HORMONAL REGULATION OF THE ONSET OF PUBERTY IN PUREBRED AND CROSSBRED HOLSTEIN AND JERSEY HEIFERS

Karen Elizabeth Getzewich

Thesis submitted to the faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
in
Dairy Science
(Reproductive Physiology)

APPROVED:

Dr. Raymond L. Nebel, Chair
Dr. Bennet Cassell
Dr. R. Michael Akers, Department Head

July 19, 2005

Blacksburg, VA

Key words: Puberty, Crossbreeding, Holstein X Jersey, GnRH

Hormonal Regulation of the Onset of Puberty in Purebred and Crossbred Holstein and Jersey Heifers

K. E. Getzewich

ABSTRACT

The objective of this study was to determine the onset of puberty by using progesterone profiles and anterior pituitary gonadotropin releasing hormone (GnRH) challenges in purebred and crossbred Holstein and Jersey heifers. In experiment 1, fifty prepubertal heifers by four sire - dam classifications (18 HH, 11 JJ, 10 JH, and 11 HJ) were used. The HH heifers attained puberty at an older age than the HJ, JH or JJ heifers, and these three classifications were not different from each other. There were significant differences in weight and wither height at puberty and average daily weight gain (ADWG) between all four sire - dam classifications. Season of birth had a significant effect on age and weight at puberty. In experiment 2, four prepubertal heifers from each sire - dam classification in experiment 1 were used at 3, 6, 9 and 12 mo of age to determine the effects of administering 200 µg GnRH on LH secretion. The effects of breed, age at challenge, and their interaction were only significant for time to LH peak. In conclusion, age at puberty in crossbred Holstein X Jersey heifers is regulated by breed and season of birth, and further research with larger sample sizes is needed to establish the relationship of pituitary maturation and the capacity to secrete LH in response to GnRH stimulation as related to onset of puberty in purebred and crossbred dairy heifers.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Ray Nebel, for all of your help with my thesis project. Your professional help and comic relief helped me get through graduate school. Thank you for your guidance while at the same time helping me learn to be self-sufficient and independent. I wish you all the best of luck at Select Sires in Ohio, but don't forget your Hokie roots!

I would like to thank my committee members, Dr. Bennet Cassell and Dr. R.M. Akers for your help and guidance through the study. For those professors that were not on my committee but still took the time out of their busy schedules to help me, especially Dr. Michael McGilliard, I also thank you.

Throughout the study, I had many undergraduate students that helped me with my data collection and lab work, and spent numerous weekdays and weekends out at the Dairy barn in all types of weather and temperatures. I owe gratitude to Carrington Knox, Julia Nagel, Jennifer Norris, Kimberly Moore and especially to Jeffrey Cornwell, a former graduate student.

Many thanks to Patricia Boyle for all of your technical and laboratory help, and Lee Johnson in the Animal Science Department for input on laboratory procedures. Many thanks to the staff of the Dairy Science Department. Your help with ordering supplies, making overheads for my classes, helping with assistantship funds, and all the help with paperwork (that it takes for a graduate student to do anything) was greatly appreciated.

I would also like to thank the Virginia Tech Dairy center employees for your help and patience on my study, and as well as for your friendship. Working at the Dairy Center as an undergraduate student taught me many aspects of the Dairy industry, and you were all very patient with me when I either got a tractor stuck in the mud or left a gate open somewhere. Thank you for helping a girl from Northern Virginia develop a love for the dairy cow; I will miss all of you.

Most importantly, I would like to thank my friends and family for all the emotional and financial support you have given me over the past years, and not just in graduate school. My parents Jim and Kathleen have played an important role throughout my life as my mentors and my friends. They have taught me that family is one of the most important things in this life, and that you can never forget where you came from. I can honestly say that I would not be the person I am today if it were not for the love and support that my parents have given me. Thank you for everything.

And last but certainly not least, I want to thank my wonderful boyfriend Garrett. You have supported me through everything I've been through, and despite the hard times in school you still managed to put a smile on my face each and every day. I can't wait to start our life together!

