Tech supports this with the HJ (142.9 to 146 d) having fewer days open than the HH (168.6 to 170.3 d) and the JH (127.2 to 129 d) having fewer days open than the JJ (132.1 to 133.3 d). The data presented from Heins et al. (2006b), are supported by the current finding that Jersey bulls achieved higher conception rates than those matings involving purebred Holsteins.

The genetic group differences continued to be seen in the analysis of progesterone. The HH and HJ cows had a higher incidence of a delayed progesterone secretion. Around 60% of these cows were in the 'delayed' progesterone category as opposed to the JH and JJ in which only 30% of these animals were 'delayed'. Petersson and colleagues (2006) found that cows that had atypical progesterone profiles in the previous lactation were at a high risk of displaying the same profile again in subsequent lactations. Our lactation number by week of lactation interaction showed different patterns of progesterone change in support. Therefore, those HH and HJ cows would have a higher incidence of having a 'delayed' progesterone profile in later lactations. The HH and HJ in the Virginia Tech portion of the study supported the previous statement. The HH and HJ had a higher incidence of 'delayed' progesterone profile in both first and second lactation compared to the JH and the JJ. The JJ had the smallest percentage of animals in this category in both first and second lactation. The JJ had the highest percentage of cows in the 'normal' and 'short' progesterone profiles.

Almost 70% of the JJ cows had either an 'early', 'normal', or 'short' progesterone profile meaning that these cows started to cycle within the first 30 DIM which would allow them to have a shorter days open period. Days open in the JJ did not differ from the HJ or the JH, only from the HH. This will also allow the JJ cows to become primed and ready to start breeding. The JH had the highest percentage of cows in the 'early' progesterone profile. Slightly over 60% of the JH cows fell into the progesterone categories of 'early', 'normal', or 'short'. The HH and HJ had a little less than 40% of these cows falling into the progesterone categories of 'normal', 'short', or 'early'. With the majority of cows in the delayed category, it suggests that most of the HH and HJ cows were compromised and did not resume cycling until after 30 DIM. Cows with successful pregnancies had higher concentrations of progesterone in the cycle prior to breeding (Shrestha et al., 2004). Therefore, the finding of the majority of the HH and HJ falling into the 'delayed' progesterone profile may explain fewer successful pregnancies compared with the JH and JJ. This finding supports Shrestha et al. (2004) who found that cows with abnormal ovarian cycles including prolonged luteal phase and anovulation, during the pre-service period had a reduced reproductive performance than those cows that experienced normal ovarian cycles.

NEFA concentrations were higher in second lactation for all genetic groups compared to first lactation. Animals entering second lactation are more mature cows and have higher energy demands for milk production compared to when they were entering first lactation. NEFA concentrations gradually decline over time as cows consume more of the energy needed for all of their activities. This agrees with Doepel and colleagues (2002) who found that NEFA concentrations decreased as DMI intake increased after calving. NEFA are

formed in early lactation because the cow is not consuming the amount of feed required to meet all of their energy demands (Bauman and Currie, 1989; Bell, 1995; Bauman, 2000; Roche et al., 2009). The HH (0.52 mEq/L) had the highest average NEFA concentration compared to the other genetic groups. This could be one of the reasons why the HH resume cycling later in lactation. NEFA concentrations tended to be lower in the hot season compared to the cold, except in 2008 where the NEFA concentration was higher in the cold season than the hot. Causes of the interaction could include management protocols, or feed quality. Nutritional environment needs to be sufficient so the lactating dairy cow can meet its energy demands from DMI and tissue mobilization is reduced (Roche et al., 2009). Feeding high-concentrate rations prepartum may supply more glucogenic metabolites like propionate, increase ruminal papillae development and capacity to absorb VFA, and decrease the NEFA accumulation near parturition (Grummer, 1995).

Insulin concentrations started high (0.81 ng/mL) and then decreased at the second week (0.72 ng/mL) and then gradually increased throughout the rest of early lactation. This finding contrasts with Doepel and colleagues (2002) who found insulin concentrations low during early postpartum. The cows in the study had insulin concentrations above the starting week at wk 7, 8, and 10 of lactation. However, our results agreed with Tanaka et al. (2008) that the insulin concentrations decrease from wk 1 to wk 2. Insulin concentrations were lower in second lactation than first lactation (0.72 vs. 0.81 ng/mL) and could be attributed to the dairy cows producing more milk in second lactation ($r = -0.28$), which has been shown to have greater insulin resistance (Chagas et al., 2009). Our results support this statement as our cows had significantly lower insulin concentrations in second lactation compared with first

lactation. That is due to maturity primarily because selection on first lactation yield was minor. Hypo-insulinemia and reduced insulin responsiveness of skeletal muscle and adipose tissue were shown occur concurrently in early lactation (Bell and Bauman, 1997; Vernon and Pond, 1997).

IGF-I concentrations were low during the first week of sampling (65.5 ng/mL) and gradually increased as the lactation progressed. This finding supports Ferguson (2005) where IGF-I activity in the blood decreased in lactating cows with high milk production. The JH had lower IGF-I concentrations in second lactation (68.3ng/mL) than in first lactation HH (90.3 ng/mL) which could be associated with the Ferguson (2005) assertion that IGF-1 concentrations decrease with nutrient restriction. Overall, second lactation had lower IGF-I concentrations (76.8 ng/mL) compared with first lactation (78.1 ng/mL). This could be because the cows in second lactation are under more lactational stress than those in first lactation. Spicer et al (1990) reported that IGF-I secretion decreased during a NEB state. The NEB could also explain why the IGF-I concentrations were low. IGF-I is an essential hormone that links nutrition with growth. Plasma IGF-I decreases with nutrient restriction, and is connected with interference with normal ovarian cycling and inhibition of folliculogenesis and ovulation (Ferguson, 2005). The decreased IGF-I concentrations could be associated with the NEB of the cows in early lactation, which could prevent the cows returning to estrus. According to Spicer et al. (1990) decreased IGF-I secretion caused by NEB could alter ovarian follicular estradiol production, thereby suppressing the expression of estrus. The IGF-I concentrations in the blood appear influenced by the nutritional status of the cow, and the cows with higher milk production undergo greater nutritional stress (Spicer

et al., 1990). According to Zulu and colleagues (2002) plasma IGF-I concentrations are directly related to energy balance, and IGF-I was cited as a potential hormone mediator or nutritional control of fertility.

The actual 305 d milk yield was highest in the HH (10,348 kg) followed by the JH (9,384 kg), HJ (9,129 kg), and last the JJ (7,080 kg). The actual 305 d milk yield does not account for energy in fat and protein. The actual milk yield could be an indicator of why the HH genetic group did not resume cycling before 30 DIM. Milk production was higher in second lactation (9,676 kg) compared to first lactation (8,295 kg). This could be why IGF-I concentrations are low and NEFA concentrations are higher in second lactation compared to first lactation. Garmo and colleagues (2009) found that the level of milk production had the greatest negative effect on reproductive performance, suggesting that reduced fertility is caused by the inability to meet the nutritional requirements. Olson and colleagues (2010) showed that net energy for production was not significantly different among HH, HJ, and JH in first lactation. The HJ and JH were numerically higher than HH (Olson et al., 2010). The HH had the highest milk production and decreased reproductive performance supporting Lucy and colleagues (1992a), who found that NEB was associated with reduced expression of estrus and lessened responses to procedures for synchronization of estrus. This may be due to the inconsistent growth and development of ovarian follicles.

Clinical conditions varied among genetic groups as well as the lactation number. Mastitis tended to occur more frequently in JJ (10.3%), the HJ (9.4%), and the JH (8.1%) compared with HH (1.1%) as well as in first lactation (17.6%) compared with second lactation (1.6%). Metritis occurred more frequently in HH (16.9%) compared to the HJ

(4.8%), the JH (6.8%), and the JJ (0.0%). The higher incidence of metritis occurring in the HH could be one of the reasons why the HH have greater days open and number of services per conception. There was a higher incidence of cows developing metritis in first lactation (0.4%) compared with second lactation (0.2%). The transition period is a high risk area for cows developing mastitis (Chagunda et al., 2006). Cows that had one or more reproductive, nutritional, or health related issue prior to 30 DIM had eaten 19 % less feed during the periparturient period (Zamet et al., 1979). Cows with metabolic disturbances or health problems occurring during early lactation produced less milk than healthy cows, resulting in economic losses for dairy farmers (Drackley, 1999). Cows in a severe NEB state during the transition period are characterized by the increased NEFA concentrations circulating, which may lead to the contribution of the suppression of the immune system. Therefore, cows exhibiting a severe NEB during the transition period are more susceptible to mastitis than those cows experiencing a moderate NEB. NEFA concentrations were highest for the purebreds followed by the crossbreds. However, in our study it was the HJ and the JJ with the higher incidence of developing mastitis.

