ABSTRACT

WILLIAMS, CHRISTINA MICHELLE. Effects of Crossbreeding on Puberty, Postpartum Cyclicity, and Fertility in Pasture-Based Dairy Cattle. (Under the direction of Dr. Steven P. Washburn.)

There were two objectives set forth in this study: 1) To determine if breed differences existed and what effects crossbreeding had on postpartum (**PP**) cyclicity and fertility in fall calving, pasture-based dairy cows and 2) To evaluate the effects of crossbreeding concerning the obtainment of puberty in dairy heifers in a pasture-based system. Towards the first objective, milk samples were collected from purebred Holstein, purebred Jersey, and Holstein/Jersey crossbred cows twice weekly after calving in the 2005 calving season and in 3 sets of 2 samples at 10 d intervals for the 2006 calving season. Skim milk samples were analyzed for Progesterone (P_4) to determine when they returned to cyclicity. Holstein cows weighed the most at dry-off, calving, 30 d and 60 d PP, followed by the crossbred cows; Jersey cows were the lightest at each time point (P <0.01). No differences were seen among the 3 breed groups in regards to BCS ($P = 0.23$). At 30 d, 60 d, and 90 d PP over both calving seasons Holstein and crossbred cows had greater milk production than Jersey cows ($P < 0.01$) and Energy Corrected Milk (**ECM**) tended to be higher for Holstein and crossbred cows ($P = 0.06$). A year effect was seen in milk production and ECM such that cows calving in 2006 had higher production and ECM values than those that calved in 2005 ($P < 0.05$). Fewer Holstein cows were cyclic at 30 d, 60 d, and 90 d PP when compared with purebred Jersey and crossbred cows ($P < 0.05$). No statistical differences were seen for mean anestrous interval, but differences may exist as not all Holsteins had reached cyclicity by 90 d PP and were therefore excluded from calculation of breed anestrous interval. Holstein cows also had lower first

service conception rates and 90 d pregnancy rates than either Jersey or crossbred cows; Jersey and crossbred cows had similar conception and pregnancy rates.

Purebred Holstein, Jersey and Holstein/Jersey crossbred heifers born in the fall of 2005 were sampled weekly from 4 mo until puberty was reached and serum was analyzed for P_4 to determine age at puberty. Serum samples were also analyzed for the hormone Leptin to elucidate its relationship to onset of puberty. Heifers were categorized as 100% Holstein (100% H), >50% Holstein (>50% H), 50% Holstein/ Jersey (50% HJ), >50% Jersey (>50% J), and 100% Jersey (100% J). Age at puberty was positively and linearly correlated to percent Holstein of a heifer, with the greater percentage Holstein of a heifer the older the heifer will be at puberty ($P < 0.01$). 100% H and >50% H heifers were heavier at birth than 100% J and >50% J heifers and stayed heavier as time progressed and at puberty ($P < 0.01$). There was no effect of breed composition on heifer BCS at anytime from 4 mo to 14 mo of age. Differences in wither heights were seen with 100% H heifers being the tallest by >50% H, and both were taller than 100% J, and >50% J, respectively ($P < 0.01$). No differences in serum Leptin concentrations were seen regarding percentage Holstein of a heifer from 12wks before puberty up to the week of puberty. Changes in Leptin concentrations over time were not significant and revealed no interesting relationship to onset of puberty.

Effects of Crossbreeding on Puberty, Postpartum Cyclicity, and Fertility in

Pasture-Based Dairy Cattle

by

Christina M. Williams

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Approved by:

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Dr. Steven P. Washburn
Chair of Advisory Committee

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Dr. Scott Whisnant   Dr. Mark Alley
Advisory Committee Member   Advisory Committee Member

DEDICATION

To Chris, without whose love and support this would not have been possible.

BIOGRAPHY

Christina was born in Gainesville, Florida and grew up in the small town of Sachse, Texas. Her family moved to North Carolina in 1998. After graduating high school in 2001, Christina went on to pursue a Bachelor of Science degree in Biology, with minors in Chemistry and Psychology, at the University of North Carolina at Greensboro. In the fall of 2005, she began work on her Master of Science degree at North Carolina State University in the Animal Science Department under the direction of Dr. Steven P. Washburn.

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d : day(s)

mo : month(s)

min : minute(s)

h : hour(s)

P_4 : Progesterone

BCS : Body Condition Score

RPM : rotations per minute

BW : Body weight

ECM : Energy Corrected Milk

PP : Postpartum

SEM : Standard error of means

H : Holstein

J : Jersey

NEB : Negative Energy Balance

CHAPTER 1

INTRODUCTION

Limited research has been conducted regarding crossbreeding in dairy cattle in the US. Genetic selection for high milk production, type and appearance for the last 50 years has suppressed secondary traits such as reproductive performance, productive life, health, and survivability in the breed. The economic importance of these secondary traits in dairy production systems are the basis for the interest seen in crossbreeding (McAllister, 2002). Crossbreeding, despite its potential ability to improve dairy production, remains under-utilized here in the United States with only about 0.5% of milk recorded cows being crossbred animals (McAllister, 2002). Crossbreeding has been utilized quite successfully in countries other than the US. Australia, Canada, India, and especially New Zealand have established practices involving crossbreeding (Lopez-Villalobos et al, 2000; McAllister, 2002; VanRaden and Sanders, 2003). In the past few years, there has been increasing interest in crossbreeding in US dairy herds which is likely due to changes in milk pricing rewarding high fat and protein percentages, farmer concerns regarding female fertility, calving ease, animal health, and survival of Holsteins as well as rapid increases in inbreeding levels of all major dairy breeds (Weigel and Barlass, 2003).

Following parturition, there are numerous physical and hormonal changes cows must undergo in order for reproductive function to be restored. Uterine involution and resumption of ovarian activity must be achieved for the recommencement of reproductive ability. The uterus must return to a pre-pregnancy state and ovarian activity must be restored (Senger 2nd ed, 2003). However, any number of things can extend the duration of postpartum anestrus in dairy cattle. Nutrition and the corresponding energy balance play a large role in the ability of a cow to achieve early postpartum cyclicity (Roche et al., 2000). Health is another critical

modifier of when reproductive functionality resumes in the postpartum cow. Any time a dairy cow is in a diseased or sickened state, energy is being diverted from regaining reproductive ability, as well as from production, to combating the malady (Roche et al, 2000). Higher genetic merit cows have greater risk of reproductive failure due to their selection for greater production and the high genetic correlations between yield traits and return to luteal activity and fertility (Veerkamp et al, 1999). Season, management practices, and parity also play vital roles in regaining reproductive capabilities (Darwash et al., 1997; Lucy, 2001).

Puberty in heifers is a culmination of a long and gradual process that begins before birth and continues all the way until puberty commences; it involves a great deal of interaction between reproductive tissues and endocrine signals (Sejrsen, 1994). It is important for heifers to reach puberty before 15 mo of age so they can be bred to calve at 2 yrs of age. Puberty tends to occur between 8 to 24 mo and depends on a multitude of factors (Patterson et al, 1992). Body size is a critical factor that will influence age at puberty such that a heifer has to be large enough to gestate and give birth to a calf as well as have an optimal level of body energy stores to meet the demands of gestation and subsequent lactation (Senger 2nd ed, 2003). The breed or genetics of a heifer will affect when puberty is reached with larger breeds of cattle taking longer to become pubertal than smaller breeds (Laster et al, 1976; Baker et al, 1988). Heifers born in the fall reach puberty at a younger age than those born in other seasons demonstrating the role of season in pubertal development (Schillo et al, 1983). Good health of the heifer aids in the obtainment of puberty in a timely manner. Illness and disease slow growth and can impair reproductive development and disrupt endocrine regulation (Sejrsen , 1994). Nutrition is key in determining when puberty

will occur in dairy cattle. Poor nutrition during prepubertal growth will increase the time it takes to reach the required weight for puberty; excessive feed will allow for puberty to commence sooner but at the cost of future production (Macdonald et al, 2004).

Leptin is a protein hormone that is secreted in correlation to the amount of body fat and adipocyte size present in an animal (Ahima and Flier, 2000). Leptin is able to cross the blood-brain barrier and can exert its effect on the regulation of feeding and energy balance, body maintenance, reproductive development, as well as a response to stress or starvation (Flier, 1998). Discovered in sterile *ob/ob* mice, Leptin may aid in obtainment of puberty and reproductive function (Williams et al, 2000). Positively correlated with BW and plane of nutrition, Leptin increases linearly before puberty such that increasing BW and nutrition result in high levels of Leptin. Higher Leptin concentrations are associated with younger age at the onset of puberty in dairy cattle (Block et al, 2003). Concerning its effects on reproductive function, Leptin has been associated with expression of estrus and concentrations vary with the estrous cycle (Leifers et al, 2003; Garcia et al, 2002b). The manner in which Leptin links nutrition and the reproductive system may be directly or indirectly through the GnRH-estradiol negative feed back system (Williams et al, 2000).

LITERATURE REVIEW

Crossbreeding

By simple definition, crossbreeding is the mating of animals from 2 different breeds. More explicitly, as defined by VanRaden and Sanders (2003), crossbreeding is "a method to increase the health and efficiency of animals by introducing favorable genes from other breeds, removing inbreeding depression and by maintaining the gene interactions that result in heterosis." The latter definition regards crossbreeding as more of a breeding system which can include any and all of the following practices: crisscrossing of 2 breeds, rotational crossing of 3 or more breeds as well as crosses of a purebred male to a purebred female, the literal interpretation of the former definition. Heterosis, also referred to as hybrid vigor, is the better performance than the average of the parent breeds of a crossbred animal. Production traits range from 0-10% heterosis whereas fertility and reproductive traits have greater heterosis at 5%-25% (Swan and Kinghorn, 1992).

Dairy Cattle Crossbreeding

Among farm animals, less experimental work has been done with crossbreeding in dairy cattle. Currently, approximately 94% of dairy cows in the United States are purebred or grade Holstein. Holsteins comprise this vast majority of the dairy industry due to their ability and selection for high lactation milk yields (McAllister, 2002). This under-utilization of crossbreds is due to the lack of use of U.S. dairy genetics, dairy production conditions and market values involved in crossbreeding research studies, as well as the absence of multi-generation economic comparisons of Holstein and crossbred populations (McAllister, 2002).

While not highly popular in the U.S., dairy crossbreeding has been employed successfully in other countries. In Australia, 5% of cows are crossbred, whereas in New Zealand herds, 20% of the cows were crossbred. Both countries use predominantly Holstein-Friesian and Jersey breeds in their crossbreeding (VanRaden and Sanders, 2003). A study conducted by Lopez-Villalobos et al. (2000) sought to evaluate the profitability of crossbreeding systems in New Zealand. It predicted higher first lactation milk yields for the Holstein-Friesian/ Jersey crossbred cows than purebred Friesian cows. Economic analysis showed a large advantage in net income per hectare for the crossbred over the purebred animals. Canada has also experimented with crossbred dairy systems. Purebred Holsteins, purebred Ayrshire, and Holstein-Ayrshire crossbred cows were compared. Crossbred animals netted higher returns than the purebred Holsteins and generated more replacement animals than both purebred Holstein and Ayshire (McAllister, 2002). The success of crossbreeding can be influenced by several factors and implementation in different dairy production systems may not be applicable. Climate, temperature, region-specific breeds, on-farm conditions, nutrition, and breeding and management practices can affect the results of crossbreeding. For these reasons, results from other countries may not apply to the dairy industry in the United States (McAllister, 2002).

Although not mainstream, there has been increasing interest in crossbreeding in US dairy herds in recent years. This is attributed largely to changes in milk pricing rewarding high fat and protein percentages, farmer concerns regarding female fertility, calving ease, animal health, and survival of Holsteins as well as rapid increases in inbreeding levels of all major dairy breeds (Weigel and Barlass, 2003). In 1997, Wiggans et al reported an average inbreeding of 4.7% in Ayrshire cows, 3.0% in Guernseys, 2.6% in Holsteins, 3.3% in Jerseys,

and 3.0% in the Brown Swiss breed. A study by Sewalem et al (2004) calculated that the mean inbreeding index for Holsteins, Jerseys, and Ayshires were 5.04%, 5.00%, and 6.04% respectively.

Weigel and Barlass (2003) surveyed dairy producers currently using crossbreeding in their herds (n=50). The most common first generation crosses (F_1) involved Holstein cows mated to Jersey or Brown Swiss bulls, and the second generation (F_2) being backcrosses to one of the parental breeds. Nearly all producers claimed improvements in fertility, calving ease and milk composition in the crossbreds. Crosses involving Jersey and Brown Swiss breeds had advantages in longevity and conception rates over purebred Holstein cows. Other advantages cited included improved feet and legs, animal temperament, grazing performance, and reduced body size. Some disadvantages were reported in the surveys, which included difficult marketing for crossbred breeding stock and bull calves, lack of herd uniformity, difficulty with mate selection, and reduced milk volume.

Other studies have also shown benefits to crossbreeding in the U. S. Touchberry (1992) combined measures of survival, growth, milk yield, and reproduction into an index of income per cow for Holstein and Guernsey breeds and their crosses in a 20 yr study conducted in Illinois. Crossbred cows were 14.9% greater than the average of the purebreds based on income per lactation and greater by 11.4% based on income per cow per year. The value of animals sold was also higher for the crossbred animals. Crossbred cows had increased survivability over both purebreds, and a higher percentage of crossbred cows had first and second calvings. This suggests that crossbreds are less susceptible to disease, sickness, and reproductive difficulties than their purebred counterparts in Touchberry's study.

VanRaden and Sanders (2003) evaluated crossbred cows of various breed compositions involving Holstein, Jersey, Ayshire, Brown Swiss, Guernsey, and Milking Shorthorn. Matings of Jersey and Brown Swiss with Holstein produced animals that stayed in the herd as long as or longer than purebred Holstein cows. Protein and fat yields of Jersey/Holstein and Brown Swiss /Holstein crosses equaled if not exceeded that of purebred Holstein. These same crosses also had higher net merit dollars and cheese merit dollars compared to purebred Holsteins. (VanRaden and Sanders, 2003). Additionally, crosses involving Brown Swiss and Jersey were reported to have less dystocia than purebred Holsteins (Cole et al, 2005).

Heins et al (2006a, 2006b, 2006c) worked in conjunction with 7 commercial dairies in California to evaluate the effects of crossing purebred Holstein cows with Normande, Montbeliarde, and Scandinavian Red (Swedish and Norwegian Reds combined) breeds. They reported that Holstein cows produced greater yields of milk and protein than the crossbreds but not were different from the Scandinavian Red/Holstein crosses for fat production. Also, the Scandinavian Red/Holstein crosses were not different from purebred Holsteins for fat plus protein production, although the other crosses had significantly lower fat plus protein production (Heins et al, 2006c). Heins et al (2006a) noted that crossing Holstein cows with Scandinavian Red or Brown Swiss bulls significantly lowered calving difficulty for first-parity Holstein heifers and Scandinavian-sired calves had lower calving difficulty in multiparous Holstein cows as well and fewer stillbirths in all parity dams. All groups of crossbred cows in the Heins study (2006a) had lower dystocia than purebred Holstein cows. Scandinavian Red/Holstein and Montbeliarde/Holstein crossed cows had lower stillbirth rates at first calving than purebred Holstein cows (Heins et al, 2006a). They

also looked at fertility and survivability measures of purebred Holstein cows and of Montbeliarde, Normande, Scandinavian Red crossed with Holstein. The crossbred cows had significantly fewer days to first breeding and fewer days open. The crosses also had higher conception rates and had greater survivability at 30 d, 150 d, and 305 d postpartum than did Holstein cows (Heins et al, 2006b).