TABLE OF CONTENTS

List of Abbreviations……...v

List of Tables……..vi

List of Figures……...….vii

Introduction……..1

Literature Review

Crossbreeding in the Dairy Industry……...…....2
Endocrine Regulation of Puberty……..…4
Onset of Puberty……..7
Negative Feedback of Estradiol-17ß……9
Gonadotropin Secretion in Prepubertal Heifers…….13
Photoperiod and season of birth…….……...16

Materials and Methods……...20

Results……....27

Discussion……..40

Conclusions……48

Literature Cited……..50

Appendix A. Statistical Analysis of Changes in LH Variables by Age at Challenge and Breed……..……..57

Vita…….62

LIST OF ABBREVIATIONS

ADWG: Average daily weight gain (from birth to 6 mo of age)

AUC: Area under the curve

BW: Body weight

CL: corpus luteum

d: day(s)

FSH: Follicle-stimulating hormone

GnRH: Gonatotropin-releasing hormone

h: hour(s)

HPG-axis: Hypothalamic-Pituitary-Gonadal Axis

i.m.: intramuscularly

HH: heifer born from a (H)olstein sire and (H)olstein dam

HJ: heifer born from a (H)olstein sire and (J)ersey dam

i.v.: intravenously

JH: heifer born from a (J)ersey sire and (H)olstein dam

JJ: heifer born from a (J)ersey sire and (J)ersey dam

LH: Luteinizing hormone

mo: month(s)

min: minute(s)

P_4: Progesterone

wk: week(s)

LIST OF TABLES

Table 1. Coefficients for estimates of additive, maternal and heterosis genetic effects……....22

Table 2. Effect of breed of sire and dam on age, weight and wither height at puberty and ADWG for four different sire - dam classifications[a] ...…….....28

Table 3. Effect of season of birth on age and weight at puberty for four different seasons[a]……...…….……28

Table 4. Prediction equations for additive, maternal, and heterosis components for age, weight and wither height at puberty and ADWG for all four sire - dam classifications…….31

Table 5. Characteristics of changes in LH levels in response to GnRH treatment in prepubertal heifers of four different sire - dam classifications at different ages[a] ……...….……32

Table 6. Characteristics of changes in LH levels in response to GnRH treatment in prepubertal heifers of four different sire - dam classifications over all ages[a]…….….33

Table 7. Prediction equations for additive, maternal, and heterosis components for baseline LH, peak LH, time to LH peak, return to baseline and AUC for all sire – dam classifications and age at challenge……..37

Table 8. Analysis of Variance for Baseline LH; fixed effects from PROC MIXED……...57

Table 9. Analysis of Variance for Peak LH; fixed effects from PROC MIXED……...58

Table 10. Analysis of Variance for Time to LH peak; fixed effects from PROC MIXED……..….59

Table 11. Analysis of Variance for Return to baseline; fixed effects from PROC MIXED……...60

Table 12. Analysis of Variance for AUC; fixed effects from PROC MIXED……..61

LIST OF FIGURES

Figure 1. Schematic illustration of the hypothalamic-pituitary-gonadal axis……..….5

Figure 2. Area under LH curve (amount of LH measured in 6.5 h minus baseline)..…….……...……...…….……25

Figure 3. Distribution of the onset of puberty for the four sire - dam classifications by month of age.……..…29

Figure 4. Distribution of the season of birth for the four sire - dam classifications by season……30

Figure 5. Baseline LH for the four sire - dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge interaction……...33

Figure 6. Peak LH for the four sire - dam classifications (breed, Holstein (H), Jersey (J) by age at challenge interaction……34

Figure 7. Time to LH peak for the four sire - dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge interaction……...34

Figure 8. AUC for the four sire – dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge interaction……..…...35

Figure 9. Peak LH for all four sire - dam classifications at different age at challenge.....…35

Figure 10. Return to baseline for all four sire - dam classifications at different age at challenge……..…….36

Figure 11. AUC for all four sire - dam classifications at different age at challenge……...…...……36

Figure 12. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the HH sire - dam classification.……...38

Figure 13. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the HJ sire - dam classification.……....38

Figure 14. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the JH sire - dam classification.……39

Figure 15. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the JJ sire - dam classification.……39