The purpose of this research was to determine if genetic groups differed in the resumption of ovarian activity as well as evaluating whether crossbreds will benefit producers by maximizing milk production from the Holsteins and maximizing the reproductive efficiency found in the Jerseys.

CONCLUSIONS

The crossbreds are better overall than their respective purebreds at the reproductive traits at Virginia Tech. The Kentucky and North Carolina data have not been analyzed. The JH have fewer days open and require fewer services per conception than the HH, HJ, or the JJ. The JH genetic group produced more milk than the JJ or the HJ, but not more than the HH. There could be a potential sire effect that allows the JJ and JH cows to return to cycling in the first 30 d of lactation; however, there were only 4 JJ sires and 4 HH sires were used. Second lactation has a beneficial effect on returning to estrus based on progesterone changes. Milk production and NEFA concentrations are higher in second lactation and IGF-I concentrations are lower. These three components together could be one of the main reasons why cows take longer to come back into estrus. Progesterone concentrations were lower in second lactation compared to first lactation. Year-season affected the concentrations of progesterone, NEFA, insulin, IGF-I concentrations. However, this study cannot identify what factors within the year-season variable influenced these concentrations. Year-season is comprised of management factors, environmental factors, as well as the quality of the feed that is being provided to our animals. However, when the maximum daily temperatures were used to analyze the blood traits, only progesterone, IGF-1, and insulin concentrations were affected. NEFA concentrations were not affected by the maximum daily temperature. This does not mean that it was only the environmental temperature that affected these cows, it could be a combination. Further studies should look at the external factors more closely to see how they affect cows. Also, studies are needed to show how much the sire influences

reproductive ability. However, it is hard to judge the fertility of the different genetic groups without having all of the data from all three locations.

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Table 2.1. Significant correlations for days open, milk yield, service number, lactation number, NEFA, insulin, progesterone (P4), and IGF-I. Days open, milk yield, service number, and lactation number correlations with NEFA, insulin, P4, and IGF-I are with the blood measure of the first sampling at wk 1.

	Days Open	**Milk Yield**	**Service #**	**Lactation #**	**NEFA**	**Insulin**	**P4**	**IGF-I**
Days Open	XXX	0.14 0.0266 241	0.76 <.0001 264	----	----	----	----	----
Milk Yield	0.14 0.0266 241	XXX	---	0.44 <.0001 241	0.17 0.0121 217	-0.28 <.0001 217	---	----
Service #	0.76 <.0001 264	----	XXX	----	----	----	----	----
Lactation #	----	0.44 <.0001 241	----	XXX	0.22 0.0005 237	-0.31 <.0001 237	----	----
NEFA	----	0.17 0.0121 217	---	0.22 0.0005 237	XXX	-0.31 <.0001 2632	-0.12 <.0001 2628	-0.10 <.0001 2632
Insulin	----	-0.28 <.0001 217	----	-0.31 <.0001 237	-0.31 <.0001 2632	XXX	----	0.12 <.0001 2631
Progesterone	----	----	----	-----	-0.12 <.0001 2628	----	XXX	----
IGF-I	----	----	----	---	-0.10 <.0001 2632	0.12 <.0001 2631	----	XXX

Table 2.2. Genetic group percentages and number of cows within each progesterone profile categories of delayed, early, normal, and short during first and second lactation in purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	**Profile**				
Frequency Percentage	Delayed	Early	Normal	Short	Total
HH	50 60.24%	14 16.87%	11 13.25%	8 9.64%	83
HJ	35 61.40%	7 12.28%	9 15.79%	6 10.53%	57
JH	35 37.04%	25 30.86%	14 17.28%	12 14.81%	81
JJ	14 32.56%	12 27.91%	8 18.60%	9 20.93%	43
Total	129 48.86%	58 21.97%	42 15.91%	35 13.26%	264 100.00%

Delayed: No increase in progesterone concentrations above 1 ng/mL by 30 DIM.

Early: An increase in progesterone concentrations above 1 ng/mL before 28 DIM and remained above 1ng/mL for 2 to 3 weeks before decreasing below 1 ng/mL.

Normal: An increase in progesterone concentrations above 1 ng/mL between 28 to 30 DIM and remained above 1 ng/mL for 2 to 3 weeks before decreasing below 1 ng/mL.

Short: Increases in progesterone concentrations above 1 ng/mL between 28 to 30 DIM, but decreased below 1 ng/mL at the next sample.

Table 2.3. The model including progesterone concentrations greater than 1ng/mL in the plasma sample by 30 DIM on days open means (±SE) for purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	Days Open	SE
HH	168.6[a]	8.12
HJ	142.9[b]	9.30
JH	127.2[b]	7.45
JJ	132.1[b]	10.01

Overall effect *P < 0.0042;* [ab] *P < 0.0486 (HH vs HJ); P < 0.0006 (HH vs JH); P <0.0107 (HH vs JJ).* The crosses were not different from each other or from the purebred JJ.

Table 2.4. The model including progesterone concentrations greater than 1 ng/mL plasma sample by 30 DIM on service number means (± SE) for purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	Service Number	SE
HH	2.4[a]	0.1
HJ	2.1[ac]	0.2
JH	1.9[bc]	0.1
JJ	2.1[ac]	0.2

Overall *P < 0.0458;* [ab] *P < 0.0245 (HH vs JH)*

Table 2.5. The model including progesterone concentrations greater than 3ng/mL plasma sample by 30 DIM on days open means (± SE) for purebred Holstein (HH) and Jersey (JJ), and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	Days open	SE
HH	170.3[a]	8.7
HJ	146.0[ac]	10.4
JH	129.0[bc]	8.5
JJ	133.3[bc]	11.3

Overall *P < 0.0039;* [ab] *P < 0.0033 (HH vs JH); P < 0.0456 (HH vs JJ)*. There were no differences between the HH and the HJ, between the crosses, or the crosses with the purebred JJ.

Table 2.6. The model including progesterone concentrations greater than 3ng/mL plasma sample by 30 DIM on service number means (± SE) for purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic Group	Service number	SE
HH	2.3[a]	0.1
HJ	2.1[ac]	0.2
JH	1.8[bc]	0.1
JJ	2.1[ac]	0.2

Overall $P < 0.0477$; [ab] $P < 0.0257$. The HH are not different from the HJ or the JJ. The crossbred cattle are not different from each other or from the purebred JJ.

Table 2.7. Genetic group means (± SE) of plasma progesterone concentrations from samples collected weekly of the cows that had an increase above 1 ng/mL within the first 30 DIM of first and second lactation in purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	% above 1ng/mL	SE
HH	42.2%[a]	5.4%
HJ	43.9%[ac]	6.6%
JH	61.7%[ac]	5.4%
JJ	67.4% [bc]	7.2%

Overall $P < 0.0097$; [ab] $P < 0.0427$*(HH vs JJ)*. The crossbreds are not different from each other or the purebred JJ.

Table 2.8. Genetic group means (± SE) of plasma insulin concentrations from samples collected weekly for the first 10 wk postpartum in first and second lactation in purebred Holstein (HH) and Jersey (JJ) and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	Insulin ng/mL	SE
HH	0.83[a]	0.03
HJ	0.87[a]	0.04
JH	0.76[ac]	0.03
JJ	0.66[bc]	0.04

Overall $P < 0.004$; [ab] $P < 0.003$*(HH vs JJ)* ; $P < 0.0007$ *(HJ vs JJ).* The JH and JJ were not different from each other and the crosses are not different from each other.