Postpartum Cyclicity and Fertility

Following parturition, cows must undergo numerous physical and hormonal changes in order for reproductive function to be restored. Uterine involution and resumption of ovarian activity must be achieved for the resumption of reproductive function. In dairy cattle, this process can take up to 60d (Senger 2nd ed, 2003). The uterus must decrease in both size and volume. This requires a decrease in uterine vasculature, expulsion of tissues and fluids associated with pregnancy, and repair of endometrial tissue. Unlike most other animals, dairy cows are not continually suckled, decreasing Oxytocin pulse frequency, therefore reducing uterine contractions and prolonging the time it takes for discharge of remaining pregnancy tissue and fluids (Senger 2nd ed, 2003). Timely uterine involution is important in dairy cow. Without a complete return of the uterus to non-pregnant state, a subsequent pregnancy can not develop and continuation of lactation is prevented.

The other important facet leading to the resumption of reproductive functionality is the return of ovarian activity. Pregnancy is dominated by high levels of Progesterone (P_4) which prevents follicular growth and ovulation. Just before parturition, P_4 levels decline sharply which allows secretion of gonadatropin-releasing hormone (GnRH) from the anterior pituitary and leading to resumption of follicular activity (Senger 2nd ed, 2003). Follicle-

Stimulating Hormone (FSH) increases shortly after parturition in transient waves in response to increasing GnRH levels. This promotes the commencement of the first postpartum follicular wave. From this follicular wave, several follicles are recruited and begin to grow and produce estradiol. Only one of the recruited follicles continues growing and becomes the dominant follicle (DF), and suppresses FSH levels (Roche and Diskin, 1999). Once high enough, estradiol levels trigger a surge in lutenizing hormone (LH) in the anterior pituitary. This LH surge causes the follicle to rupture, releasing the ovum; this follicular lysis is known as "ovulation." The first postpartum ovulation signals a return to cyclicity and luteal activity in the form of progesterone (P_4) production (Senger 2nd ed, 2003).

Factors affecting return to luteal activity

High reproductive efficiency requires cows to resume normal cycles as quickly as possible post-calving (Roche and Diskin, 1999). Early cyclicity increases the probability of earlier insemination, and resulting in fewer days open. However, return to postpartum luteal activity does not always occur in an ideal time frame, and many dairy cows experience prolonged postpartum anestrus. Any number of things can extend the duration of anestrus post-calving in dairy cattle. Discussed here will be the most notable and common causes for postpartum cyclicity failure which include nutrition and energy balance, animal health, genetics, season, farm management practices, and parity of the cow.

Nutrition and the corresponding energy balance play a large role in the ability of a cow to achieve early postpartum cyclicity. Once a cow has given birth to her calf, there is a 4 to 6 x increase in dry-matter intake (DMI) needed to meet energy demands of lactation (Roche et al., 2000). The cow is unable to consume enough DM to meet the requirements of

early lactation so the cow has to mobilize energy stores in its body and partition that energy into milk production. Using body fat as an energy source lowers BCS, and the cow usually enters a negative energy balance (NEB) at the same time she is expected to resume ovarian cyclicity (Royal et al, 2000). A NEB affects postpartum resumption of ovarian function through suppression of both GnRH concentrations and LH pulse frequency (Zurek et al, 1995). Prolonged NEB is not only associated with low LH frequency, it also results in smaller diameter of the DF, lower IGF-I, and increased non-esterified fatty-acids. All of these changes lower estradiol production thereby preventing ovulation of the DF. Higher milk production in early lactation has been associated with longer intervals to luteal activity, lower likelihood of estrous expression and successful pregnancy due to the larger increase in energy demand, and extended amount of time in NEB seen in high-producing cows (Westwood et al, 2002). A cow will remain anestrus until its NEB begins to return to a positive state, so the severity and duration of the NEB is negatively correlated to estradiol production and resumption of postpartum cyclicity (Roche and Diskin, 1999).

Health is another critical modifier of when reproductive functionality resumes in the postpartum cow. Anytime a dairy cow is in a diseased or sickened state, energy is being diverted from regaining reproductive ability, as well as production, to combating the illness or malady. Body condition is a visual indicator of dairy cow's health and has been shown to affect the anestrous interval. Rhodes et al. (1999) reported a negative correlation between postpartum interval and BCS at calving in pasture systems such that cows with higher BCS return to ovarian functionality sooner than those at low BCS. This is because the animal has more body stores to use after calving and such cows do not experience as great a NEB as those that do not have the extra available energy. However, excessive body condition

increased incidence of fatty liver, decreased DMI, and negatively affected return to cyclicity and subsequent fertility (Roche et al, 200).   Lameness decreases reproductive performance by prolonging the anestrous interval, increasing the number of inseminations per conception, and reducing pregnancy rates. Mastitis affects postpartum cyclicity similarly to lameness in that it extends the anestrous interval (Petersson et al, 2006). Reproductive abnormalities such as cystic ovaries and retained placenta may also delay or prevent uterine involution as well as prolonging the postpartum anestrous period. Cystic ovaries are present in approximately 10% of dairy cows in the US, and its incidence is increased in high producing cows (Lucy 2001). Follicular cysts are formed when DFs either do not ovulate or fail to regress or therefore continue to secrete estradiol. The inability of the DF to ovulate or regress prevents further postpartum ovarian function until it eventually regresses (Roche and Diskin, 1999). Retained placentas delay uterine involution and therefore return to luteal activity. Retention of the placenta also increases the likelihood that the cow will become ketotic, further delaying resumption of reproductive processes (Roche et al, 2000).

Multiple studies have shown that higher genetic merit cows have greater risk of reproductive failure due to their selection for greater production and that the genetic correlation between yield and reproduction is a negative one (Veerkamp et al., 1999; Darwash et al, 1997). Also, cows of high genetic merit are less likely to show signs of estrus at first ovulation (Westwood et all, 2002). A study by Veerkamp et al (1999) noted high genetic correlations between yield traits and return to luteal activity which were equivalent to correlations between yield and fertility. The heritability for commencement of luteal activity was greater ($h^2 = 0.13$ to 0.28) than those for traditional fertility measures such as days open, calving interval, and services per conception ($h^2 < 0.09$) (Darwash et al, 1999). Due to only

50% of variation for return to luteal activity being explained by genetic variation in yield, the possibility of selection for both luteal activity and milk production is still possible (Veerkamp et al, 1999). Energy balance, BCS, and live weight had positive genetic correlations with the return to cyclicity, such that selection for those measures may prevent further decline in fertility without negative impact on yield (Royal et al, 2000).

Management practices on the farm contribute largely to the length of time in which a cow will return to luteal activity. The form of housing used on the farm impacts anestrous intervals. Intervals to first ovulation are longer for cows kept in tie-stalls than those in free-stalls and longer for cows kept in confinement versus those on pasture. Fertility is also lower for cows in confined spaces compared to those in more open areas (Petersson et al, 2006). Larger farms tend to have longer intervals of postpartum anestrus and lower conception and pregnancy rates than smaller farms. This is most likely due to increase in time needed for estrous detection and amount of labor required on large dairy farms (Lucy, 2001). Estrous detection practices greatly determine subsequent number of services and conception rates with efficiency of detection increasing with detection frequency and use of aids like tail chalk, etc (Roche et al, 2000; Roche and Diskin, 1999). Insemination at spontaneous estrus requires fewer services per conception and produces higher pregnancy rates than inseminations resulting from timed AI procedures (Lucy, 2001). Also, the administration of additional hormones (i.e. bST) negatively impact reproductive performance by increasing milk yield (Lucy, 2001).

Season and parity are additional factors that can affect when reproductive activity will resume. The time until resumption of cyclicity is greatly affected by season. Petersson et al (2006) reported that cows calving in the winter season had longer postpartum intervals than

those calving in other seasons in Sweden, while Darwash et al (1997) noted spring calving

cows tended to have longer intervals until first ovulation. Both studies agreed that cows

calving in summer and fall had shorter anestrous intervals, with the Darwash et al study

showing fall calving cows with the shortest interval of all 4 seasons. Conflicting reports on

the effect of parity on postpartum anestrus are present. According to Lucy (2001) and

Petersson et al (2006), first parity cows had prolonged intervals to the start of postpartum

cyclicity compared to later parities. This delay in cyclicity is attributed to more severe NEB

experienced by first lactation cows. Darwash et al (1997), however, reported that the time

until first postpartum ovulation increased linearly with increasing parity.

Obtainment of Puberty

Puberty in heifers is a culmination of a long and gradual process that begins before

birth and continues until the animal is sexually mature (Sejrsen, 1994). The onset of puberty

can be defined as the age at first estrus, age at first ovulation, or age at which pregnancy can

be supported without any deleterious effects (Senger 2nd ed, 2003). To meet any of these

definitions, a heifer must have fully developed reproductive organs and functioning hormonal

signaling.

The reproductive system is the last major organ system to develop. Once the tissues

of the reproductive organs are fully developed, the hormonal regulation of puberty may begin

(Nakada et al, 2000). Secretion of GnRH from the hypothalamus is the fundamental

requirement for production of the gonadotropins responsible for the onset of puberty (Senger

2nd edition, 2003). During early growth and development, GnRH is secreted in low "tonic"

levels and at a low frequency. Just before puberty, full neural activity of the GnRH "surge

center" in the hypothalamus is achieved when the tonic center begins increasing GnRH production. This increase in GnRH from the tonic center is produced by a decrease in estradiol sensitivity to the low but continual estradiol production from the maturing ovaries. This increase in GnRH stimulates an increase in FSH and LH release (Senger 2nd ed, 2003).

With FSH and LH secretions from the anterior pituitary, the interaction between reproductive hormones and reproductive tissues has become sufficient enough to initiate puberty. Both FSH and LH begin to stimulate more follicles and their growth, creating greater release of estradiol by the ovaries (Nakada, et al, 200). Through positive feedback, estradiol increases GnRH which increases LH levels until estradiol concentrations are high enough to produce a GnRH surge. This preovulatory GnRH surge triggers an LH surge that initiates a heifer's first ovulation (Senger 2nd ed, 2003; Nakada et al, 2000).

Factors affecting age at puberty

Age at puberty is an important production trait for dairy heifers, especially if they are to be bred to calve at 24 mo of age. Calving by 24 mo is required for maximum lifetime productivity in both beef and dairy heifers (Patterson et al, 1992). Age at puberty can range anywhere from 8 to 24 mo depending on breed, nutrition, environment, management and other factors that may be unknown. On average, Holstein and Jersey dairy heifers reach puberty at 9 to 11 mo of age, with larger breeds generally taking longer to reach puberty (Sejrsen, 1994). A certain body size is also required for the onset of puberty to occur in addition to organ and hormonal competency. A heifer has to be large enough to gestate and give birth to a calf as well as have an optimal level of body energy stores to meet the demands of gestation and subsequent lactation (Senger 2nd ed, 2003). The required weight

to reach puberty is approximately 40% of the adult weight for dairy heifers (Hafez 4th ed, 1980).

Numerous factors determine when heifers will achieve puberty. Genetics, nutrition, season, and health are just a few that will be discussed here. The breed of the heifer will influence when it reaches puberty. As already mentioned, larger breeds of cattle take longer to reach the minimum weight required to become pubertal than smaller breeds. Laster et al (1976) crossed Hereford and Angus dams with Hereford, Angus, Jersey, South Devon, Limousin, Charolais, and Simmental sires. Age and weight at onset of puberty were evaluated for heifers of each cross. Although lightest at puberty, Jersey crosses reached puberty earlier and at a lighter weight. Charolais, Limousin, and South Devon crosses were the heaviest heifers at puberty, as well as the oldest at onset. Baker et.al. (1988) examined relationships among puberty and growth characters for diallel mating of Angus, Brahman, Hereford, Holstein and Jersey. They reported that Jersey heifers and Jersey crossed heifers were the youngest and lightest at puberty compared to all other breeds and crosses.

Heifers experience a seasonal effect for onset of puberty. Heifers born in the fall are younger at puberty than those born in the spring. Schillo et al. (1983) reported that effects of seasons are more pronounced during the second 6mo of life. Angus X Holstein heifers were reared under natural conditions until 6 mo of age, after which time they were reared in environmental chambers used to simulate all four seasons of the year. Heifers born in September reached puberty at a younger age than those born in March and exposure to Spring to Fall conditions hastened the onset of puberty, versus those exposed to Fall to Spring conditions.

Heifers must be in good health to reach puberty in a timely manner. Illness and disease slow growth and can impair reproductive development and disrupt endocrine regulation (Sejrsen , 1994). Sick or diseased heifers have lower weight and tend to have smaller mature weights and size. Due to this and the energy required to combat sicknesses, puberty in such heifers is at an older age than average (Patterson et al, 1992). Stress, including heat stress will also increase age at puberty and lowers the immune system, increasing the risk of infection or illness.

Nutrition is by far the most critical factor affecting the age of onset of puberty in dairy heifers. A good plane of nutrition is required for heifers to reach the optimal weight when onset of puberty can occur and to develop suitable fat stores of energy (Patterson et al, 1992). Macdonald et al (2005) examined the effects of feeding to achieve 3 different growth rates in Holstein and Jersey heifers. In this study, heifers that received the high feed allowance were heavier, taller, and had larger heart girth circumferences than the other growth rates. These animals also had younger ages at puberty than the slower developing heifers. All heifers reached puberty at similar BW whithin breed. The same was seen by Sejrsen (1994) with and increase in growth rate from 400g/d to that of 850g/d decreased age at first estrus from 16.6 mo to 8.4 mo.

Patterson et al (1992) reported lower planes of nutrition delayed the onset of \puberty by inhibiting maturation of the endocrine system. Also contributing to the increased age at puberty was the inability to allocate energy into fat deposits. An excess of feed will decrease age at puberty but may have deleterious effects on future production such that accelerated prepubertal growth reduced mammary development due to fat deposits in the mammary

glands impairing milk secretary cell development (Macdonald et al, 2005; Luna-Pinto and Cronje, 2000).

Leptin

Leptin was discovered in 1994 by J. M. Friedman and colleagues at Rockefeller University in the *ob/ob* mouse, a genetic line of obese mice. It is a 16 kDa glycosolated protein hormone, 126 amino acids in length, produced by adipose tissue (Wiesner et. al, 1999). The level of leptin present in circulation is positively correlated to the amount of body fat stored and adipocyte size. Secretion of leptin occurs in a diurnal, circadian rhythm, with pulses taking place at different times of the day depending on species (Ahima et al, 2000, Weise et al, 1999).

Once synthesized in the adipose tissue, it is released into the bloodstream and transported to other parts of the body to cause its effects. Leptin is able to cross the blood-brain barrier and thus have nueroendocrine effects within the nervous system as well as general endocrine effects in peripheral tissues (Flier, 1998). Leptin has been implicated in the regulation of feeding and energy balance, stress response, reproductive development and maintenance, as well as a neuroendocrine response to starvation (Ahima et. al, 1998). Plasma leptin levels may also be influenced by gender, changes in energy balance, and other hormones (Wiesner et al, 1999).