INTRODUCTION

Modern interest in crossbreeding dairy cattle has developed in response to concerns dairy producers have regarding milk components, fertility, calving ease, and rising inbreeding levels seen in today's primarily Holstein herds (Weigel and Barlass, 2003). The first scientific trials using crossbred dairy cattle date back as early as 1906 in Denmark using Jersey and Red Danish breeds (Touchberry, 1992). Touchberry (1992) examined a trial that was conducted at the Illinois Agricultural Experiment Station from 1949 to 1969 using Holstein X Guernsey crosses. Touchberry (1992) reported that crossbred animals had greater survivability, weight gain, milk yields and components (fat, protein, solids-non-fat (SNF)) than purebred animals. This trial, among many early trials, measured total performance of crossbreds in terms of heterosis for milk yields and components, and on a smaller scale other measures such as survival and growth rate past first breeding weight. What was not examined in these trials, and has not been examined to this date, is total performance of crossbreds at and before puberty, using age at puberty and weight gain prior to puberty.

The onset of puberty is dependent on many factors including (but not limited to) photoperiod, nutrition, management and breed of the heifer (Rorie et al., 2002). Heifers reach puberty when they have attained 40% of their adult BW, and on average this occurs at 9 to 11 mo of age in Jersey and Holstein heifers, respectively. Few studies have been published regarding age at puberty either for Jersey or Holstein X Jersey crossbred heifers. Age at puberty is an important economic trait because the cost of feeding animals that are not productive reduces efficiency and profit (Schillo, 2003). By understanding the mechanisms that regulate onset of puberty and when puberty occurs, especially for farms utilizing crossbreeding, producers can maximize the reproductive efficiency of their heifers.

Studies have investigated the role of the hypothalamic-pituitary-gonadal axis (HPG-axis) and its regulation of the onset of puberty. Administering exogenous gonadotropin-releasing hormone (GnRH) overrides the negative feedback effects of estradiol that inhibit estrus and cyclicity prior to puberty. These studies give insight to the changes in pituitary maturation months before puberty occurs. Nakada et al. (2002) reported that after administering exogenous GnRH to prepubertal heifers (1, 2, 6, 8 mo of

age) luteinizing hormone (LH) accumulation in the pituitary gland and the responsiveness to GnRH increased with age. The development of the capacity to secrete LH from the pituitary gland in response to GnRH is a determining factor that regulates the onset of puberty (Nakada et al., 2002).

LITERATURE REVIEW

Crossbreeding in the Dairy Industry

Crossbreeding in the dairy industry has the greatest potential for improving the quality of dairy production systems regarding health, reproduction and survival (McAllister, 2002). According to McAllister in 2002, fewer than 5% of dairy cattle in the US were not purebred or grade Holsteins. This is due to the genetic development and selection in Holsteins for high lactation yields. However, it is likely secondary traits to milk yield such as reproduction, productive life, health and survival have increased interest in crossbreeding systems (McAllister, 2002). Crossbreeding increases heterosis (a vigor or capacity for growth) in crosses by introducing favorable genes from other breeds, removing inbreeding depression and maintaining gene interactions (VanRaden and Sanders, 2003). Despite the potential of crossbreeding, multiple-generation lifetime performance on an array of purebreds and crossbreds under US conditions does not exist, and due to the complexity of a crossbreeding system it may always remain low in popularity (McAllister, 2002).

Interest in crossbreeding of dairy cattle in the US is increasing. However, it has been very popular in other countries. In New Zealand approximately 20% of the cows participating in milk recording programs were crossbred Holsteins and Jerseys, and in Australia this number is about 5% (VanRaden and Sanders, 2003). A study conducted in Canada consisting of two crossbred groups had lifetime yields, net milk value and dollar returns equivalent to Holsteins (McAllister, 2002). A New Zealand study of Friesian-Jersey crossbred cows was predicted to have higher first-lactation yields than Friesian purebred cows (McAllister, 2002). These and other studies have shown that outcomes from crossbreeding of dairy cattle can be positive. However, there are many factors that could influence the results of crossbreeding studies and the country where the research was conducted can be a major factor. Commonly cited factors that influence