Table 2.9. Genetic group means (± SE) of milk production (actual milk in first 305 d of lactation) in first and second lactation in purebred Holstein (HH) and Jersey (JJ) cattle and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Genetic group	Milk (kg)	Std Error
HH	10,348[a]	207.2
HJ	9,129[b]	229.8
JH	9,384[b]	189.6
JJ	7,080[c]	239.8

Overall genetic group effect *P < 0.0001*; [ab] *P < 0.0001 (HH vs HJ); P < 0.0020 (HH vs JH); P < 0.0001 (HH vs JJ);* [cd] *P < 0.0001 (HJ vs JJ); P < 0.0001 (JH vs JJ).* The crossbreds were not different from each other.

Table 2.10. The Glimmix model showing clinical condition means (± SE) occurring during the sample collection period of the first 10 wk after parturition in first and second lactation in purebred Holstein (HH) and Jersey (JJ), and crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH) cattle.

Clinical Condition				
Genetic group	**Mastitis**	**Metritis**	**Ketosis**	**Displaced Abomasum**
HH	1.1 ± 0.9%[a]	16.9 ± 4.2%	11 ± 3.5%	14.5 ± 3.9 %
HJ	9.4 ± 4.1%[b]	4.8 ± 2.7%	10.8 ± 4.2%	7.1 ± 3.4%
JH	8.1 ± 3.4%[b]	6.8 ± 2.8%	8.7 ± 3.1%	7.4 ± 2.9%
JJ	10.3 ± 4.7%[b]	0.0 ± 0.01%	9.9 ± 4.7%	2.4 ± 2.3%
Lactation #				
1	17.6 ± 3.6%[c]	0.4 ± 0.4%	7.6 ± 2.1%	6.4 ± 2.2%
2	1.6 ± 1.1%[d]	0.2 ±0.2%	13.1 ± 3.4%	6.9 ± 2.6%

Overall genetic group effect for mastitis $P < 0.0344$ and overall lactation effect for mastitis $P < 0.0006$; [ab] $P < 0.0322$ *(HH vs HJ);* $P < 0.0488$ *(HH vs JH);* $P < 0.0256$ *(HH vs JJ).* The crossbreds were not different from each other or from the purebred JJ. [cd] $P < 0.0006$.

Overall genetic group effect for metritis was $P < 0.0817$. There were only 24 observations of metritis in the study, however, this shows a trend and with the HH having a higher incidence of metritis could be a reason why they have a hard time getting bred back.

There was no significant genetic group or lactation effect seen in ketosis and displaced abomasums.

Table 2.11. Overall genetic group and lactation number means for the independent variables in the purebred Holstein (HH) and Jersey (JJ) and for the crossbred Holstein-Jersey (HJ) and Jersey-Holstein (JH).

Variable	Genetic group means			
	HH	**HJ**	**JH**	**JJ**
P4 (ng/mL)	1.55 ± 0.12	1.46 ± 0.12	1.79 ± 0.10	1.82 ± 0.13
NEFA (mEq/L)	0.52 ± 0.02	0.47 ± 0.03	0.46 ± 0.02	0.48 ± 0.03
Insulin (ng/mL)	0.83 ± 0.03	0.87 ± 0.04	0.76 ± 0.03	0.66 ± 0.04
IGF-1 (ng/mL)	89.1 ± 7.42	76.4 ± 8.84	75.4 ± 7.13	69.5 ± 9.40
Actual 305 d milk yield (kg)	10,348 ± 208	9,129 ± 230	9,384 ± 192	7,080 ± 261
Variable	**Lactation number means**			
	First lactation		**Second lactation**	
P4 (ng/mL)	1.65 ± 0.09		1.66 ± 0.08	
NEFA (mEq/L)	0.45 ±0.02		0.52 ± 0.02	
Insulin (ng/mL)	0.81 ± 0.02		0.75 ± 0.02	
IGF-1	78.6 ± 4.91		76.6 ± 4.44	
Actual 305 d milk yield (kg)	8,295 ± 160		9,676 ± 163	

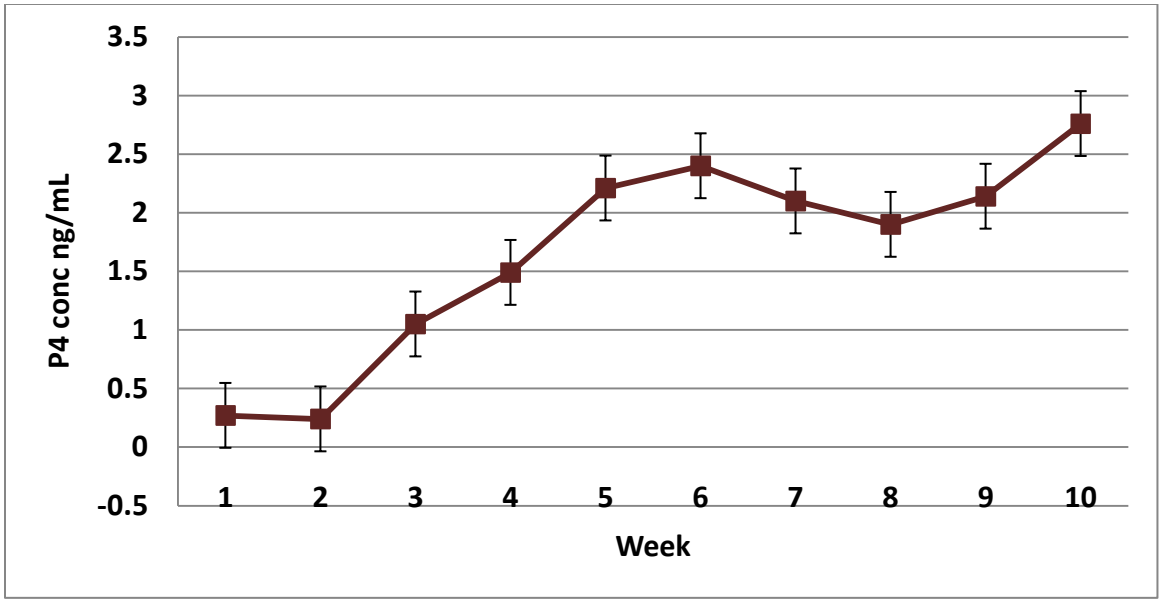

Figure 2.1. Average progesterone concentration (± SE) over the first 10 wk of lactation of both first and second lactation. Week was a significant effect *P < 0.0001*.