Leptin effects on reproduction

The effects of Leptin on reproduction were first seen in the *ob/ob* mouse. *Ob/ob* mice are not only obese, they also suffer from sterility and infertility. Administration of

exogenous Leptin was found to restore puberty and reproductive function in *ob/ob* mice, and accelerate puberty in the wild-type mouse (Ahima and Flier, 2000). From this, it was hypothesized that leptin was acting as a metabolic signal to initiate the onset of puberty and may be necessary for maturation of the reproductive system (Williams et al, 2002).

Leptin has been shown to be circulating at high levels a few weeks after birth, after which it remains at a fairly constant level (Block et al, 2003). A rise in Leptin concentrations then occurs in the months just before reaching puberty. The prepubertal increase in Leptin is linear and closely associated with growth and BW (Garcia et al, 2002b). As previously discussed, age at puberty is largely dependent on BW and plane of nutrition of the heifer (Block et al, 2003). The same relationship seems to hold for Leptin.

Nutritionally, feed or dietary energy restrictions have been shown to decrease Leptin levels. Heifers receiving inadequate energy from their feed have lower fat stores and would therefore produce less Leptin. These heifers also weigh less at the same age and grow slower than better fed animals (Block et al, 2003; Luna-Pinto and Cronje, 2000). Heifers receiving good nutrition or high fat feed reached puberty at an earlier age than those getting poor nutrition or low fat feed, but they weighed the same at puberty (Garcia et al, 2002a). Studies done by Garcia et al (2002a) and Williams et al (2002) on heifers concerning puberty found that, while most variation could be associated with BW, Leptin was the most predictive indicator of onset of puberty in the absence of BW, regardless of season in which puberty was reached.

Leptin has also been associated with the estrous cycle and reproduction in mature cattle. Leptin levels are high during pregnancy and decline as energy balance becomes more negative (Liefers et al, 2003). Liefers et al (2003) also noted an association between Leptin

concentrations and first observed estrus; no relationship was seen between Leptin and return to postpartum luteal activity. The relationship between Leptin and first observable estrus strengthens the hypothesis that Leptin plays a role in the estrous cycle. Further supporting this idea are findings from studies conducted by Garcia et al (2002b) in which circulating Leptin levels decreased during the late luteal and early follicular phase of the cycle and increase during the mid-luteal phase.

Leptin may be the metabolic link between available bodily energy and the hypothalamic-pituitary-gonadal axis. As a mediator of nutrition and reproduction, Leptin has been seen to stimulate the release of gonaotropins and inhibit IGF-mediated release of estradiol (Ahima and Flier 2000). This suggests that Leptin is signaling the amount of available energy to the GnRH-gonadotropin system, directly or indirectly, causing enhancement of LH secretion and decreasing the negative feedback sensitivity between estradiol and GnRH (Flier, 1998; Williams et al, 2002).

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CHAPTER 2

BREED DIFFERENCES IN POSTPARTUM CYCLICITY AND FERTILITY OF FALL

CALVING, PASTURE-BASED DAIRY COWS

ABSTRACT

Milk samples were collected from purebred Holstein, purebred Jersey, and Holstein/Jersey crossbred cows twice weekly after calving in both the 2005 (n=150) and on November 20 and 30, December 11 and 21, and January 5 and 16 for 2006 (n=102) calving season. Skim milk samples were analyzed for Progesterone (P_4) to determine when cows returned to cyclicity. Holstein cows weighed the most at dry-off, calving, 30 d and 60 d postpartum (PP), followed by the crossbred cows; Jersey cows were the lightest at each time point ($P < 0.01$). No differences were detected among breed groups in BCS before calving or postpartum ($P = 0.23$). At 30 d, 60 d, and 90 d PP over both calving seasons, Holstein and crossbred cows had greater milk production than Jersey cows ($P < 0.01$) and Energy Corrected Milk (ECM) tended to be higher for Holstein and crossbred cows ($P = 0.06$). A year effect was seen in milk production and ECM such that cows calving in 2006 had higher production and ECM values than those that calved in 2005 ($P < 0.05$).

Fewer Holstein cows were cyclic at 30 d, 60 d, and 90 d PP when compared to purebred Jersey and crossbred cows ($P < 0.05$). No statistical differences were seen for anestrous interval, but may be present as not all Holsteins had reached cyclicity by 90 d PP and were therefore excluded from calculation of breed anestrous interval. Holstein cows also had lower first service conception rates and 90d pregnancy rates than either Jersey or crossbred cows ($P < 0.05$). Crossbred cows preformed at an equivalent level to Jersey cows for reproductive measures. There was a year effect such that cows calving in 2006 had higher conception and pregnancy rates than cows calving in 2005 ($P < 0.05$).

INTRODUCTION

Declines in the reproductive efficiency of dairy cattle have been reported in countries around the world, including the US. First service conception rates have dropped from 65% in 1951 to 40% in 1996 and are continuing to decrease (Lucy 2001). Washburn et al (2002) reported days to first breeding had increased between 1985 and 1999 from 84d to 100d in dairy herds in the southeastern US; days open increased from 124d to 168d between 1976 and 1999. Services per conception increased to almost 3 services by 1996, whereas detection of estrus declined between 1985 though 1999 (Washburn et al, 2002). This reproductive decline coincides with increasing milk production per cow and the shift to larger herds and confinement dairying (Lucy, 2001). Research has been done to determine what factors may be contributing to the decline in dairy fertility. Genetics, nutrition, season, temperature, parity, farm size and management, health, and other physiological or environmental factors have been implicated (Lucy, 2001).

It has been reported that early postpartum cyclicity in pasture-based dairy cows is associated with higher fertility and increases the likelihood of an earlier insemination (Roche and Diskin, 1999). There is a highly negative correlation between yield traits and return to luteal activity, an early measure of reproductive viability. Cows selected for greater milk production have been shown to have a greater risk of reproductive culling (Darwash et al 1997; Veerkamp et al, 1999). The heritability for commencement of luteal activity was greater (h^2 = 0.13 to 0.28) than heritability estimates for traditional fertility measures such as days open, calving interval, and services per conception (h^2 < 0.09) (Darwash et al, 1997). Rhodes et al (1999) reported a negative correlation between postpartum interval and BCS at

calving in pasture systems such that cows with higher BCS return to ovarian functionality sooner than those at low BCS.

There has also been increasing interest in crossbreeding in US dairy herds in recent years due to concerns regarding female fertility, calving ease, health, longevity of Holsteins, and increased inbreeding levels within all major dairy breeds (Weigel and Barlass, 2003). Crossbred cows seem to be less susceptible to disease, sickness, and reproductive difficulties than their purebred counterparts (Touchberry, 1992). Heins et al (2006b) reported that crossbred cows had significantly fewer days to first breeding, fewer days open, had higher conception rates, and greater survivability at 30 d, 150 d, and 305 d postpartum than purebred Holstein cows. Crossbred animals tended to be equivalent to their purebred counterparts in production traits (Heins et al, 2006c; VanRaden and Sanders, 2003) but have higher fertility and improved reproductive health than purebred Holsteins (Heins et al, 2006a, Cole et al, 2005).

Fertility and reproductive ability have been shown to depend greatly on management practices of the dairy farm. Delay until first postpartum ovulation is longer for cows kept in tie-stalls than those in free-stalls and longer for cows kept in confinement versus those on pasture. This delay is the result of decreased display of estrus and lower estrus detection rates (Petersson et al, 2006; Lucy, 2001). Regulation of reproduction in dairy cattle is also affected by season with cows calving in summer and fall returning to cyclicity sooner than those that gave birth in spring or winter (Darwash et al, 1997; Petersson et al, 2006). Little work has been done examining the effects of interactions between calving season, crossbreeding, and pasturing on resumption of postpartum cyclicity and subsequent fertility

in dairy cows. The purpose of this study was to examine breed differences and the effects of crossbreeding on postpartum cyclicity and fertility of fall calving, pasture-based dairy cows.

MATERIALS AND METHODS

Animals and Treatments

This experiment was conducted at the Dairy Unit of the Center for Environmental Farming Systems (Goldsboro, NC). During the fall calving season in 2005, freshened cows (n=150) were sampled consisting of 46 purebred Holstein, 50 purebred Jersey, and 54 various Holstein - Jersey crossbreds. The breed make up of crossbreds was 1/2 Holstein/Jersey (n=28), 1/4 Holstein (n=6), 3/4 Holstein (n=13), 5/8 Holstein (n=6) and 3/8 Holstein (n=1), for an average breed make-up of 54.4% Holstein for crossbred cows. Sampling was conducted again in the 2006 calving season (n= 102) with 24 Holstein cows, 34 Jersey cows, and 44 Holstein-Jersey cows. The breed make-up of the crossbred cows was 1/2 Holstein/Jersey (n=21), 1/4 Holstein (n=7), 3/4 Holstein (n=12), and 3/8 Holstein (n=4), for an average breed make-up of 51.7% Holstein for crossbred cows.

The cows were maintained on pasture with water ad libitum and received supplemental feed before milkings. 0A portion of the cows in the 2005 sampling were part of an ongoing trial examining the effects of high and low stocking rates. The high stocking rate was 3.7 cows/ha with 1.5x supplementation of 6 to 12 kg of concentrate per head per day. The low stocking rate was 2.5 cows/ha with 1x supplementation of 4 to 8 kg of concentrate per head per day. Amounts of concentrate varied depending on quantities and quality of pasture or round bale haylage (fed when pasture was limited or unavailable) and consisted of ground corn, whole cottonseed, soybean meal, and minerals. When lush pasture

was available the relative proportion of soybean meal in the supplement was reduced as were total amounts of concentrate. There were also cows included in this study that were not part of the stocking rate trial. Those cows received pasture plus a corn silage-based TMR during late fall and winter.

Sample collection

Animal weights and BCS were taken by farm personnel at dry-off, calving and monthly thereafter in the 2005 calving season. No weights or BCS were taken in the 2006 calving season. Milk production per cow and Energy Corrected Milk (ECM) were measure on the farm's monthly test day in both the 2005 and 2006 calving seasons.

Milk samples were collected twice weekly from late October 2005 to the beginning of February 2006 for the 2005 calving season. Samples were collected again on November 20 and 30, December 11 and 21, and January 5 and 16 for the 2006 calving season. Samples were taken during the PM milking of volumes at least 25 ml per sample. All samples were centrifuged at 3000 RPM at 4°C for at least 15 min to obtain the skim milk portion. The skim milk was stored frozen at -20°C until analysis for Progesterone (P_4) concentrations to determine the onset of postpartum cyclicity. Return to cyclicity was defined for this study as the first day P_4 levels were at least 1 ng/ml for 2 consecutive samples or 2ng/ml for one sample. Length of anestrous was calculated for cyclic cows only in the 2005 sampling as the length from calving until the 1st day P_4 levels were at least 1 ng/ml.

Hormone Concentration Analysis

Analysis of P_4 consisted of a Coat-a-Count solid-phase radioimmunoassay (Diagnostic Products, Los Angeles, CA). The standard curve was determined from seven points (0, 0.1, 0.5, 2, 10, 20, and 40 ng/ml) in duplicate. A skim milk sample of 100 µl

volume and a positive control from a known pregnant cow were pipetted into provided Progesterone Ab-Coated tubes. Next, 1 ml of [125]I Progesterone was added to all tubes. Tubes were vortexed and then incubated at 25°C for 3h. After the incubation period, the supernatant was decanted. The tubes were then blotted and allowed to dry overnight. The antibody-bound fraction of the ^{125}I-Progesterone was quantified in a gamma counter for 1 min. Samples were assayed in duplicate and interassay CVs were between 5.6 and 9.7%.

Statistical Analyses

Categorical data, i.e. cyclicity percentages, conception and pregnancy rates, were evaluated using Frequency Tables and contingency Chi-square (SAS, Cary, NC). The GenMod procedure with type 3 Sums of Squares was also used to verify the accuracy of the Chi-square analysis. Due to the time frame in which sampling took place in this study, not all cows were included in each postpartum time point. Cows were only included in postpartum cyclicity time points for which they reached within the sampling time frame (late October to early February in the 2005 calving season and November 20 through January 16 for the 2006 calving season). Cows that reached 30 d or 60 d PP before the onset of sampling were not included in 30 d or 60 d cyclicity calculations. Cows that did not reach 60 d or 90 d PP were excluded from 60 d or 90 d cyclicity calculations.

The General Linear Model (GLM) procedure (SAS, Cary, NC) was used to analyze anestrous interval, milk production, ECM, BW and BCS at dry-off, calving, 30, 60, and 90 d postpartum and form LSMeans for these variables. In the preliminary model, breed, parity, year and stocking rate effects were tested for significance as well as all two-way and three-way interactions. Stocking rate had no influence on the other variables and was therefore omitted from the model. The final model contained significant effects for breed, parity, and

year. The model used to evaluate anestrous interval, milk production, ECM, BW and BCS was:

$$Y_{ijk} = \mu + B_i + P_j + S_k + BP_{ij} + BS_{ik} + BPS_{ijk} + E_{ijkl}$$

where:

Y_{ijk} = Anestrous interval, milk production, ECM, BW and BCS

μ = Mean (Y_{ijk})

B_i = Effect of ith breed group

P_j = Effect of jth parity

S_k = Effect of kth year (2005, 2006)

BP_{ij} = Effect of ith breed and j^{th} parity

BS_{ik} = Effect of ith breed and k^{th} year

BPS_{ijk} = Effect of ith breed, j^{th} parity, and k^{th} year

E_{ijk} = Residual

Tukey-Kramer Multiple Comparison tests were used to compare Least Squares Means (LSM) for anestrous interval, milk production, ECM, BW and BCS.

RESULTS

Weight and BCS

Similar patterns of weight loss and gain from dry-off to 60 d postpartum were seen for all breeds (Figure 1). Holstein cows had more pronounced weight changes over time than the Jersey or crossbred cows, however, these weight fluctuations were not significantly different by breed group when evaluated as a factor of BW ($P = 0.16$). At dry off, Holstein cows weighed 612 ± 14.5 kg, Jersey cows weighed 433 ± 13.8 kg, and crossbred cows

weighed 558 ± 14.3 kg. BW decreased at calving to 570 ± 13.9 kg, 403 ± 13.3 kg, and 518 ± 13.7 kg for Holstein, Jersey and crossbred cows respectively. Weight loss continued into 30 d postpartum with Holstein cows weighing 500 ± 11.6 kg, Jersey cows weighing 354 ± 12.6 kg, and crossbred cows weighing 456 ± 10.9 kg. By 60 d postpartum all breeds began to regain weight such that Holstein cows weighed 533 ± 12.3 kg, Jersey cows were 62 ± 11.5 kg, and crossbred cows weighed 479 ± 15.1 kg. At all 4 times, Jersey cows weighed significantly less when compared to either Holsteins or crossbred cows ($P < 0.01$). Crossbred cows had weights statistically similar to purebred Holstein cows.