crossbreeding studies are temperature, climate, region-specific breeds, farm conditions, breeding and management practices and nutrition. Results from countries abroad may not be applicable to the US dairy industry and crossbreeding programs for three major reasons (McAllister, 2002). First, no crossbreeding study has directly involved current US genetics for any of the breeds studied except the current composition of New Zealand Holstein-Friesian. Second, none of the crossbreeding studies have involved current US dairy production conditions, including market values. Third, no crossbreeding studies have involved a multi-generation economic comparison of Holstein and contemporary crossbred populations. Commonly, either Jersey or Brown Swiss sires were mated to Holstein cows to form the first generation. The idea of calving ease and lower birth weight for calves sired by breeds of smaller stature was the primary factor given for these matings. Matings between these breeds produced crossbred progeny that on average stayed in the herd as long as or longer than purebred Holstein offspring (VanRaden and Sanders, 2003).

In a study by Weigel and Barlass in 2003, surveys were sent to 528 US dairy producers who were implementing crossbreeding in their herd. These surveys indicated that the most common first generation crosses involved Jersey and Brown Swiss bulls mated to Holstein cows, and second generation crosses included backcrosses to one of these parental breeds. Advantages of crossbreeding stated in responses to the survey were improvements in fertility, calving ease, longevity, component percentages, improvement in feet and legs, temperament, grazing performance, body size and less inbreeding. Producers also listed many disadvantages to crossbreeding, such as, decreased marketability of slaughter animals and bull calves, lack of uniformity in the herd, difficulty in choosing mates for the next generation, and reduced milk volume. Respondents indicated that first generation offspring involving Jersey and Brown Swiss breeds had a clear advantage in longevity relative to purebred Holsteins, and conception rates for first generation offspring from Jersey or Brown Swiss sires and Holstein cows were similar to the (high) conception rates typically achieved in purebred Jersey matings. These findings were similar to Hocking et al. (1988) that determined that crossbred Holstein and Aryshire lines had a clear advantage of longevity in the herd. McDowell (1985) indicated that crosses by Holstein sires have proven superior to crosses from

other breeds but problems arise when determining what parental breed to mate back to the first generation animals. Sufficient guidelines have not been developed to determine the best breed; however, common practice has been to backcross to the improved parent breed for milk production for the second-generation offspring.

The higher favor and opinion of crossbreeding has been contradicted by previous researchers (Freitas et al., 1998; Alexander et al., 1984) observing European-Zebu crossbred cows in tropical regions. First generation offspring have performed well for milk yields; however, for the F2 milk production per cow declined, fertility was lower, and calf mortality increased after the F1 because of loss of heterosis or environmental deterioration (i.e. feeding and management) (Freitas et al., 1998). These conflicting views reiterate the ideals that success of crossbreeding is dependent on many factors. Further studies in the US with crossbreeding schemes and systems need to be implemented using current genetics and market values to determine if crossbreeding is a viable recommendation under specific conditions for today's dairy industry.

Endocrine Regulation of Puberty

At the onset of puberty, a surge of GnRH causes a surge of LH release from the anterior pituitary. This surge of LH stimulates ovulation of a fully developed follicle from the ovary. The HPG-axis (Figure 1) acts as the regulating system of reproduction, with the final component, for regulating the time of the onset of puberty in heifers, being the hypothalamus (Nakada et al., 2002). Estradiol-17ß and inhibin directly and indirectly regulate GnRH and gonadotrophin secretion in prepubertal heifers (Nakada et al., 2002; Dodson et al., 1988 and Day et al., 1987). Terasawa and Fernandez in 2001 defined puberty as the age when the regulating system for gonadotropin secretion becomes desensitized to steroid feedback during sexual maturation and the shift in sensitivity to steroids permits gonadotropin secretion. This secretion then results in ovarian follicle maturation and ovulation. The mechanism of the onset of puberty was defined by Harris (1955) as the Gonadostat hypothesis or Central Restraint Theory. According to this theory, the HPG- axis is fully functional at birth, yet activation of this axis does not take place until a defined age dependent on the species (Kuenzel, 2000). Gonadotropin secretion involves release of LH and follicle stimulating hormone (FSH) from the