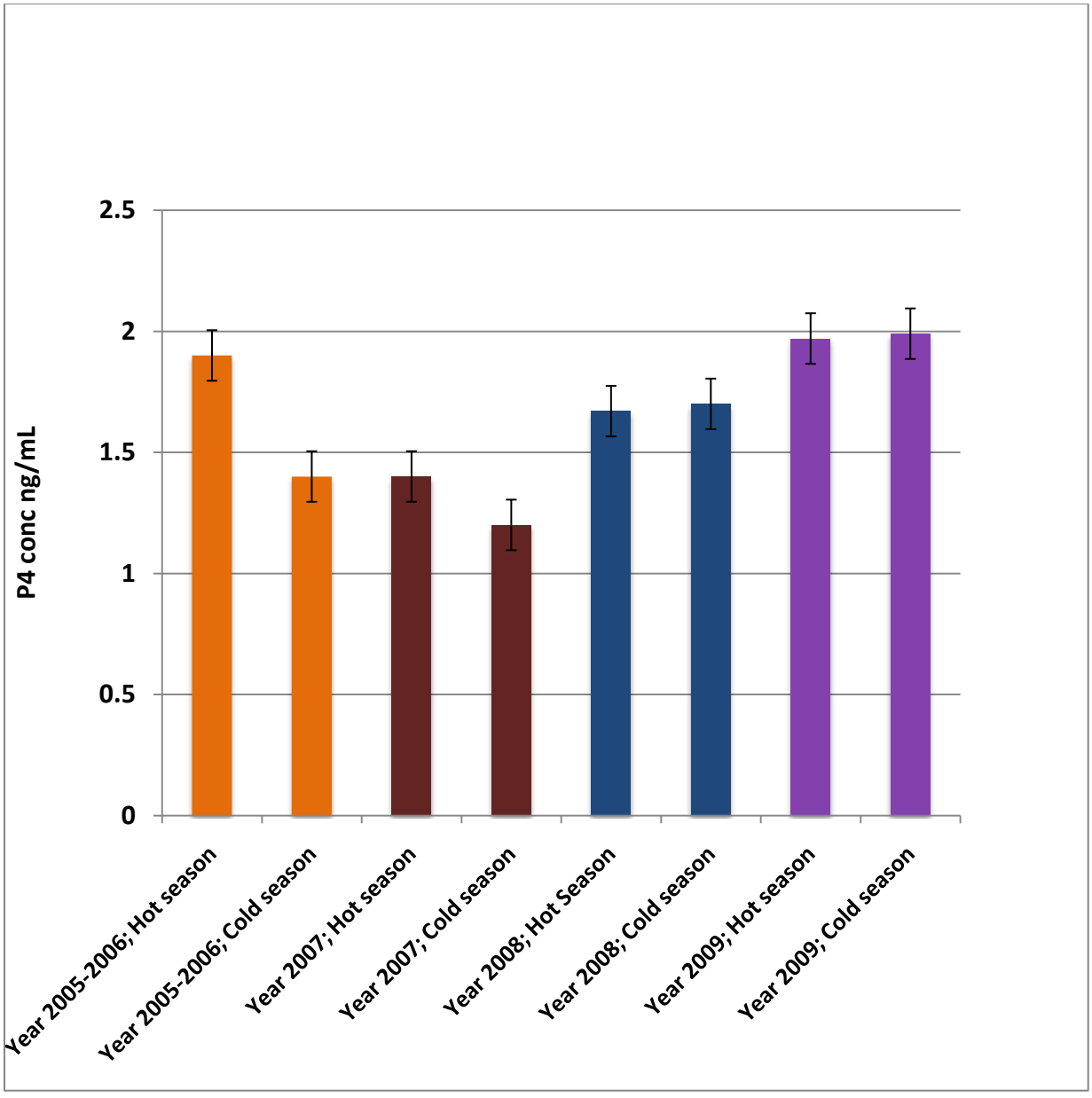

Figure 2.2. The relationship between the progesterone concentrations means (± SE) and the year-season effect. This effect was significant with $P < 0.0005$.

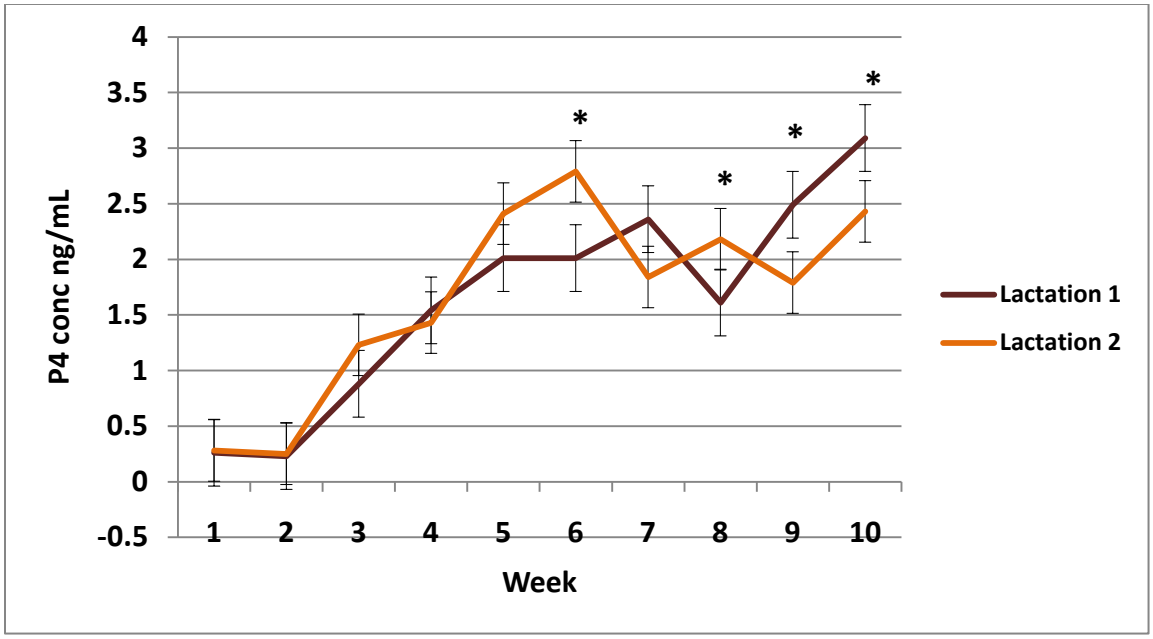

Figure 2.3. The interaction between first and second lactation progesterone means (± SE) and the week of lactation on all four genetic groups. The overall interaction was significant $P < 0.0003$. The slice analysis revealed that the change between lactation at wk 6 ($P < 0.0075$), 8 ($P < 0.0469$), 9 ($P < 0.0105$), and 10 ($P < 0.0254$) were significant.

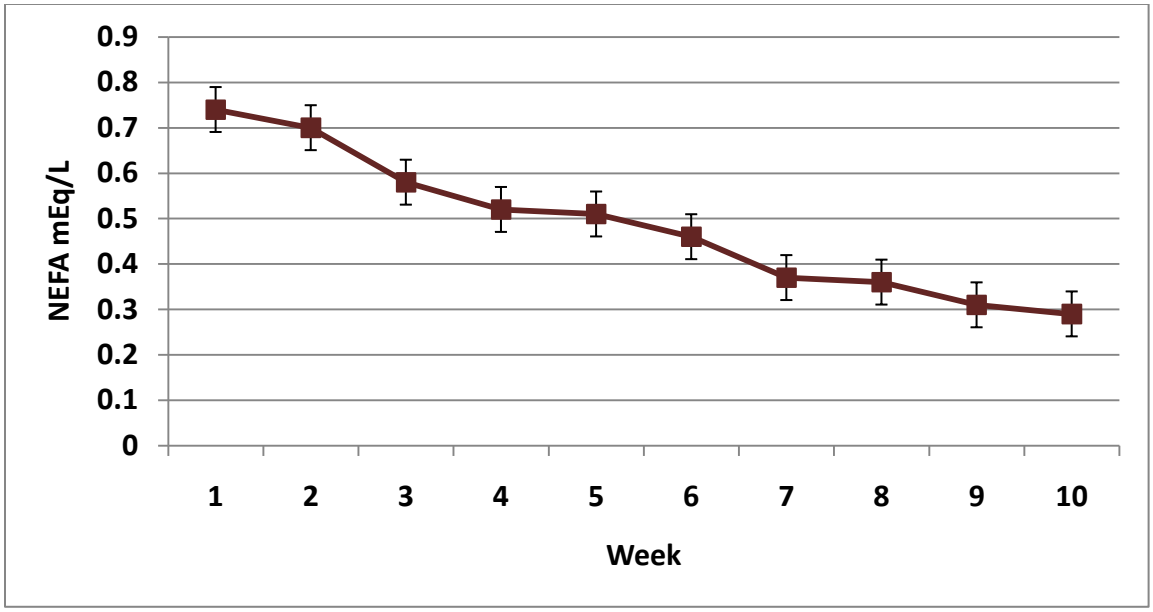

Figure 2.4. The change in NEFA concentrations means (± SE) during the first 10 wk of lactation across all four genetic groups. The week effect was significant with *P < 0.0001*.