Parity had a significant effect on BW at all 4 times ($P < 0.01$; Figure 2). Third and greater parity cows were significantly heavier than second parity cows which were significantly heavier than first parity cows at all 4 time points ($P < 0.01$). At dry-off, third and greater parity cows weighed 596 ± 11.2 kg vs 528 ± 17.4 kg for second parity cows vs 464 ± 13.3 kg for first parity cows. At calving, later parity cows decreased in weight to 553 ± 10.7 kg vs 494 ± 16.7 kg for second parity vs 431 ± 12.8 kg for first. By 30 d postpartum, weights had decreased to 509 ± 11.8 kg for later parity cows, 465 ± 14.0 kg for second parity cows and 386 ± 8.7 kg for first. Cow weights had increased with third and greater parity cows weighing 505 ± 10.1 kg, 453 ± 14.5 kg for second parity, and 379 ± 14.2 kg for first. Similar changes in BW were seen across all parities. There was not a significant breed by parity interaction ($P = 0.62$).

No differences in BCS were seen among breed groups ($P = 0.23$; Figure 3), although there was a tendency ($P = 0.06$) for Holstein cows to have a higher BCS than Jersey cows at dry-off and 30 d postpartum. Little change was seen regarding BCS from dry-off to 60 d postpartum) for Holstein, Jersey, and crossbred cows (2.86 to 2.65, 2.77 to 2.60, and 2.81 to

2.63 respectively) but all 3 breed groups had a similar pattern of change in BCS. There were not differences in BCS between first, second or third and greater parity cows ($P = 0.63$; Figure 4). All parities experienced similar and small changes in BCS from dry-off through 60 d postpartum (2.79 to 2.61, 2.85 to 2.63, and 2.82 to 2.64 respectively). The breed by parity interaction for BCS was not statistically significant ($P = 0.91$).

Milk production and ECM

Breed differences were seen in milk production at 30 d, 60 d and 90 d postpartum in the 2005 calving season (Table 1). Holstein cows produced more milk than either Jersey cows or crossbred cows. This difference was only significant at 30 d and 60 d for Holstein versus Jersey cows and 60 d versus crossbred cows ($P < 0.05$, $P < 0.01$ respectively). All breeds had similar production when ECM values were incorporated. Across all breeds and at all time points, primiparous cows had lower milk production and ECM than multiparous cows ($P < 0.01$).

During the 2006 calving season , Holstein cows and crossbred cows had equivalent milk production levels at 30 d, 60 d, and 90 d postpartum (22.8 kg, 27.8 kg, and 31.0 kg; 21.0 kg, 24.4. kg, and 27.5 kg respectively; Table 2). Both breeds produced more milk than Jersey cows at all time points (15.5 kg, 19.1 kg, and 20.4 kg; $P < 0.01$). The same was seen for ECM at all 3 postpartum time points to a lesser extent ($P = 0.03$, $P = 0.04$, and $P = 0.05$ respectively). Multiparous cows produced more milk and had higher ECM values than primiparous cows at 30 d, 60 d, and 90 d postpartum ($P < 0.01$).

When summarized over both 2005 and 2006 calving seasons, breed differences were apparent at 30 d and 60 d postpartum time points for both milk production and ECM values

(*P* <0.01; Table 3). Holstein and crossbred cows performed at similar production levels and at a greater level than Jersey cows. At 90 d postpartum, Holstein and crossbred cows had higher production and ECM but it was not statistically significant (*P* = 0.06). Primiparous cows had lower milk production and ECM than multiparous cows across all breeds and time points (*P* < 0.01). When examining both calving seasons, a significant year effect (*P* = <0.05) was observed with all breed groups having higher milk production and ECM values in the 2006calving season than in 2005.

Cyclicity

For the 2005 calving season, more Jersey and crossbred cows were cyclic by 30 d, 60 d, and 90 d postpartum (P < 0.05; Figure 5). At 30 d, 21.9% of Holstein cows (7 of 32), 43.2% of Jersey cows (16 of 37) and 42.5% of crossbred cows (17 of 40) were cyclic. At 60d, percentage cycling increased to 58.9% for Holstein cows (23 of 39), 94.1% for Jersey cows (32 of 34), and 95.1% for crossbred cows (39 of 41). By 90 d postpartum, only 26 of 34 Holstein cows (76.5%) had initiated cycles compared to 29 of 30 Jersey cows (96.7%) and all 33 crossbred cows (100%).

In the 2006 calving seasons, breed differences were only seen at 90 d postpartum (*P* < 0.05; Figure 6). 100% of Jersey cows (24 of 24) and 97.4% of crossbred cows (37 of 38) had returned to cyclicity whereas 83.3% of Holstein cows (15 of 18) were cycling. More Jersey and crossbred cows were cyclic at 30 d (15 of 30 and 23 of 44) and 60 d postpartum (30 of 34 and 38 of 44) than Holstein cows (9 of 22 and 18 of 24, respectively), but differences were not statistically significant (30d - *P* = 0.68, 60d - *P* =0.34).

Breed differences in postpartum cyclicity were seen at 30 d, 60 d, and 90 d when summarized across both years ($P < 0.05$; Figure 7). At each time point, significantly fewer Holstein cows (31.4%, 67.0%, and 79.9%) were cyclic compared to Jersey cows (46.6%, 91.2%, and 98.4%) and crossbred cows (47.4%, 90.8%, and 98.7%). Differences were more significant at 60 d and 90d postpartum ($P < 0.01$ and $P = 0.01$) than at 30 d postpartum ($P = 0.03$). No statistical differences were observed for the effect of parity on return to cyclicity (Figure 8). There was no breed by parity interaction at any postpartum time point.

No significant differences were found regarding mean anestrous intervals between breeds for the 2005 calving season ($P = 0.19$). The means for estimated days to first ovulation for each breed are as follows: 35.8 ± 2.8d for Holstein cows, 31.2 ± 2.1d for Jersey cows, and 33.7 ± 1.9d for crossbred cows. This may not be reflective of true anestrous interval as 23% of Holsteins (8 cows) were not followed all the way until they started cycling; sampling stopped once the breeding season started. Parity did not have an effect on anestrous interval either. The interval for first parity cows was 35.9 ± 2.1d, 29.5 ± 2.5d for second parity cows, and 35.2 ± 1.9d for third and greater parity cows ($P = 0.11$). No breed by parity interaction was seen regarding postpartum anestrous intervals.

Fertility Measures

Lower first service conception and 90 d pregnancy rates were seen for Holstein cows compared to Jersey and crossbred cows for the 2005 calving seasons ($P < 0.05$; Figure 9). For 1st service, Holstein cows had a 34.4% (11 of 32) conception rate while 61.4% (27 of 44) Jersey cows and 53.7% (22 of 52) crossbred cows conceived from their first insemination. By 90 d of the breeding season, only 58.8% (23 of 39) of Holstein cows were confirmed

pregnant by rectal palpation, whereas 84.1% (38 of 45) of Jersey cows and 83.7% (41 of 49) of crossbred cow were pregnant ($P < 0.05$).

No statistical differences were seen between breeds in the 2006 calving season regarding first service conception or 90d pregnancy rates ($P = 0.14$; Figure 10). While not statistically different, a similar trend was seen for the cows that calved in 2006 as those that calved in 2005. Fewer Holstein cows were able to conceive on their first service compared to both Jersey and crossbred cows (45.8% vs 65.6%, and 63.6% respectively). The 90 d pregnancy rate for Holstein cows (81.8%) was less than that of Jersey cows (96.2%), and crossbred cows were intermediate (87.8%).

When summarized over both calving seasons, fertility differences between breeds were apparent ($P < 0.05$; Figure 11). After first service, 63.5% of Jersey cows and 58.6% of crossbred cows were confirmed pregnant compared to only 40.1% of Holstein cows. By 90 d of the breeding season, 90.1% of Jersey cows and 85.8% of crossbred cows were confirmed to be pregnant compared to 70.3% of Holstein cows. There was a year effect such that cows calving in 2006 having higher conception and pregnancy rates than those cows calving 2005 ($P < 0.05$). Parity did not affect conception on first service or 90 d pregnancy rates ($P \= 0.26$, $P = 0.89$ respectively; Figure 12). No breed by parity interaction was seen for first service conception or 90d pregnancy rates.

DISCUSSION

Weight and BCS

Overall in this study, Holstein cows weighed more than either the crossbred or Jersey cows. However, weight differences between Holstein and crossbred cows were not

significant. These findings agree with those reported by Auldist et al (In Press). In the 4 herds they studied, 2 herds had Holstein cows that were heavier than Jersey/Holstein crossbred cows whereas in the other 2 herds, crossbred cows were not statistically different in weight from purebred Holsteins. Results from Touchberry's Illinois Experiment (1992) show the opposite; Holstein/Guernsey crossbred cows exceeded the weights of either crossbred from 18 mo to 48 mo of age. The differences reported between these 3 studies can most likely be attributed to the breeds used in the crossings. Holstein and Guernsey breeds are larger in size than the Jersey breed, so mating to large animals produces large animals. Auldist et al (In press) did not see differences in BCS between Holstein cows and crosses. BCS change over time did not differ as well, though all cows did lose some condition postpartum (In Press). Similar results regarding BCS were seen in this study. For these 2 studies, BCS and BW were not significant factors in the resumption of postpartum cyclicity.

Milk production and ECM

Holstein cows had the highest milk production, but differences were not significant versus crossbred cows. These results are similar to those found by Auldist et al (In Press). In studies by Heins et al (2006c) and VanRaden and Sanders (2003), Holstein cows produced significantly more milk than their crossbred counterparts. For fat and protein yields, crossbred cows equaled or outperformed purebred Holstein cows (Heins et al, 2006c; VanRaden and Sanders, 2003; Auldist et al, In Press; Touchberry, 1992). In this study, fat and protein production were incorporated into ECM values, which were not different between Holstein and crossbred cows. From this, it can be concluded that milk production

did not contribute a great deal to the suppressed reproductive ability seen in Holstein cows in this study.

Fertility Measures

Heins et al (2006b) and Auldist et al (In Press) report higher first service conception rates for Holstein/Normande, Holstein/Montbeliarde, and Holstein/Scandinavian Red and Holstein/Jersey crossbred cows, respectively, when compared to purebred Holstein cows. Farmers reported higher conception rates for Holstein/Jersey and Holstein/Brown Swiss crosses, almost equal to those of purebred Jersey cows (Weigel and Barlass, 2003). These results are in agreement with breed differences in first service conception rates seen in this study; Crossbred cows had higher conception rates than Holstein cows. However, conception rates in this study were much higher for all breeds than those reported by Heins et al and slightly greater than those of Auldist et al (in press). The findings from this research also concur with those from Westwood et al (2002). Westwood et al noted that cows with prolonged anestrus intervals had lower conception rates by 150d postpartum; such was seen in the current study regarding Holstein cows. Touchberry's Illinois Experiment produced results that were contrary to those of this study and those of Heins et al and Auldist et al; purebreds did not differ from crossbred cows for first service conception rate. However, the data from Touchberry is over 30 years ago and is likely outdated.

Westwood et al (2002) saw a parallel relationship between anestrus and likelihood of pregnancy by 150 d postpartum as was seen between anestrous interval and conception by 150 d postpartum. Crossbred cows maintain pregnancy over time more so than purebred Holstein cows and had lower not-in-calf rates compared to Holstein cows (Auldist et al, In

Press).   These results coincide with those seen in this study; more crossbred cows were pregnant compared to Holstein cows by 90 d of the seasonal breeding season of the research farm.

CONCLUSIONS

Breed differences in postpartum cyclicity were evident in this study. Fewer Holstein cows initiated estrous cycles when compared to Jersey and crossbred cows at 30 d, 60 d and 90 d postpartum. Also, Holstein cows had lower conception and pregnancy rates compared to Jersey and crossbred cows. Crossbred cows were intermediate to the purebreds for production and body weight, but were most similar to Jersey cows regarding reproductive measures. Also, of the major influences on postpartum cyclicity and reproduction examined in this research, only genetics seem to explain the lower cyclicity and reduced fertility seen in Holstein cows in this study. Having reproductive performance similar to Jerseys with milk production levels similar to Holsteins could make crossbred cows more profitable than either purebred. More analyses are needed, however, to determine the relative merits, both economical and practical, of crossbred dairy cows in various dairy systems.