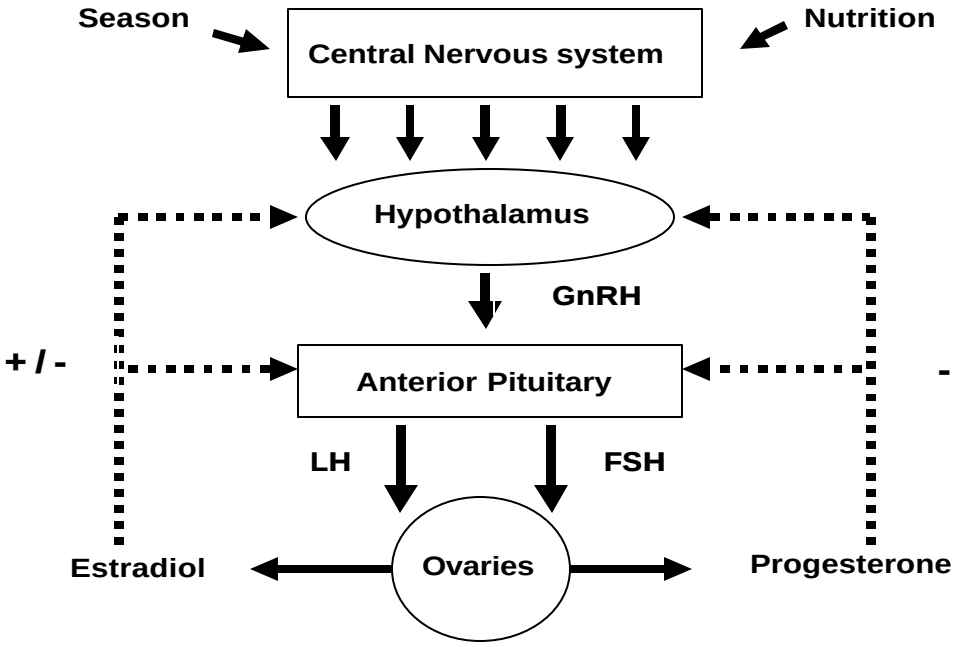

Figure 1. Schematic illustration of hypothalamic-pituitary-gonadal axis.

anterior pituitary gland. This escape of suppressed LH secretion by gonadal steroids is the trigger for the onset of puberty. However, there have been noted differences between species regarding the onset of puberty. In ruminants, the onset of puberty is influenced by the negative feedback and presence of gonadal steroids; whereas, in primates, GnRH release is independent of these ovarian steroids. The degree to which prepubertal GnRH release is influenced by gonadal steroids has not been fully assessed (Terasawa and Fernandez, 2001). In peripubertal female rats, a decrease in sensitivity to estradiol negative feedback did not occur until after the first preovulatory surge and it is a consequence of pubertal onset, rather than a controlling mechanism (Andrews et al., 1981; Day et al., 1984). Therefore, certain areas of the "gonadostat hypothesis" may not be applicable to all species.

Previous studies on calves have shown that heifer calves as young as 2 wk of age exhibit growth of ovarian follicles in a wave-like manner consistent with results seen in adult cattle (Evans et al., 1994b). Previous endocrinological studies have also noted differences in the serum concentrations of LH and FSH from birth to puberty. These

differences may be due to infrequent blood sampling or the influence of season of birth (Dodson et al., 1988). Starting with the use of specific bioassays for LH and FSH in the bovine in the 1960's there has been conflicting evidence regarding endocrine status from birth to puberty in the heifer (Desjardins and Hafs, 1968). While some researchers have reported the pulsatile increase of LH from birth to puberty (Rodrigues et al., 2002; Day et al., 1987; Day et al., 1984 and Schillo et al., 1982), others have reported no detectable changes in LH (McLeod et al., 1984), or in both LH and FSH (Dodson et al., 1988). One study found that serum LH and FSH decrease from birth to 24 wk, and then increase to 39 wk of age (Dodson et al., 1988), while others note that concentrations increase from 4 to 14 wk of age and then decrease at 6 mo of age (Evans et al., 1994). Anderson et al. (1985) reported the inhibition of pulsatile LH secretion is established by 3 mo of age.