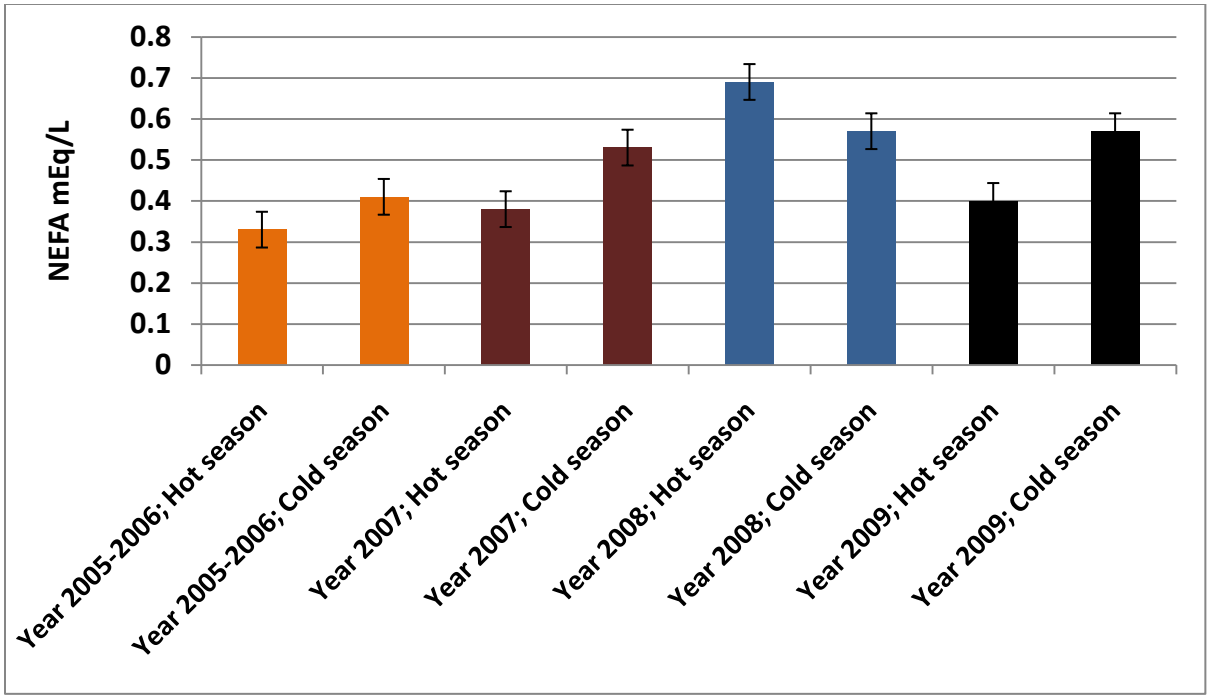

Figure 2.5. The change in NEFA concentration means (± SE) by the year-season effect. This effect was significant with $P < 0.0001$.

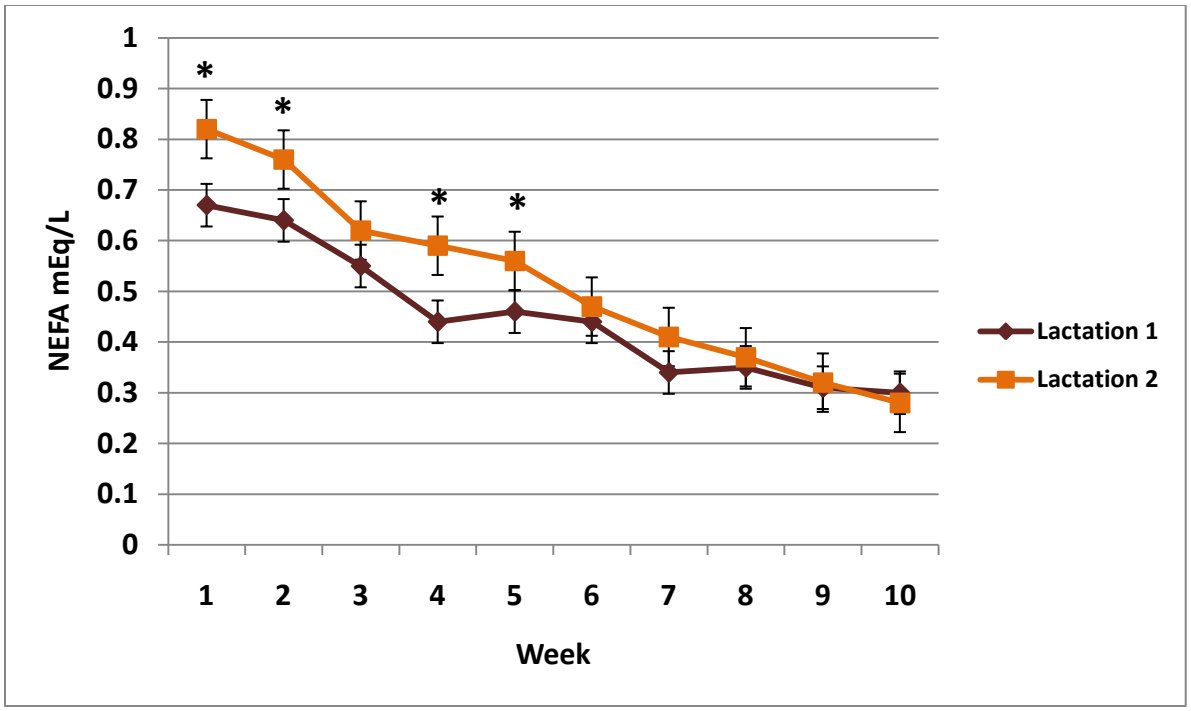

Figure 2.6. The interaction between NEFA concentrations means (± SE) and the first 10 wk of lactation for first and second lactation. This is a significant interaction with $P < 0.0058$. The slice analysis revealed a significant change between lactations at weeks 1 ($P < 0.0002$), 2 ($P < 0.004$), 4 ($P < 0.0001$), and 5 ($P < 0.007$).

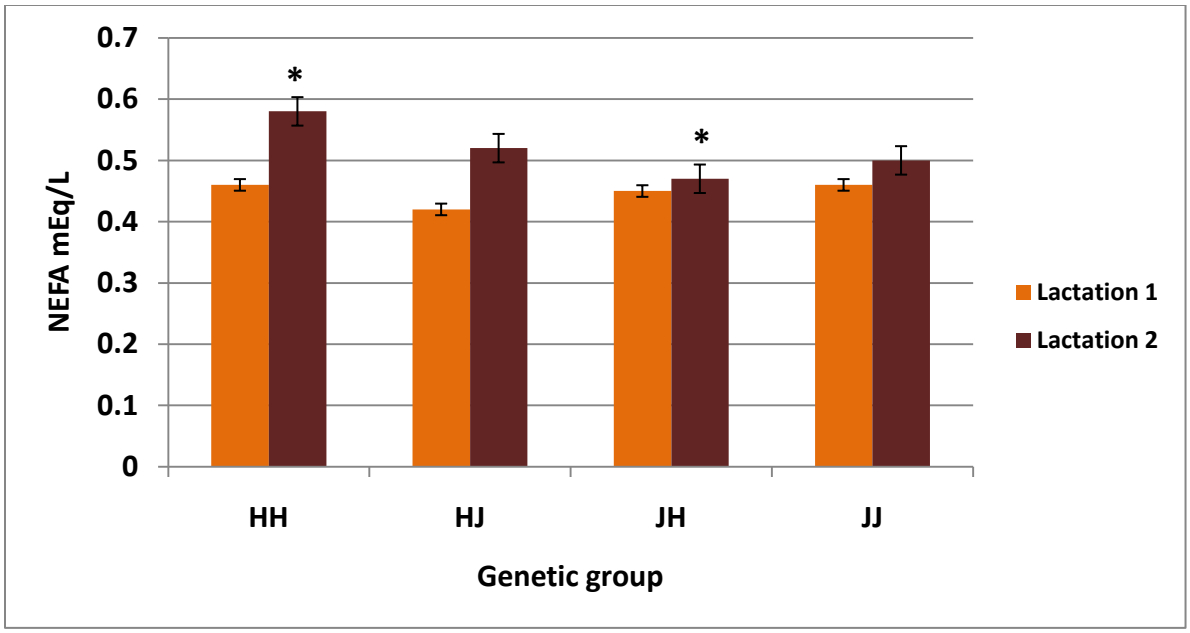

Figure 2.7. The genetic group NEFA concentration means (± SE) by lactation number interaction. The overall effect is significant $P < 0.01$. The contrasts revealed that the difference occurs between the HH in second lactation compared to the JH in second lactation $P < 0.004$.