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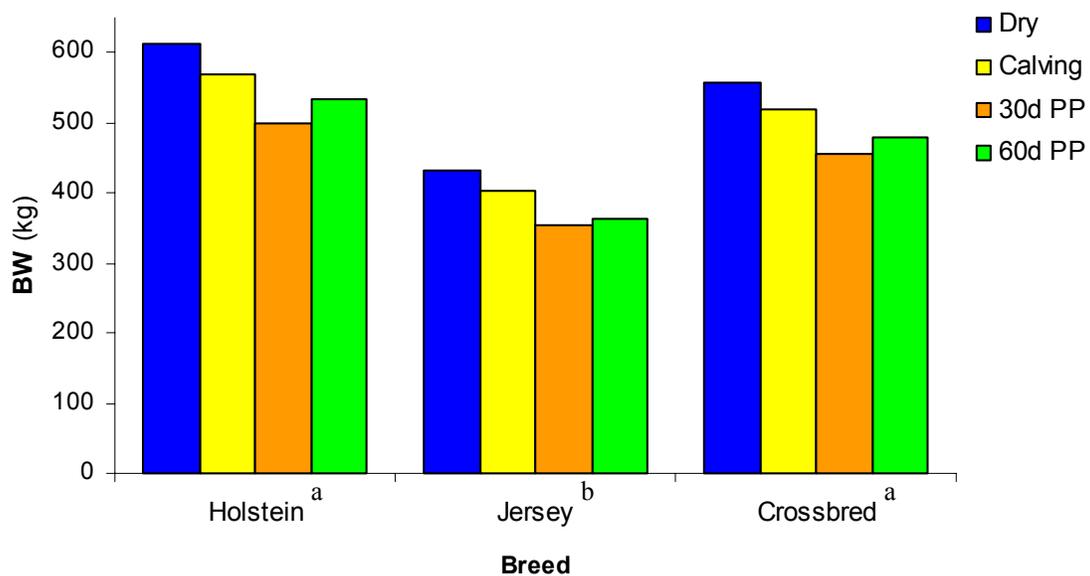
Figure 1. LSMean body weights by breed at dry-off, calving, 30 d and 60 d postpartum. Breed groups with differing superscripts are significant at *P* <0.01. Changes over time were also significant at *P* <0.05. S.E. ranged from 10.9 to 14.5 kg.
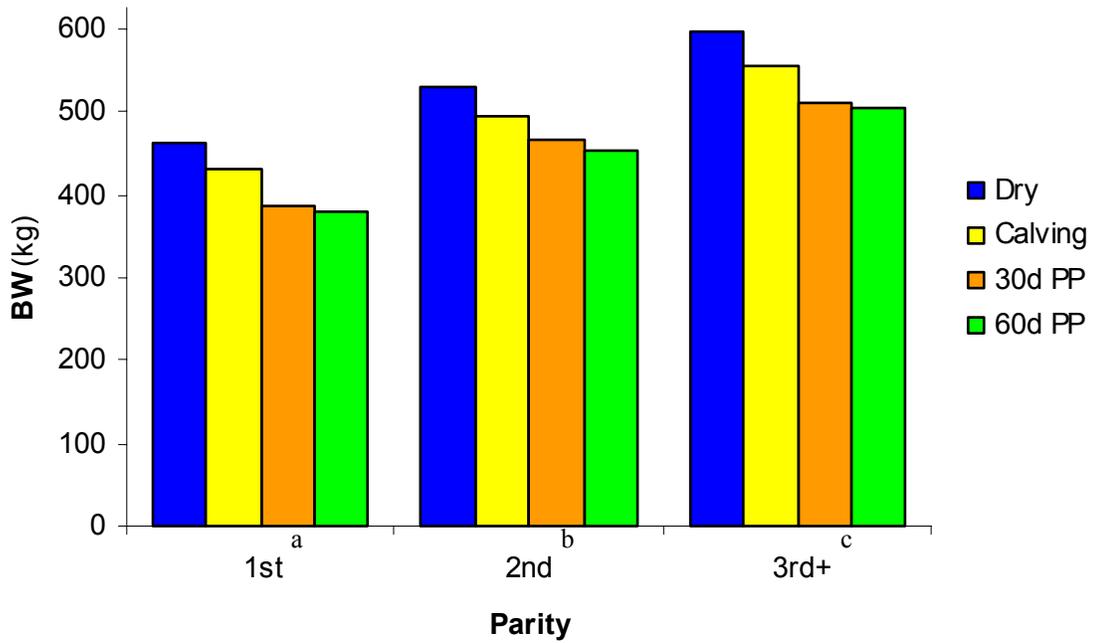
Figure 2. LSMean body weights by parity at dry-off, calving, 30d and 60d postpartum. Differing superscripts are significant at *P* <0.01. Changes over time were also significant at *P* <0.05. S.E. ranged from 8.7 to 17.4 kg.
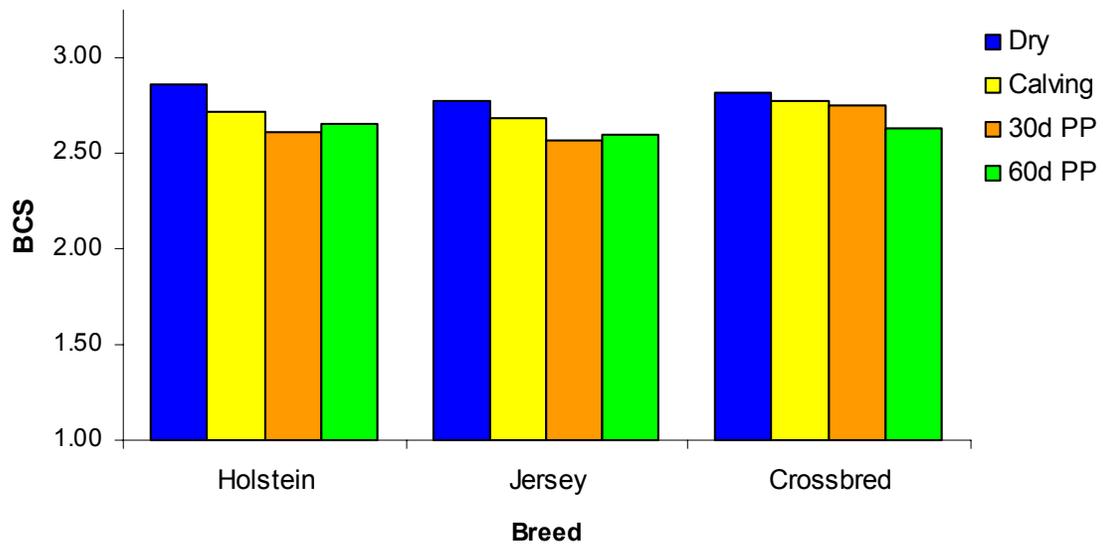
Figure 3. LSMean body condition scores by breed at dry-off, calving, 30d and 60d postpartum. No statistical differences among breed groups were seen. Changes over time were not significant.
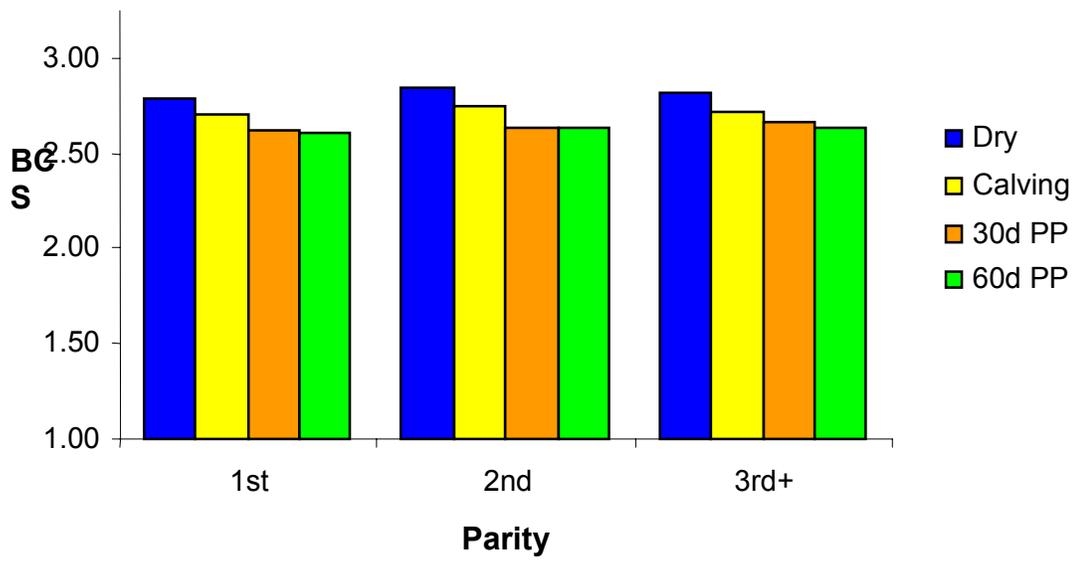
Figure 4. LSMean body condition scores by parity at dry-off, calving, 30d and 60d postpartum. No statistical differences among breed groups were seen. Changes over time were not significant.

Table 1. Milk Production and Energy Corrected Milk (ECM) by Breed Groupat 30, 60, and 90 d Postpartum for 2005 Calving Season

	Holstein	Jersey	Crossbred	SEM	*P*-value
Milk Production (kg)[1]					
30d	24.0[a]	19.4[b]	21.8[ab]	1.2	0.04
60d	23.9[a]	18.4[c]	20.6[b]	0.9	<0.01
90d	24.1	20.5	21.0	1.5	0.17
ECM (kg)[1]					
30d	22.2	19.9	22.3	1.3	0.35
60d	22.3	19.3	20.4	0.9	0.08
90d	23.1	20.6	20.6	1.4	0.35

[a-c] Breed group means with differing superscripts within postpartum period differ (*P* <0.05)
[1] Primiparous cows had lower milk production and ECM than multiparous cows across all breeds (*P* <0.001).

Table 2. Milk Production and Energy Corrected Milk (ECM) by Breed Group at 30, 60, and 90 d Postpartum for 2006 Calving Season

	Holstein	Jersey	Crossbred	SEM	*P*-value
Milk Production (kg)[1]					
30d	22.8[a]	15.5[b]	21.0[a]	1.0	<0.01
60d	27.8[a]	19.1[b]	24.4[a]	1.3	<0.01
90d	31.0[a]	20.4[b]	27.5[a]	1.2	<0.01
ECM (kg)[1]					
30d	25.7[a]	15.8[b]	21.2[a]	1.6	0.03
60d	30.6[a]	21.2[b]	25.4[ab]	1.5	0.04
90d	29.6[a]	24.0[b]	28.7[a]	1.4	0.05

[a-c] Breed group means with differing superscripts within postpartum period differ (*P* <0.05)
[1] Primiparous cows had lower milk production and ECM than multiparous cows across all breeds (*P* <0.01).

Table 3. Milk production and Energy-Corrected Milk (ECM) by breed at 30, 60, and 90 d postpartum across both 2005 and 2006 calving seasons

	Holstein	Jersey	Crossbred	SEM	*P*-value
Milk Production (kg)[1,2]					
30d	23.6[a]	17.4[b]	21.4[a]	0.8	<0.01
60d	25.8[a]	18.7[b]	22.5[a]	0.8	<0.01
90d	27.5[a]	20.5[b]	24.2[c]	1.0	<0.01
ECM (kg)[1,2]					
30d	23.9[a]	17.9[b]	21.7[a]	1.0	<0.01
60d	26.4[a]	20.2[b]	22.9[a]	0.8	<0.01
90d	26.3	22.3	24.6	1.1	0.06

[a-c] Breed group means with differing superscripts within postpartum period differ (*P* <0.05)

[1] Primiparous cows had lower milk production and ECM than multiparous cows across all breeds (*P* <0.001).