Recent research conducted by Nakada et al. (2000) examined changes in plasma concentrations of reproductive hormones in Holstein-Friesian heifers from birth to puberty. Nakada et al. (2000) reported concentrations of LH and FSH rapidly increased from birth to 7 d in heifers and was thought to be induced by the decrease in the plasma concentrations of steroid hormones. This increase in FSH after birth may have a role in triggering follicular development that continues during sexual maturation. In late prepubertal heifers after maintaining relatively constant levels until 16 wk before puberty, concentrations of LH increased to peak at puberty (0.66 $\pm$ 0.29 ng/ml). Concentrations of FSH reached a peak by 4 wk of age (0.27 ng/ml) and then decreased at 4 wk before puberty (0.13 ng/ml) before again increasing to 0.17 ng/ml at puberty. Heifers had a greater concentration of plasma estradiol-17ß (23.10 $\pm$ 4.70 pg/ml) at birth than at any other age until puberty, while there were no changes in plasma concentrations of progesterone from birth to puberty with levels being continuously low (0.05 $\pm$ 0.01 to 0.18 $\pm$ 0.05 ng/ml). After examining these results, the authors noted that there were three brief periods in which dramatic changes occur regarding hormone profiles between birth and puberty. The first period was the week after birth, during which a reciprocal relationship between steroid hormones and gonadotropins was observed. The second was at 4 wk of age when there was an increase in concentrations of LH and estradiol-17ß. The third was the last 5 wk before ovulation due to increases of the previously noted hormones in the second period. The authors concluded that regular hormone changes

start from 10 d after birth and that the periods from birth to 4 wk of age and the last 5 wk before the first ovulation in heifers are important to the development of reproductive functions before puberty (Nakada et al., 2000).

Onset of Puberty

The onset of puberty has many definitions. Those include the age at first estrus, age at first ovulation, and age at which a female can support pregnancy without deleterious effects (Senger, 1999). There are many factors that influence the onset of puberty and estrus, which can include (but are not limited to) environmental temperature, health of heifer, proper feeding and management (nutrition), growth rates, breed, and the number of animals in estrus simultaneously (Rorie et al., 2002). Heifers generally reach puberty when they have reached 40% of their adult body weight. On average, Holsteins and Jerseys heifers reach puberty at 9 to 11 mo of age. However, age at puberty can range anywhere from 8 to 24 mo depending on breed, nutrition, environment, management and other factors that may be unknown. In a study of 20 prepubertal Holstein heifers body weight and age of first estrus was determined to be 247.6$\pm$ 4.8 kg and 304.0 $\pm$ 7.5 d (10 mo), respectively with an average estrous length of 18 to 24 d (Vecchio et al., 1992). These numbers correlate closely to growth charts for average weights at certain ages for Holstein heifers developed by Hoffman in 1997. Nelsen et al. (1982) conducted a study using Angus, Brahman, Hereford, Holstein and Jersey breeds and all possible two-breed crosses. Puberty was defined by the presence of a corpus luteum by rectal palpation for a 21 d interval starting at 7 mo of age. Age at puberty for the purebred Holstein heifers was 328 $\pm$ 15 d, for the purebred Jersey heifers was 363 $\pm$ 20 d, and for the crossbred heifers was 355 $\pm$ 14 d. Therefore there was an intermediate effect of age at puberty for the crossbred heifers, however the age at puberty was not tested against the means of the purebred heifers, and therefore significance was not determined. Age at puberty for the purebred Holstein and Jerseys heifers was not significantly different. Weight at puberty for the purebred Holstein heifers was 237 $\pm$ 10 kg, for the purebred Jersey heifers was 168 $\pm$ 13 kg, and for the crossbred heifers was 235 $\pm$ 9 kg. There was a significant difference for weight at puberty for the purebred Holstein and Jersey heifers, and the mean for the crossbred heifers was not tested against