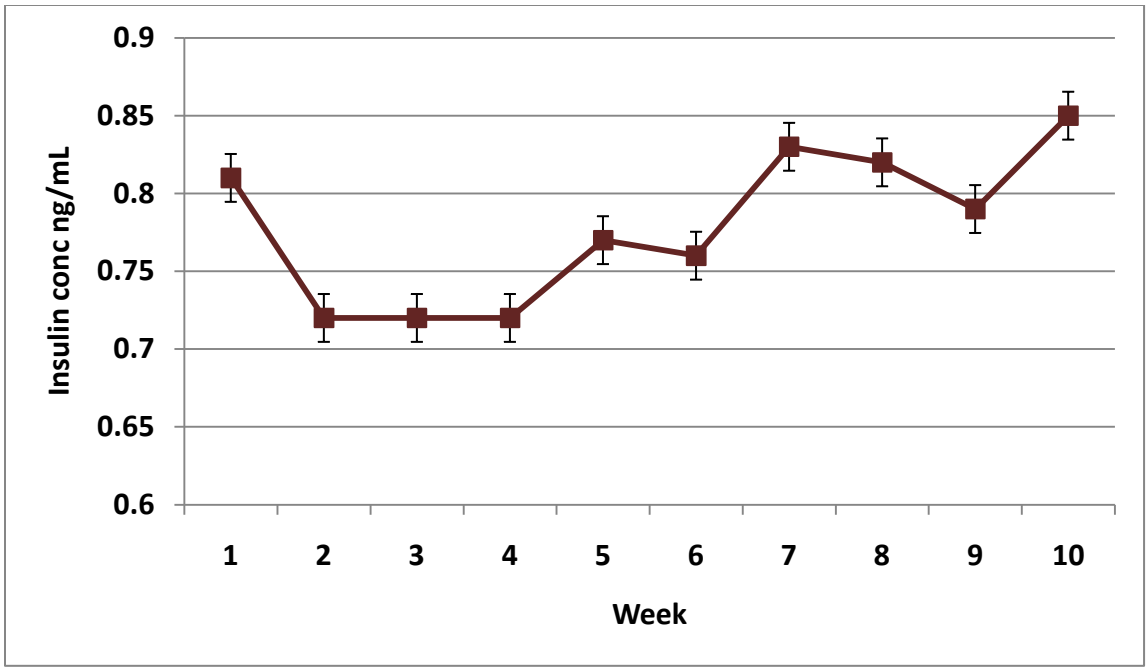

Figure 2.8. The change in insulin concentration means of all four genetic groups (± SE) over the first 10 wk of lactation. Week had a significant effect on insulin concentration with $P < 0.0001$.

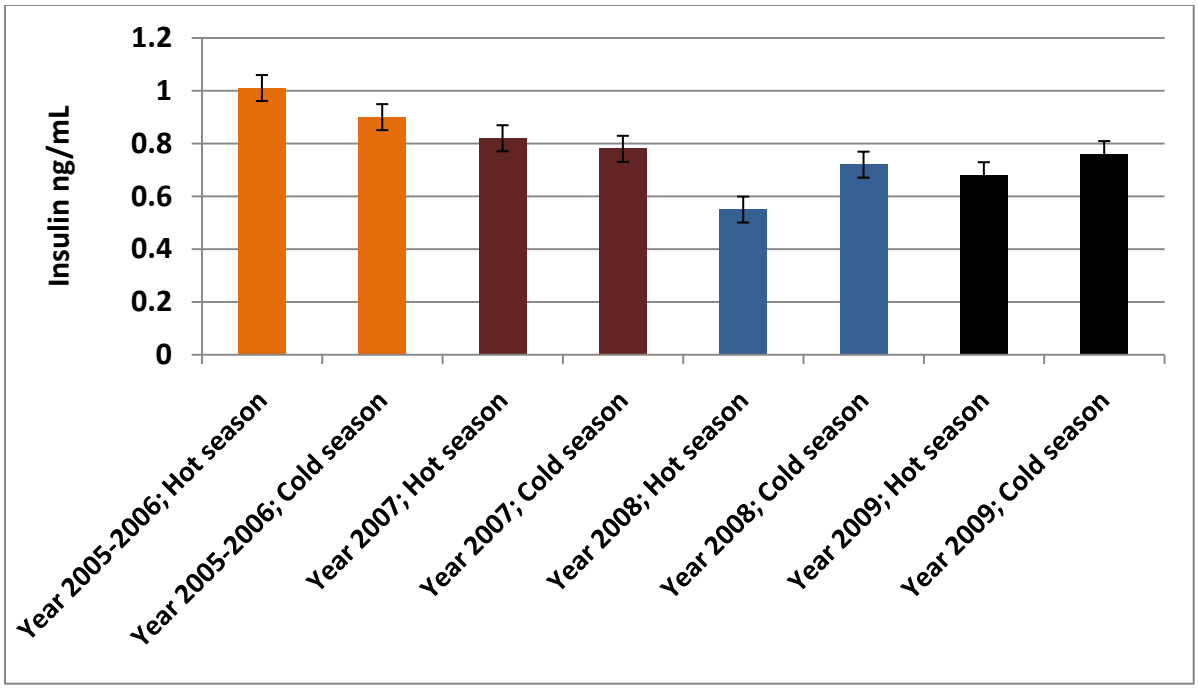

Figure 2.9. The relationship between insulin concentrations means (± SE) and the effect of year-season. Year-season had a significant effect on insulin concentrations with $P < 0.0001$.

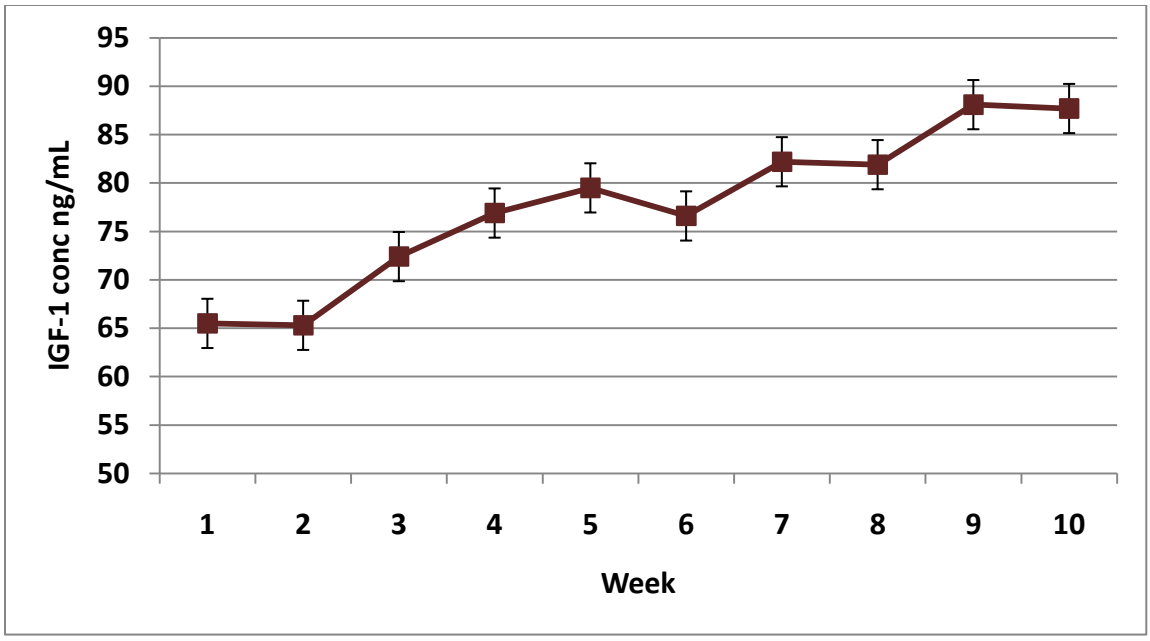

Figure 2.10. The change in IGF-1 concentration means (± SE) of all four genetic groups over the first 10 wk of lactation. Week was a significant effect on IGF-1 concentrations with $P < 0.0001$.