[2] Cows in 2006 calving season had higher milk production and ECM than those calving in 2005 season (*P* <0.05).
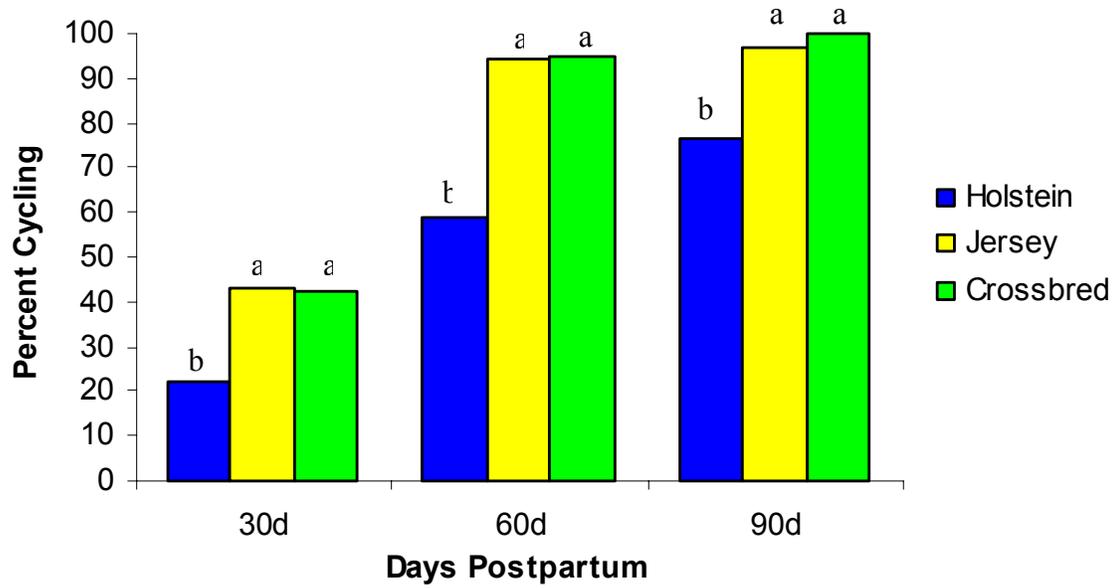
Figure 5. Percentage of cows by breed group that returned to cyclicity by 30d, 60d, and 90d postpartum for the 2005 calving season. Superscripts differ at *P* <0.05.
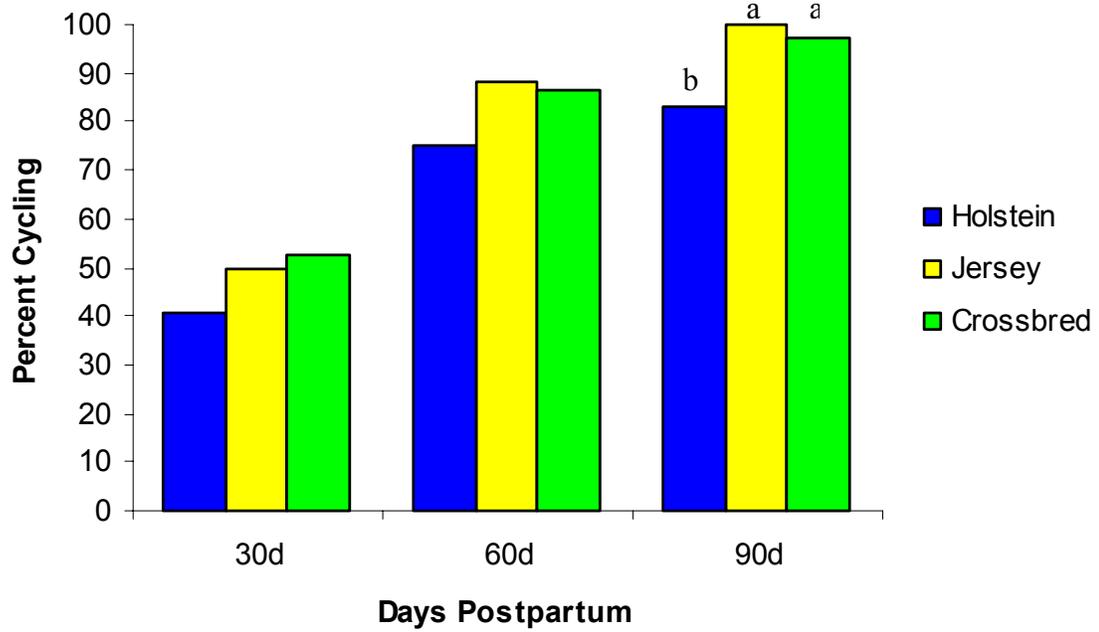
Figure 6. Percentage of cows by breed group that returned to cyclicity by 30d, 60d, and 90d postpartum for the 2006 calving season. Superscripts differ at *P* <0.05.
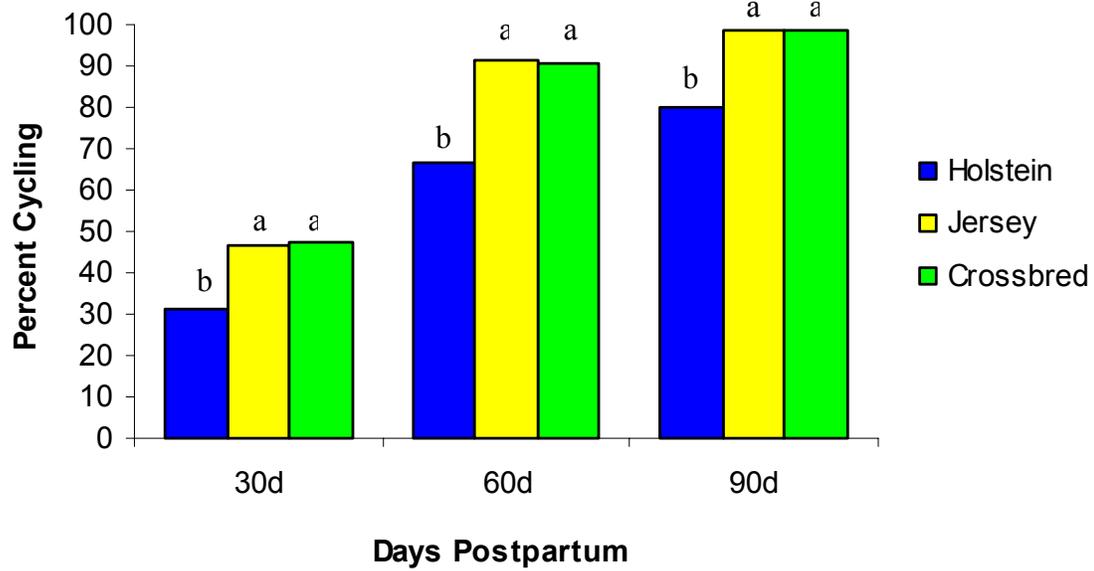
Figure 7. Percentage of cows by breed group that returned to cyclicity by 30d, 60d, and 90d postpartum averaged over both 2005 and 2006 calving seasons. Superscripts differ at *P* <0.05.
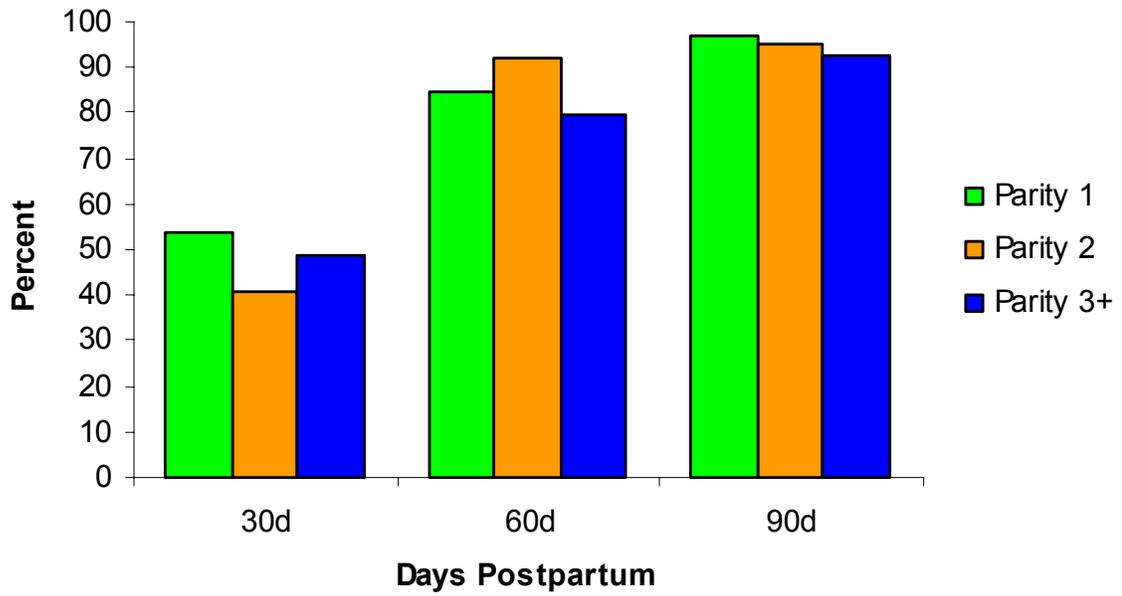
Figure 8. Percentage of cows by parity that returned to cyclicity by 30d, 60d, and 90d postpartum. No statistical differences were detected.
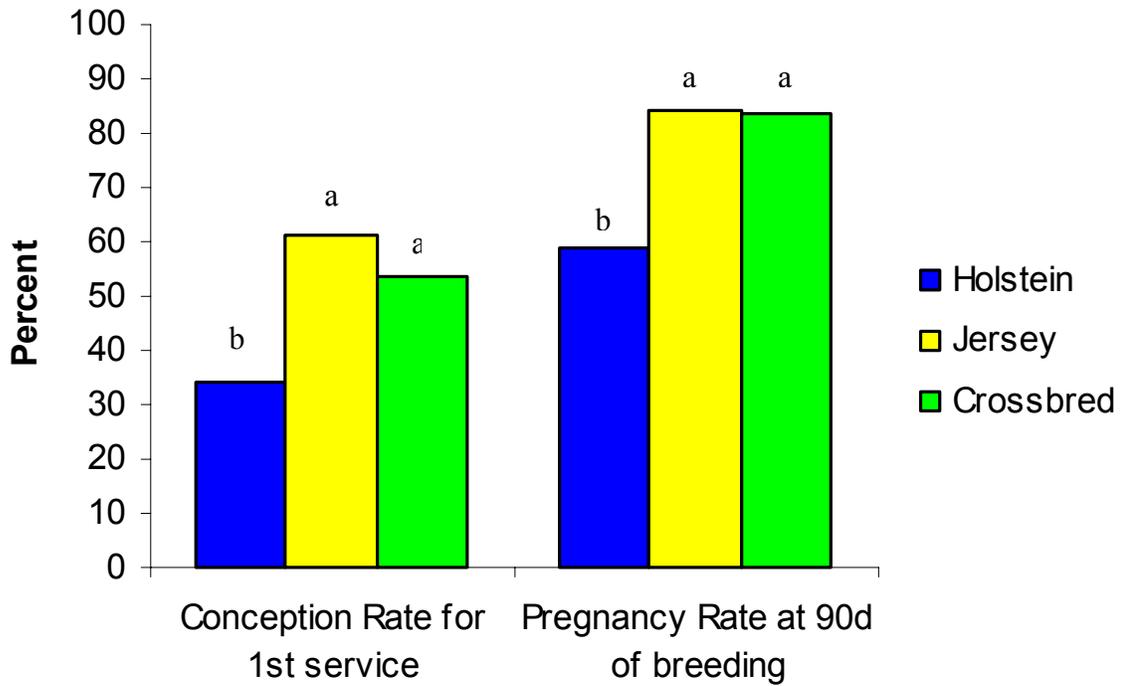
Figure 9. First service conception rate and pregnancy rate at 90d of the breeding season by breed group for the 2005 calving season. Superscripts differ at *P* <0.05.
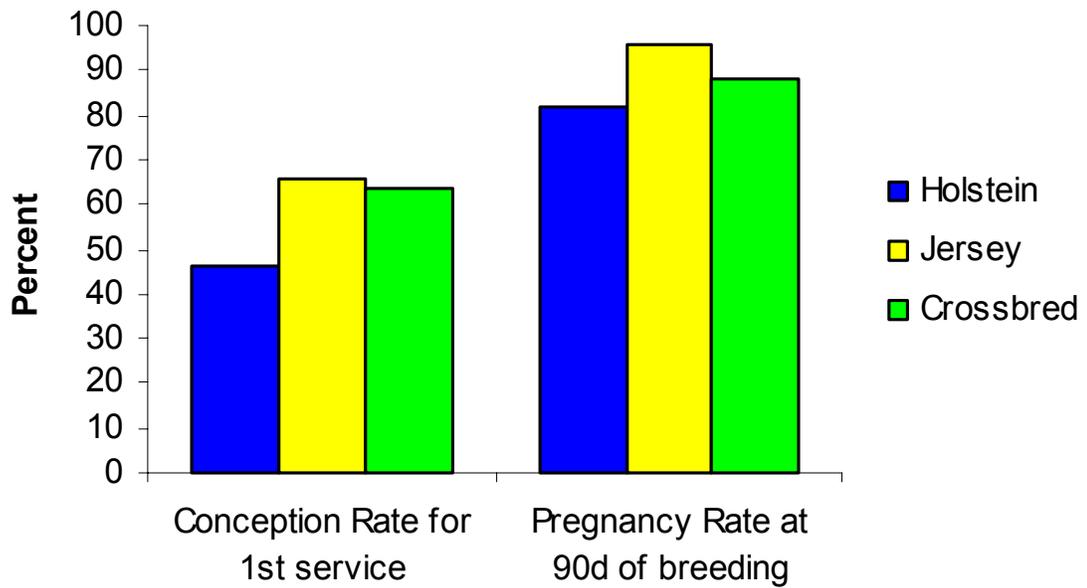
Figure 10. First service conception rate and pregnancy rate at 90d of the breeding season by breed group for the 2006 calving season. No statistical differences were detected.

Figure 11. First service conception rate and pregnancy rate at 90d of the breeding season by breed group for both 2005 and 2006 calving seasons. Differing superscripts are significant at *P* <0.05.
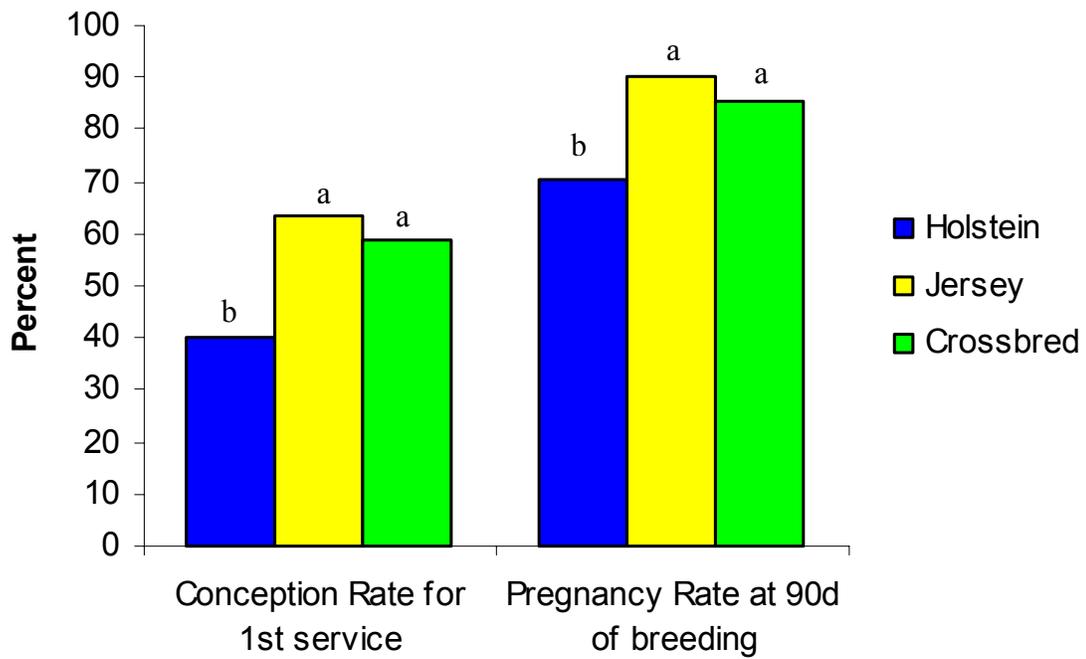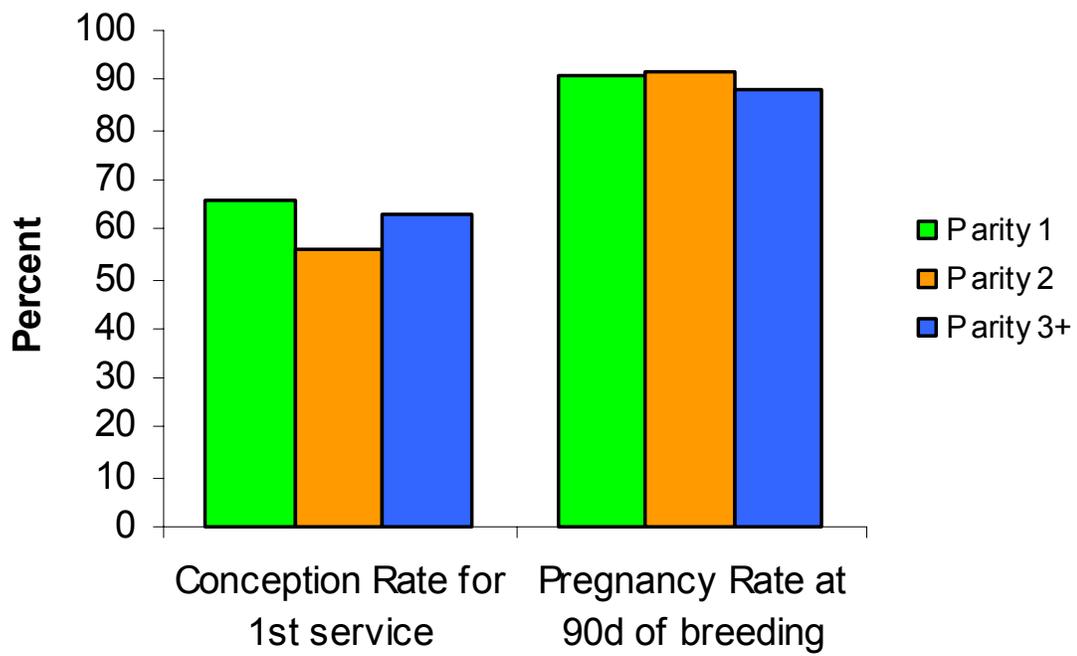
Figure 12. First service conception rate and pregnancy rate at 90d of the breeding season by parity for both 2005 and 2006 calving seasons. No statistical differences were detected.

CHAPTER 3

BREED DIFFERENCES IN PUBERTY OF PASTURE-BASED DAIRY HEIFERS

ABSTRACT

Purebred Holstein, Jersey and Holstein/Jersey crossbred heifers born in the fall of 2005 were sampled weekly from age 4 mo until puberty was reached and serum was analyzed for Progesterone (P_4) to determine age at puberty. Serum samples were also analyzed for the hormone Leptin to elucidate its role in obtainment of puberty. Heifers were categorized as 100% Holstein (100% H), >50% Holstein (>50% H), 50% Holstein/Jersey and Jersey/Holstein (50% HJ), >50% Jersey (>50% J), and 100% Jersey (100% J). Age at puberty was positively and linearly correlated to percent Holstein of a heifer, with the greater percentage Holstein, the older the heifer will be at puberty ($P < 0.01$). Ages at puberty were as follows: 404.2 ± 19.7d for 100% H heifers, 383.6 ± 15.6d for >50% H heifers, 341.0 ± 12.4d for 50% HJ heifers, 309.4 ± 19.7d for >50% J heifers, and 349.9 ± 23.5d for 100% J heifers. 100% H and >50% H heifers were heavier at birth than 100% J and >50% J heifers and stayed heavier as time progressed and at puberty ($P < 0.01$). Heifers that were 50% HJ were intermediate for weight at birth, over time, and at puberty. There was no effect of breed composition on heifer BCS at anytime from 4 mo to 14 mo of age. Differences in wither heights were seen with 100% H heifers being the tallest followed closely by >50% H, and both were taller than 100% J, and >50% J as the heifers grew as well as at puberty ($P < 0.01$). Again, 50% HJ heifers had intermediate wither heights over time and at puberty. No differences in serum Leptin concentrations were seen regarding percentage Holstein of a heifer from 12wk before puberty up to the week of puberty. Changes in Leptin concentrations over time were not significant either. An effect was seen for individual heifer differences ($P < 0.05$), but this effect was not significant as time progressed.

INTRODUCTION

Most of the literature regarding heifer reproduction focuses on first calving and first calving interval. Age at first calving is an important time point in heifer development; a large portion of the total cost of milk production comes from maintaining replacement heifers (Heinrichs, 1993). White reported that first calving in dairy heifers occurred between 24 and 30 mo of age in 1919. Age at first calving has remained fairly constant, averaging about 24 mo in present day, while calving to conception interval for cows has been increasing steadily (Garcia-Peniche et al, 2005). Age at first calving is dependant, however, on heifers reaching puberty, making age a puberty an important reproductive measurement in heifer development (Macdonald et al, 2005).

Limited research has been conducted regarding age at puberty. Age at puberty can range from 8 to 24 mo depending on breed, nutrition, environment, management and a multitude of other factors. For dairy breeds (i.e. Holstein and Jersey), heifers reach puberty at 9 to 11 mo of age, with larger breeds taking longer to reach puberty (Sejrsen, 1994). Even less has been done with the effects of crossbreeding on puberty with the majority of work completed in beef heifers (Laster et al, 1976; Long et al, 1979, and Stewart et al, 1980). It has only been in recent years that crossbreeding and pasturing dairy cattle has become a topic of interest (Wiegal and Barlass, 2003). Macdonald et al (2005) put forth the idea that if larger first calving cows will be raised in pasture-based systems, faster growth rates will need to be achieved to maintain the seasonality of the system. The objective of this study was to evaluate the effects of crossbreeding concerning the obtainment of puberty in dairy heifers in a pasture-based system.