the purebred means. Heterosis estimates for characteristics were calculated by the following equation: % heterosis = ((Crossbred mean / Straightbred mean) – 1) X 100. There was a significant heterosis effect (16.0 $\pm$ 5.9 kg) regarding weight at puberty. Wither height at puberty also showed the same genetic effect. Wither height at puberty for the purebred Holstein heifers was 113.6 $\pm$ 1.3 cm, for the purebred Jersey heifers was 103.2 $\pm$ 1.8 cm, and for the crossbred heifers was 113.0 $\pm$ 1.3 cm. There was a significant difference for wither height at puberty for the purebred Holstein and Jersey heifers, and the mean for the crossbred heifers was also not tested against the purebred means. There was also a significant heterosis effect (4.2 $\pm$ 0.5 cm) regarding wither height at puberty. Implications of this study were that crossbred Holstein X Jersey heifers attain puberty at an intermediate age between the purebred heifers, and age at puberty is not influenced by heterosis. However, weight and wither height at puberty are influenced by heterosis. Regardless of the study by Nelsen et al. (1982) few studies have been published regarding age at puberty either for Jersey or Holstein X Jersey crossbred heifers.

In a study conducted by Ringuet et al. (1994), Holstein heifers were bled weekly to determine onset of puberty by examining increases in blood progesterone levels. Sampling began when heifers reached 149 kg BW and the heifers were defined as pubertal when progesterone levels were greater than 1ng/ml for two consecutive samples. Similar procedures were used by Rodrigues et al. (2002), Tortonese and Inskeep (1992) and Melvin et al., (1999). Day et al. (1984) also used similar procedures and defined puberty when blood samples were collected every 7 d and either 1) serum progesterone levels were sustained above 1ng/ml for at least two samples; or 2) serum progesterone levels were > 2 ng/ml for at least 1 sample. By determining plasma progesterone levels in the blood, it can be assumed that either ovulation occurred or a corpus luteum was present (Honaramooz et al., 1999). The duration of estrous cycles in prepubertal heifers after the first ovulation that determines the onset of puberty are usually of shorter and abnormal length (Evans et al., 1994a; Vecchio et al., 1992). Vecchio et al. (1992) reported in 20 prepubertal Holstein heifers that the frequency of abnormal length estrous cycles was greater ($P< 0.02$) during the first (40%) and second (35%) cycles than during the third estrous cycle (0%) with the average estrous cycle

ranging from 18 to 24 d. For heifers with normal estrous cycle lengths, progesterone levels were greater in the third compared with the first estrous cycle ($P < 0.05$). Evans et al. (1994a) reported similar results in prepubertal Hereford heifers where following the first ovulation there was an ovulatory cycle of short duration (7.7 $\pm$ 0.2 d) accompanied by one wave of follicular development and then a normal ovulatory cycle (20.3 $\pm$ 0.5 d). They also noted that these follicular waves were evident in heifers at all prepubertal ages represented in the study.

Negative Feedback of Estradiol-17ß

It is thought that heifers by 1 to 3 mo of age have functionally mature-hypothalamic-pituitary-ovarian systems (Nakada et al., 2002; Barnes et al., 1980). These heifers have the capacity to undergo the cascade of endocrine events that induces estrus; however, a high sensitivity to estradiol negative feedback does not allow estrus to occur (Schillo 2003). Estradiol is an important regulator of the HPG-axis and is believed to be the primary factor responsible for induction of the pre-ovulatory surge of gonadotropins (Nett et al., 2002). Estradiol increases sensitivity of the anterior pituitary gland to GnRH, and after maximum sensitization GnRH is released into circulation and causes the pre-ovulatory LH surge. Therefore, low concentrations of estradiol exert a negative feedback effect on tonic LH release (Schillo et al., 1992). Estradiol functions by way of two mechanisms involved in causing this surge. First, it increases synthesis and insertion of GnRH receptors into the membranes of gonadotropes. Secondly, it stimulates a sustained secretion of GnRH (Nett et al., 2002). Estradiol can act directly on the pituitary gland to modify expression of the genes encoding gonadotropin subunits and to influence the amounts of LH and FSH that are secreted (Nett et al., 2002). Research has also shown that a decrease in progesterone (coupled by an increase in estradiol) plays a role in the increasing pituitary sensitivity prior to ovulation but has not been shown to directly act on the pituitary gland, but instead regulates GnRH frequency due to indirect mechanisms and causes an up-regulation of GnRH receptors (Nett et al., 2002, Vizcarra et al., 1997).