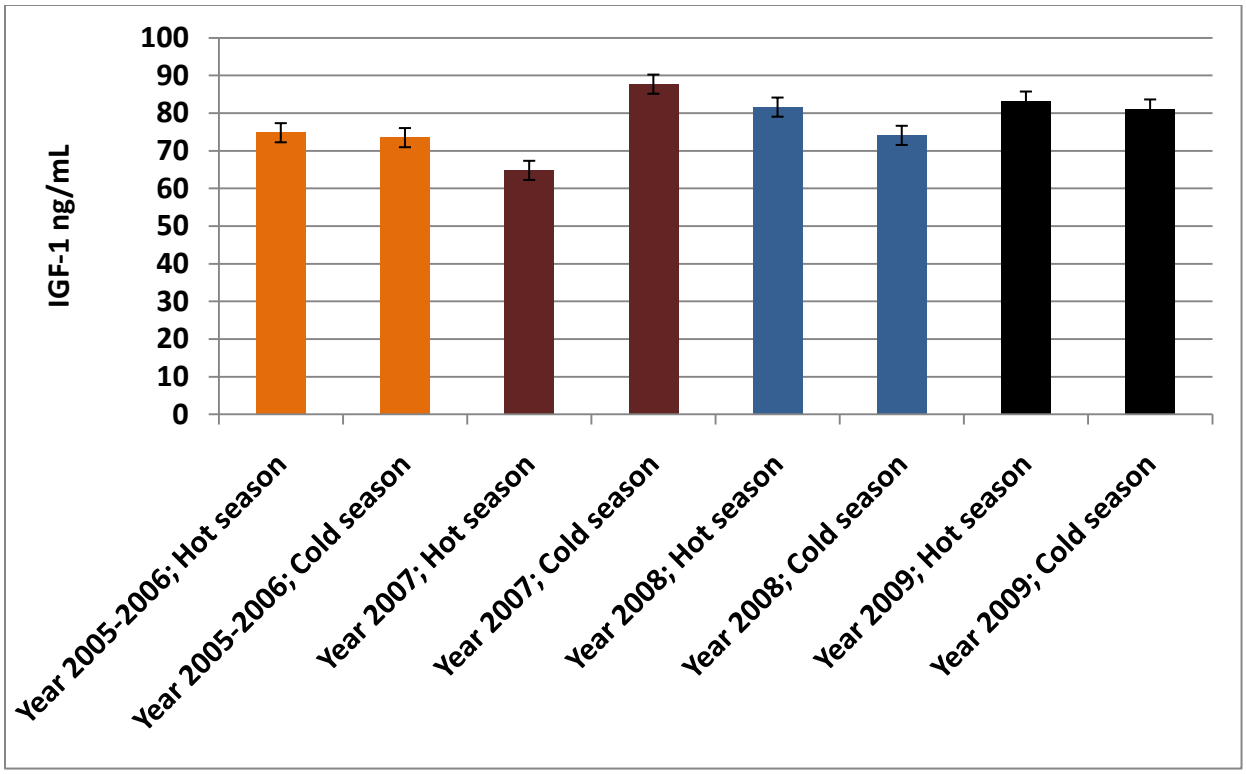

Figure 2.11. The relationship between IGF-1 concentrations (± SE) and the year-season effect. Year-season effect was significant on the IGF-1 concentrations $P < 0.0030$.

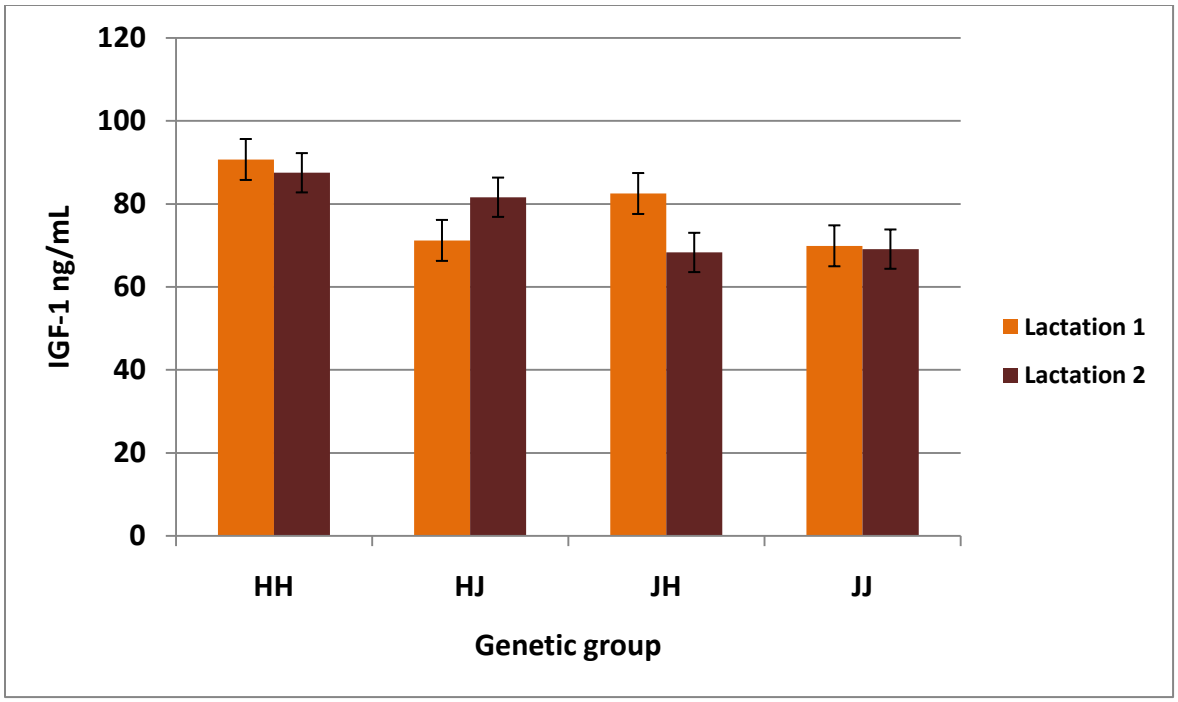

Figure 2.12. Genetic group IGF-1 concentration means (± SE) by lactation number interaction. The overall interaction between genetic group and lactation number was significant $P < 0.0018$. No contrasts between genetic groups and their lactations were found.

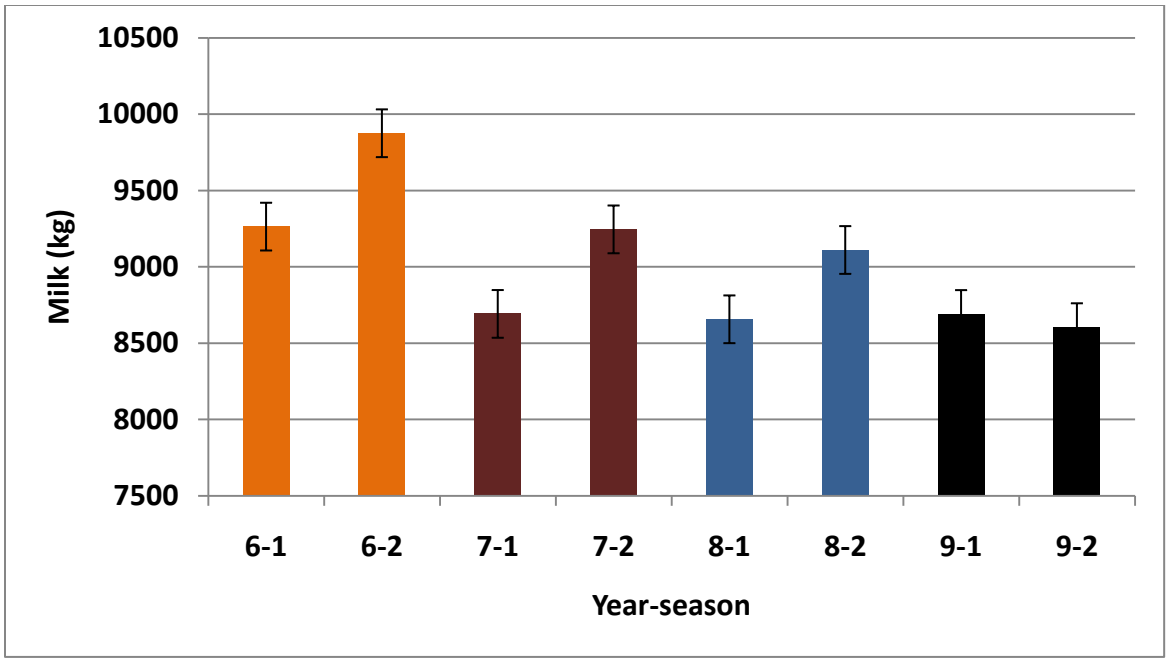

Figure 2.13. The relationship between actual 305 d milk production means (± SE) and the year-season effect. Year-season overall significantly affected 305-d milk yield (*P < 0.0143*).