MATERIALS AND METHODS

Animals and Treatments

Prepubertal heifers (n=68) of different breed compositions (10 purebred Holstein, 7 purebred Jersey, and 51 various Holstein/ Jersey crossbred) from the Dairy Unit of the Center for Environmental Farming Systems (Goldsboro, NC) were studied from February 2006 to March 2007. All heifers sampled were born between September 27 and December 27, 2005. The breed composition of the crossbred heifers was 1/2 Holstein/Jersey (n=25), 1/4 Holstein (n=5), 3/4 Holstein (n=9), 5/8 Holstein (n=5), 3/8 Holstein (n=5), and 11/16 Holstein (n=2), for a mean breed composition of 52.6% Holstein for the crossbred heifers. The heifers were rotationally grazed on pasture with water ad libitum. All heifers were given supplemental feed in the morning consisting of haylage, whole cottonseed, corn, and minerals with amount fed varying on pasture availability.

Sample Collection

Heifers were weighed at birth, at the start of the study, February 2006, and at month intervals thereafter. Wither heights and BCS were collected at the start of the study and then monthly until March 2007. All animal measurements were taken by farm personnel.

Blood samples were collected every other week from heifers starting at 5mo of age from February 2006 to April 2006. Weekly samples were taken starting May 2006 and continuing until March 2007. Heifers were no longer collected if two heats were observed or puberty was reached as determined by hormonal assay. Blood was collected via coccygeal venipuncture into 10ml evacuated serum tubes (BD Vacutainer, Franklin Lakes, NJ) and placed on ice. Samples were centrifuged for at least 30 min at 3000 RPM at 4°C. Serum was harvested and stored at -20°C until P_4 analysis for onset of puberty. Puberty was defined for

this study as the first day P_4 levels were at least 1 ng/ml for 2 consecutive samples or 2ng/ml for one sample (Day et al, 1987).

Hormone Concentration Analyses

Progesterone

P_4 analysis consisted of a Coat-a-Count solid-phase radioimmunoassay (Diagnostic Products, Los Angeles, CA). The standard curve was determined from seven points (0, 0.1, 0.5, 2, 10, 20, and 40 ng/ml) in duplicate. Serum samples of 100 µl were pipetted into provided Progesterone Ab-Coated tubes. High and low progesterone concentration controls previously validated in the laboratory were included. Next, 1 ml of [125]I Progesterone was added to all tubes. Tubes were vortexed and then incubated at 25°C for 3 h. After the incubation period, the supernatant was decanted. The tubes were then blotted and allowed to dry overnight. The antibody-bound fraction of the [125]I Progesterone was quantified in a gamma counter for 1 min. Samples were assayed in duplicate. Interassay CVs were between 5.6 and 9.7%.

Leptin

Analysis for Leptin concentrations consisted of a multi-species radioimmunoassay (Linco Research, St. Charles, MO). The standard curve was determined from eight points (0, 0.5, 1, 2, 5, 7.5, 10, and 20 ng/ml) in duplicate. Assay buffer of 100 µl volume was pipetted into 12 x 75 mm borosilicate glass tubes. Serum samples of 100 µl were pipetted into tubes as well as 100 µl of both the high and low value quality controls provided. 100 µl Multi Species Leptin antibody was pipetted into sample and control tubes. Tubes were vortexed, covered and allowed to incubate for 20 to 24 hrs at 4°C. Next, 100 µl of ^{125}I-Human Leptin

was added to all tubes which were then vortexed, recovered, and incubated again for 20-24 hrs at 4°C. A volume of 1.0 ml of 4°C Precipitating reagent was added, and all tubes were vortexed and incubated for 20 min at 4°C. Tubes were then centrifuged at 4°C for 30 min at 3000 RPM. The supernatant was immediately decanted, tubes were allowed to drain for 30 to 60 s and any excess liquid was then blotted from tubes. All tubes were placed in a gamma counter for 1 min to quantify leptin concentrations. Interassay CVs were between 6.5 and 8.7%.

Statistical Analyses

The General Linear Model (GLM) procedure (SAS Inst. Inc., Cary, NC) was used to analyze birth weight, age at puberty, wither height, weight at puberty, and BCS at puberty. Breed (% Holstein) effect was tested for significance for all measures; heifers were grouped together for statistical analysis as 100% Holstein (100% H), >50% Holstein (>50% H), 50% Holstein/ Jersey (50% HJ), >50% Jersey (>50% J), and 100% Jersey (100% J). The model used to evaluate age at puberty, wither height, BW at puberty, and BCS at puberty was:

$Y_i = \mu + B_i + E_{ij}$

where:

Y_i = birth weight, age, wither height, BW, and BCS at puberty

μ = Mean (Y_i)

B_i = Effect of ith breed (% Holstein)

E_{ij} = Residual

Tukey-Kramer Multiple Comparison tests were used to compare Least Squares Means (LSM) for age, wither height, body weight, and BCS at puberty. Linear regression comparing age at puberty to % Holstein was used to evaluate the relationship between breed

and age at puberty. Growth measures were confounded by breed and could not be directly

associated to differences in age at puberty

Statistical analysis of Leptin data consisted of the GLM procedure and was evaluated

from -12 wk to 0 wk prior to puberty. Breed (% Holstein), individual (heifer) effects and

change over time were tested for significance. Wither height and BW were confounded by

breed and could not contribute to differences in Leptin concentrations. The model used to

evaluate leptin levels and log-transformed leptin concentrations was:

$Y_{ijk} = \mu + B_i + H_j + W_k + BW_{ik} + E_{ijkl}$

where:

Y_{ijk} = leptin and log-transformed leptin concentrations

μ = Mean (Y_{ijk})

B_i = Effect of ith breed (%Holstein)

H_j = Effect of jth heifer

W_k = Effect of kth week prior to puberty

BW_{ik} = Effect of ith breed and k^{th} week prior to puberty

E_{ijkl} = Residual

RESULTS

Age at Puberty

Percent Holstein had a statistically significant effect on age at puberty in this study. a

linear correlation existed between percentage Holstein of heifers and how long it took the

animal to reach puberty with the greater the Holstein percentage, the older the heifer will be

at puberty ($P < 0.01$; Figure 13). No differences were seen between 50% Holstein/Jersey and

50% Jersey/Holstein heifers so these animals were grouped together as 50% HJ. The

LSMeans for age at puberty for the heifers are as follows: 404.2 ± 19.7 d for 100% H heifers,

383.6 ± 15.6 d for >50% H heifers, 341.0 ± 12.4 d for 50% HJ heifers, 309.4 ± 19.7 d for

>50% J heifers, and 349.9 ± 23.5 d for 100% J heifers. 100% J and >50% J heifers were

statistically younger at puberty than 100% H and >50% H heifers ($P < 0.01$) while the 50%

HJ heifers were similar to all (Table 4).

Body Weight and BCS

Heifers in the 100% H and >50% H breed groups were heavier at birth than 100% J

and >50% J heifers (33.6 ± 1.8 kg, 31.9 ± 1.5 kg vs 25.5 ± 1.6 kg, 25.4 ± 1.2 kg respectively;

$P < 0.01$). Over time, 100% H and >50% H heifers weighed significantly more than 100% J

and >50% J heifers ($P < 0.01$) except at 5 mo and 6 mo of age (Figure 14). Heifers that were

50% HJ were intermediate to both the 100% H and >50% H heifers and the 100% J and

>50% J heifers at all ages, but were most like the 100% H and >50% H heifers and different

from the 100% J and >50% J heifers from ages 9 mo to 14 mo. At puberty, BW (Table 4)

was higher for 100% H and >50% H heifers (289.5 ± 14.7 kg and 259.9 ± 23.1 kg) than that

of 100% J and >50% J heifers (192.7 ± 19.0 kg and 192.1 ± 10.4 kg; $P < 0.01$). heifers in the

50% HJ breed group were intermediate (241.8 ± 9.5 kg) and not different from all other

heifer groups for BW at puberty.

There was no effect of breed composition on heifer BCS at anytime from 4 mo to 14

mo of age (Figure 15). No statistical change in BCS over time was seen for any group of

heifers either. At puberty, BCS were not different between heifers regardless of percentage

Holstein; heifers had a mean BCS of 2.62 ± 0.04 at puberty.

Wither height

Wither heights were significantly different at all ages from 4 mo to 14 mo ($P < 0.01$; Figure 16). Heifers that were 100% H were the tallest at the withers from 4 mo to 14 mo followed by >50% H heifers. 50% HJ heifers were intermediate for wither heights and not different from 100% H, >50% H heifers, or 100% J heifers. Heifers in the >50% J group were the shortest over time and were significantly smaller than all but 100% J heifers at 4 mo and 11 mo to 14 mo ($P < 0.05$). At puberty, 100% J and >50% J heifers were the shortest at 109.6 ± 2.4 cm and 106.9 ± 2.0 cm, respectively ($P < 0.01$; Table 4). Heifers that were 50% HJ were intermediate in height at 112.9 ± 1.2 cm, but not statistically different from the predominantly Jersey heifers. Both 100% H and >50% H heifers had the tallest wither heights at 124.0 ± 2.0 cm and 102.4 ± 1.6 cm respectively.

Leptin

No differences in serum Leptin concentrations were seen regarding percentage Holstein from 12 wk prior to puberty up to the week of puberty (Figure 17). Changes in Leptin concentrations over time were not significant by breed composition either. However, 100% J heifers were the only breed composition that had any visible change in trend over time. A statistical difference was seen between individual heifers' Leptin levels ($P < 0.05$) but was variable and not significant over time. BW and BCS were not able to explain any differences in Leptin concentrations due to being confounded by percentage Holstein.

DISCUSSION

Age at Puberty

Stewart et al (1980) and Laster et al (1976) reported decreased age at puberty for crossbred heifers when compared to their purebred contemporaries. While most of the breeds used in both studies were predominantly beef breeds, Holstein and or Jersey breeds as well as their crosses with various beef breeds were observed. The purebred Holstein and Jersey heifers, Holstein crossed and Jersey crossed heifers had the youngest age at puberty compared to Brahman and Hereford heifers which were the oldest (Stewart et al, 1980). In the 1976 Laster et al study, a greater percentage of Jersey heifers reached puberty compared to the beef breeds and were young at puberty.

In a dairy setting, purebred Holstein heifers (48 wk) were older than either Holstein/Jersey crossbred (43wk) or purebred Jersey (40wk) heifers (Getzewich, 2005). All 3 studies, but especially the Holstein/Jersey crossbreeding study, are consistent with the results found in this research. Ages at puberty by Getzewich were lower than those seen in this study (338 d vs 404 d for Holsteins, 279 d vs 350 d for Jerseys, and averages of 301 d vs 345 d for crossbred heifers, respectively). This difference may easily be explained by the fact that the heifers in this study were grown on pasture while those of the Getzewich study were raised in a confinement setting. Heifers on pasture tend to be smaller and arrive at puberty later than heifers penned in confinement dairies (Stewart et al, 1980).

Body Weight and BCS

Heifers with beef genetics weighed more than Jersey heifers at puberty (Laster et al, 1976). Both Stewart et al (1980) and Long et al (1979) reported pure Brahman and Brahman crossed heifers were not only older, but heavier at puberty, and Jersey and Jersey crossed heifers were the lightest. Long et al (1979) also showed that Holstein and Holstein crossed heifers weighed significantly more than those with Jersey genetics. Holstein heifers were significantly heavier than both Holstein/Jersey crossbred and purebred Heifers (Getzewich, 2005). Findings from this study were similar to those seen by all of the aforementioned studies. Surprisingly, BW were not different between this and the Getzewich study as research comparing heifer BW at puberty in confinement versus that of heifers on pasture (Long et al, 1979; Stewart et al, 1980). No breed differences were seen in this study and was supported by Long et al; while beef breed and beef crossbred heifers had higher BCS, Holstein, Jersey and their crosses were not different.

Wither height

Baker et al (1988) reported that heifer height, once the high interdependence between age at puberty, BW and hip or wither height, was most influential on age at puberty. Crossbred heifers tended to be taller than the average of their respective purebreds; with Brahman crossed and pure Brahman heifers the tallest at puberty (Stewart et al, 1980). Long et al (1979) reported similar results with the Brahman heifers and Brahman crosses becoming taller at a younger age; Holstein and Holstein crossed heifers had the greatest heights at older ages. With regards to Getzewich's research, Holsteins were taller at puberty than Jersey heifers, with crossbred heifers being of intermediate height. The same pattern was seen in

this study with 100% H and >50% Holstein heifers being significantly larger than the other crossbred and Jersey heifers. Wither heights between these 2 studies were comparable; height did not differ between confinement and pasture settings.

Leptin

Garcia et al (2002a, 2002b) showed correlations between Leptin concentrations, BW, and onset of puberty. Block et al (2003), Williams et al (2002), as well as Luna-Pinto and Cronje (2000) all saw increases in Leptin in the weeks leading up to puberty. Because no change in Leptin serum concentrations were seen from 12 wk prior to the week of puberty, the data reported here are unexpected. Leptin levels seen in this study (2-3 ng/ml) are equivalent to those reported in other studies in younger, prepubertal heifers. If these data are correct, Leptin does not modify pubertal development. If incorrect, some error in sample collection or handling, insufficient specificity of the assay, or an unknown effect of pasturing could have occurred. Further work with dairy heifers, particular with heifers raised in pasture-based systems, needs to be conducted to explain the data presented by this research and to better understand whatever role Leptin may hold in obtainment of puberty.

CONCLUSIONS

Increasing the percentage Holstein in a heifer's genetic makeup increased the animal's respective age at puberty in this study. Purebred and predominantly Holstein heifers also weighed more and had greater wither heights at puberty when compared to purebred Jersey and predominantly Jersey crossbred heifers. The results of this study suggest that by increasing the percentage Jersey in a crossbred dairy heifer, the animal becomes

pubertal sooner, and at an intermediate weight and height. Additional research needs to be conducted to further explain differences seen between breed compositions in this study and to evaluate subsequent reproductive measures of crossbred heifers as they are bred and sustain pregnancy. Leptin did not have any relationship to puberty in this study but may in herds with less individual variation. More studies are needed to fully elucidate the role Leptin plays in dairy cattle puberty and whether it may or may not be dependent upon breed.