CHAPTER 3: General Conclusions

Reproductive issues in the bovine can be seen across genetic groups over the past 4 decades. Crossbreeding has shown that it can benefit from the reproductive ability that the Jerseys have as well as the milk production that the Holsteins are known for. Results from the Virginia Tech herd support this statement. The JH had fewer days open and services per conception compared to the HH, HJ, and JJ. The JH had the greatest amount of actual 305 d milk yield of the crossbreds and was closest to the actual 305 d milk yield that was produced by the HH. Cows that do not meet the standard of calving once a year can be problematic for producers as they will have lower lifetime milk production. However, very few cows in a high producing herd will meet this standard. It has been shown that there are multiple factors that influence the reproductive performance of dairy cows after parturition.

NEB is one of the larger issues regardless of genetic group and lactation number. NEFA has been shown to be the best indicator of the energy status of the cow as well as metabolic diseases. NEFA concentrations were greater in second lactation relative to first lactation because the demand for milk production increases and more lipid stores are used to meet that demand. NEFA concentrations were greater in the HH and the HJ compared to the JH and JJ. As the cows age, their milk production increases, but their fertility decreases. Second lactation cows had higher actual 305 d milk yield compared to those cows in first lactation. NEFA measures were negatively correlated with progesterone, insulin, and IGF-I, suggesting that energy balance impacts both reproduction and metabolic activity.

IGF-I and insulin influence both nutritional and reproductive status of the animals. The low IGF-I and insulin concentrations were related with higher milk production in early lactation. Low IGF-1 and insulin concentrations were found in the first 4 wk of lactation, which was before or when plasma progesterone concentrations started to increase above the 1 ng/mL threshold. Reproductive ability improves as the IGF-I and insulin concentrations increase later in lactation. Both IGF-I and insulin concentrations were lower in second lactation compared with first lactation. This could be a result of the increased NEFA concentrations and milk production. Increased NEFA concentrations were negatively correlated with progesterone concentrations. Progesterone and IGF-1 concentrations both start out low in the first weeks of lactation and then gradually increase over 10 wk sample time. Insulin follows the same pattern to an extent, except it is greater in the first week before dramatically decreasing prior to increasing throughout the 10 wk of lactation that was measured.

Progesterone is the hormone associated with ovulatory activity and analyzing this hormone with the other factors of IGF-I, insulin, NEFA, milk, and maximum temperature will help identify other issues that may impact the reproductive ability of the genetic groups. This will assist in future research to determine what steps should be taken next to improve the status of the dairy industry as well as making sure producers are maximizing their animals to their full potential.

Management factors as well as feed quality need evaluated in more detail to see how large of an effect they can have on the animal reproduction. Crossbreds may be the better choice than purebreds in certain parts of the United States and across the world depending on

the type of environment they thrive in. How a cow is managed can influence how certain cows or genetic groups respond reproductively. The type of nutrition that the cow is receiving can influence how quickly they escape the NEB and enter into a more positive energy balance. If animals enter into a less NEB after parturition, then their NEFA concentration should be lower which could allow for IGF-1 concentrations to increase at a faster rate. Increasing IGF-1 could stimulate the ovary to begin ovarian activity which in turn will allow ovulation and progesterone to be produced from the corpus luteum.

Fertility of the sires of the crossbreds should be looked into, to see how much they contribute to their daughter's ability to reproduce and produce milk efficiently based on the data obtained from the Virginia Tech herd. Once the rest of the data has been merged from Kentucky and North Carolina, this statement may not be valid. This information would allow producers to analyze which sires they want to breed to their animals to maximize their potential. Other research needs to be done on management factors to see if there are particular ways to manage Holsteins, Jerseys, and their crosses. Each genetic group may respond to management factors in different ways. Some management factors may be beneficial for one particular genetic group, and harmful to another.

Movement between groups during lactation could influence how they react to returning to estrus. Moving groups often could inflict stress on the cows, which could inhibit their return to estrus. Each genetic group may have different needs. Additional research needs to be done on how each genetic group handles feed. If that is found to be the case, then producers would be able to adequately feed their cows accordingly to the particular genetic group to reduce the NEB state they enter after parturition.

There are multiple directions this research could go. Trying to control one possible factor at a time could show where the actual problem lies. If researchers find where the largest problem lies, then ways to alleviate it may be found. Then smaller problems could be looked into and possibly solved.

APPENDIX A: Anova Tables for Models

Table A.1. Explanatory variables in prediction of days open with progesterone concentrations above 1ng/mL within the first 30 DIM

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
P4 > 1ng/mL	**1**	**0.6128**
Genetic group	**3**	**0.0042**
Lactation #	**1**	**0.0980**
Profile	**3**	**0.6835**
Error	**256**	

Table A.2. Explanatory variables in prediction of days open with progesterone concentrations above 3 ng/mL within the first 30 DIM

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
P4 > 3 ng/mL	**1**	**0.1365**
Genetic group	**3**	**0.0039**
Lactation #	**1**	**0.0842**
Profile	**3**	**0.6865**
Error	**256**	

Table A.3. Explanatory variables in prediction of service number with progesterone concentrations above 1 ng/mL within the first 30 DIM

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
P4 > 1 ng/mL	**1**	**0.5649**
Genetic group	**3**	**0.0458**
Lactation #	**1**	**0.2144**
Profile	**3**	**0.5234**
Error	**256**	

Table A.4 Explanatory variables in prediction of service number with progesterone concentrations above 3 ng/mL within the first 30 DIM

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
P4 > 3 ng/mL	**1**	**0.1103**
Genetic group	**3**	**0.0477**
Lactation #	**1**	**0.1755**
Profile	**3**	**0.2855**
Error	**256**	

Table A.5. Explanatory variables in prediction of progesterone

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**0.0850**
Lactation #	**1**	**0.8843**
Year-Season	**7**	**0.0005**
Week	**9**	**<.0001**
Maximum temperature	**1**	**0.0020**
Lactation # * week	**9**	**0.0003**
Error	**2600**	

Table A.6. Explanatory variables in prediction of NEFA

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**0.2657**
Lactation #	**1**	**0.0002**
Week	**9**	**<.0001**
Year-season	**7**	**<.0001**
Maximum temperature	**1**	**0.5027**
Genetic group*lactation #	**3**	**0.0114**
Lactation # * week	**9**	**0.0058**
Error	**2600**	

Table A.7 Explanatory variables in prediction of insulin

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**0.0032**
Lactation #	**1**	**0.0066**
Week	**9**	**<.0001**
Maximum temperature	**1**	**0.0062**
Year-Season	**7**	**<.0001**
Error	**2612**	

Table A.8. Explanatory variables in prediction of IGF-I

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**0.3652**
Lactation #	**1**	**0.6551**
Week	**9**	**<.0001**
Maximum temperature	**1**	**0.0489**
Year-Season	**7**	**0.0030**
Genetic group*lactation #	**3**	**0.0018**
Error	**2608**	

Table A.9. Explanatory variables in prediction of actual 305 d Milk production.

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**<.0001**
Lactation #	**1**	**<.0001**
Year-Season	**7**	**0.0143**
Insulin in week 1	**1**	**<.0001**
Genetic group * Lactation #	**3**	**0.3813**
Error	**202**	

Table A. 10. Explanatory variables in prediction of progesterone concentrations above 1 ng/mL within the first 30 DIM

Type 3 Tests of Fixed Effects		
Effect	**DF**	**Pr > F**
Genetic group	**3**	**0.0097**
Lactation number	**1**	**0.9782**
Error	**260**	