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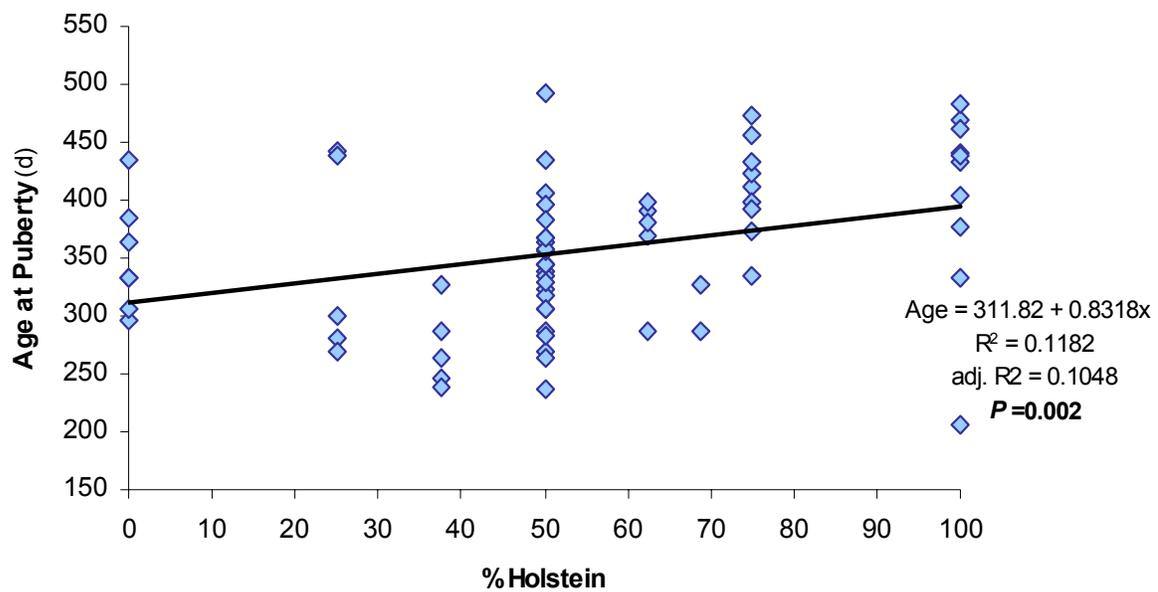
Age = 311.82 + 0.8318x
$R^2 = 0.1182$
adj. R2 = 0.1048
P =0.002

Figure 13. Linear regression of age at puberty for heifers by percent Holstein.

Table 4. LSMeans for birth weight and age, body weight, BCS, and wither height at puberty of heifers by breed.

Breed	Age at puberty (d)	SEM	Birth Weight (kg)	SEM	BW at puberty (kg)	SEM	BCS at puberty	SEM	Wither ht at puberty (cm)	SEM
100% H	404.2[a]	19.7	33.6[a]	1.8	289.5[a]	14.7	2.63	0.04	124.0[a]	2.0
>50% H	383.6[a]	15.6	31.9[a]	1.5	259.9[a]	23.1	2.63	0.03	120.4[a]	1.6
50% H/J	341.0[ab]	12.4	29.4[ab]	0.9	241.8[ab]	9.5	2.63	0.03	112.9[b]	1.2
>50% J	309.4[b]	19.7	25.4[b]	1.2	192.1[b]	10.4	2.65	0.04	106.9[b]	2.0
100% J	349.9[b]	23.5	25.5[b]	1.6	192.7[b]	19.0	2.57	0.05	109.6[b]	2.4
P-value	0.006		<0.001		<0.001		0.783		<0.001	

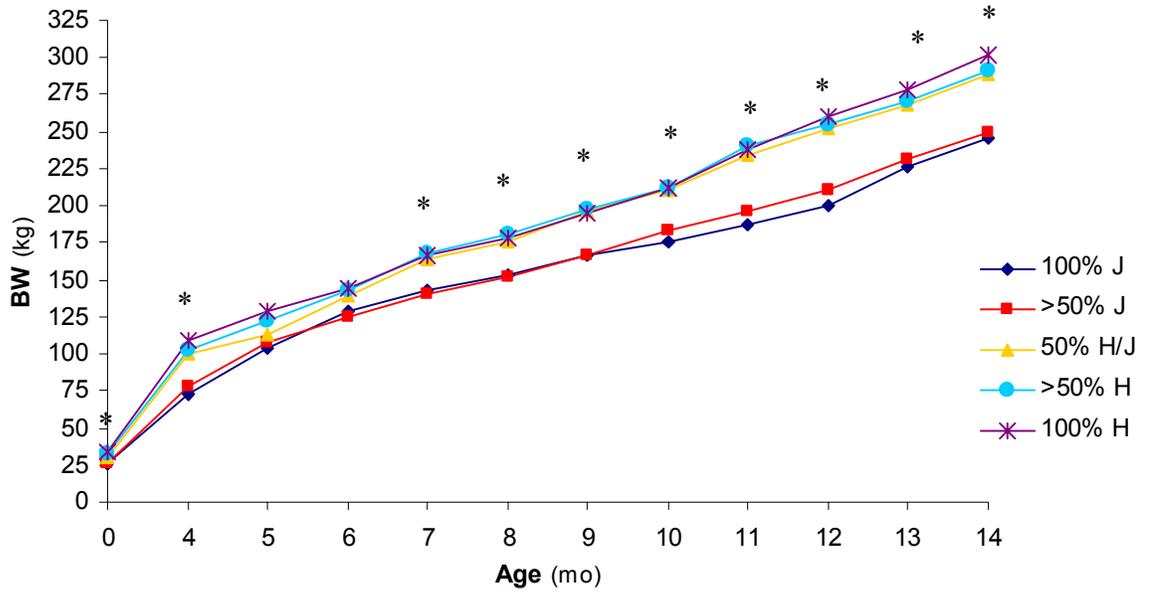[a-c] Breed means with different superscripts differ ($P < 0.05$).

Figure 14. LSMeans for heifer BW from birth to 14mo by breed. Breed differences denoted by "*" at *P* <0.01.
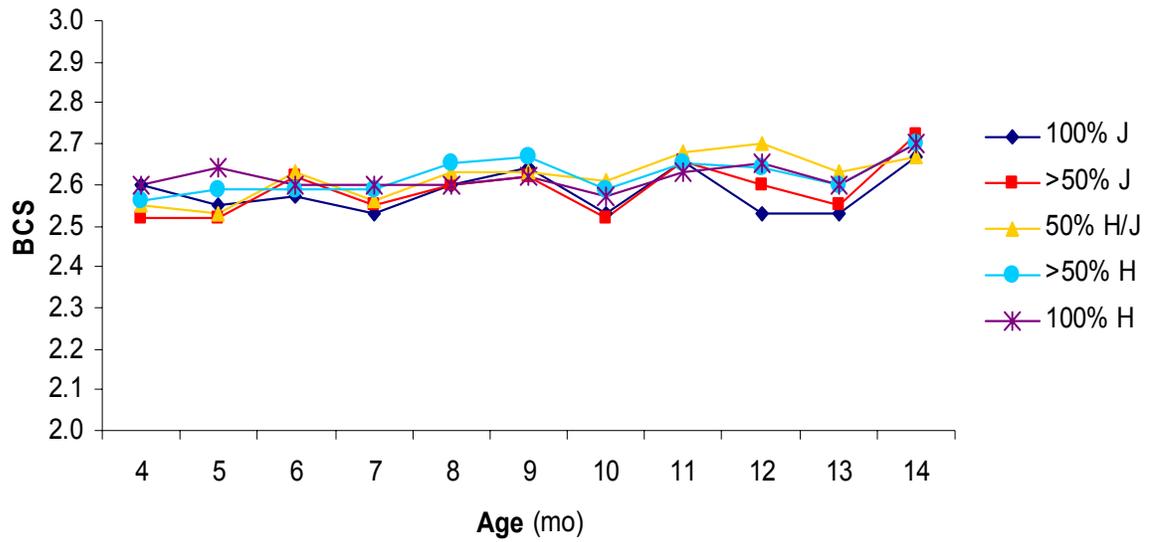
Figure 15. LSMeans for heifer BCS from 4mo to 14mo by breed. Significant differences were not seen.
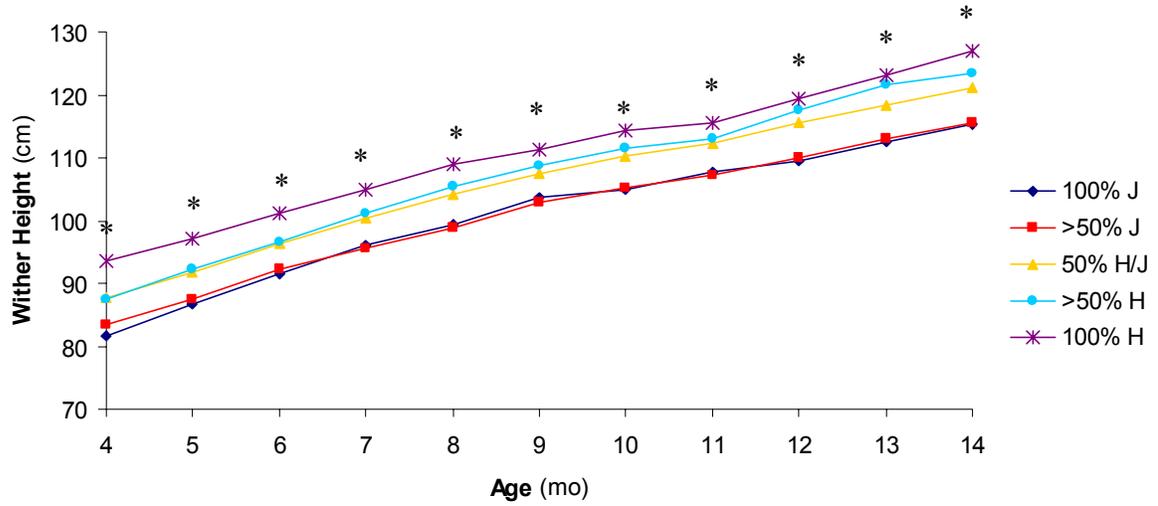
Figure 16. LSMeans for heifer wither heights from 4mo to 14mo by breed. Breed differences were significant at all time points and denoted by "*" (*P* <0.01).
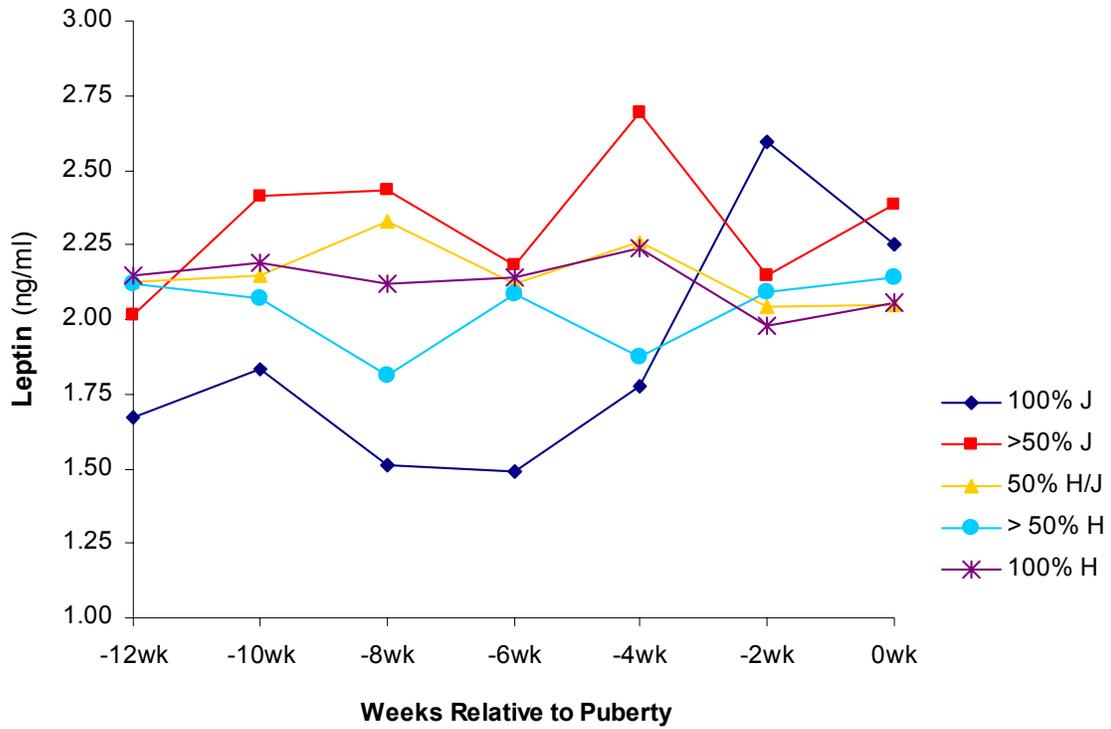
Figure 17. LSMeans for serum Leptin concentrations by breed from 12wk before puberty to the week of puberty. Statistical differences were seen between individual heifers (*P* <0.05) but not breed.

CHAPTER 4

SUMMARY

Breed differences were seen regarding both postpartum cyclicity and fertility as well as for heifer puberty. In both studies, crossbreeding had beneficial effects. Following calving, crossbred cows had milk production and ECM values on par with those of purebred Holstein cows, high proportions of cows cycling by 30 d, 60 d, and 90 d postpartum and fertility rates equal to or greater than purebred Jersey cows. Both 50% H/J and >50% J heifer groups were as young or younger at puberty as purebred Jersey heifers. Crossbred heifers tended to be intermediate for BW and wither heights. Leptin data were inconclusive for this study and will require more research to fully evaluate.

This research provides alternative systems for dairying. Cows maintained on pasture sustained an acceptable level of production and had greater than average reproductive performance. Heifers took longer to reach puberty than the study conducted in confinement, but were pubertal at a adequate age and will be able to calve prior to reaching 2 yr of age. Implementing crossbreeding in this study produced cows and heifers of intermediate size. Crossbred cows retained the higher milk production of their Holstein genetics and greater fertility from their Jersey genetics along with evidence of heterosis. Crossbred heifers were typically younger than purebred Holstein heifers but equal to Jersey at puberty. Some of the differences seen in postpartum cyclicity and puberty can attributed to the seasonality of the herd studied. The animals in this herd calved or were born in the time of year that other research has shown to have the shortest intervals to first ovulation and first ovulation postpartum.

IMPLICATIONS FOR FUTURE STUDIES

Further research is warranted involving crossbreeding in pasture-based systems. Additional values should be explored regarding postpartum breed differences. Other production measures I think to be of interest include peak milk volume, DIM, total milk yield per lactation, and fat and protein production. Measures of nutrition (DMI, digestible nutrients, metabolizable energy, etc.) would also prove interesting. More health variables should also be recorded and examined. Differences in SCS by breed and over time, incidence of mastitis, lameness, and occurrence of cystic ovaries and retained placentas could be inspected. Other indicators of reproductive performance could be included such as services per conception, pregnancy rate at time points other than 90d postpartum (16wk in-calf rate, 150d pregnancy rate, carried to term rate, etc.), interval to first observed estrus and 21d submission rate. Characterization of additional factors as to why over 20% of Holstein cows were not cyclic by 90d postpartum need be completed.

With respects to the puberty research, much more work can be done. Other growth measures can be taken and correlated with age at puberty like pelvic diameter, ADG, heart girth circumference, and weight at weaning. More hormones and their relationship to puberty could be explored, including but not limited to Ghrelin, Prolactin, IGF-1 and other adipocyte hormones. Leptin and its role in pubertal development will require more studies conducted to fully elucidate its role and interactions between adipose tissue, nutrition, and reproduction.

For both the postpartum and pubertal parts of this study, genetic analysis needs to occur to determine the amount of heterosis seen in this research and how great the effect. Also, comparisons with multiple farms with similar and different management could further

validate this project as well as bring to light factors other than those explored here that would

explain the differences seen between breeds. Inclusion of other breeds in repeat studies

would further expand possible options from which dairy farmers and managers can select