This negative feedback of estradiol maintains a low frequency mode of secretion. As puberty approaches in heifers, the negative feedback effects on the secretion of LH declines, and in turn LH secretion or pulse frequency increases dramatically during the

last few wk before puberty (Rodrigues et al., 2002; Day et al., 1987; Day et al., 1984 and Schillo et al., 1982) resulting in increased mean circulating concentrations of LH (Honamarooz et al., 1999). This increase in frequency of LH pulses has been suggested to be a prerequisite for the onset of puberty in heifers where the increase in frequency of pulses of LH are evident especially during the 50 d preceding puberty (Day et al., 1987). This increase is then accompanied by a decline in the amplitude of LH pulses. Frequency of LH pulses ranged from 1 to 4 pulses per 24 h from d -130 to -46 before puberty. From d -46 to puberty, the frequency of LH pulses increased and approached 1 pulse/h for several days before puberty. Day et al. (1986) noted that the prepubertal increase in LH may be essential to stimulate increased ovarian production of estradiol to levels high enough to induce the first preovulatory surge of gonadotropins by positive feedback. It was noted that in control heifers the experiment mean concentration of LH had a rapid quadratic increase prior to puberty, and frequency of LH pulses increased in a linear fashion.

Research has shown that estradiol increases the number of GnRH receptors as a result of increasing sensitivity of the pituitary gland to GnRH (Looper et al., 2002; Nett et al., 2002). Looper et al. (2002) used nutritionally induced anovulatory ovariectomized cows to evaluate the effects of estrogen and progesterone on gonadotropin secretion. Cows received an intravaginal insert containing either estradiol or progesterone, both progesterone and estradiol, or a sham insert. Pituitary glands were removed and collected within 1 to 2 h after removal of intravaginal insert. Looper et al. (2002) found that the number of GnRH receptors increased ($P < 0.05$) in cows treated with inserts containing estradiol; however, the number of GnRH receptors was not affected by inserts containing progesterone. These findings support evidence that treatment of anestrous cows with estradiol similar to levels found during the luteal phase of the estrous cycle may increase GnRH receptors necessary for reinitiation of cyclicity, and may play an important role before the pre-ovulatory surge of gonadotropins prior to puberty.

Estradiol influences the activity of neurotransmitter and neuropeptide systems in the brain that affect GnRH secretion (Smith and Jennes, 2001). Estradiol receptors are not present within GnRH neurons; therefore, estradiol does not inhibit GnRH neurons directly (Wolfe et al., 1991). It was thought that the actions of estradiol on GnRH

neurons were indirect; however, more recent evidence suggests that some GnRH neurons may contain estrogen receptors (Skynner et al., 1999).

Recent evidence further proves that estrogen signals GnRH neurons though direct regulation, primarily though estrogen receptors and indirectly though E_2-sensitive neurons in the anteroventral periventricular region (Petersen et al., 2003). Previous studies have suggested that opiod neuropeptides may play a role in transmitting the effects of estradiol (Wolfe et al., 1991). Neurotransmitters and neuropeptides such as ?-Aminobutyric acid, Neuropeptide Y, Neurotensin, Vasoactive intestinal polypeptide and catecholamines play a either a stimulatory role or inhibitory role in the regulation of GnRH neuronal functioning by binding to and activating specific membranes receptors that are expressed in the GnRH neurones. Smith and Jones (2001) suggested that estradiol drives the secretion of GnRH by coupling a circadian neuronal signal from the suprachiasmatic to the neurotransmitters and neuropeptides that regulate GnRH secretion. Day et al. (1987) reported that the distribution pattern of GnRH neurons in the hypothalamus during development in the embryo is well established before birth in most species, but the function, morphology, and biosynthesis is not yet mature. Also, the hypothalamic content of GnRH and the number of pituitary receptors for GnRH do not change during sexual maturation in heifers (Day et al., 1987). Therefore, regulation of the onset of puberty in prepubertal heifers is dependent on maturation of GnRH neurons and not the lack of receptors for GnRH in the anterior pituitary.

Day et al. (1987) suggested that the decline in estradiol feedback on secretion of LH during the prepubertal period in heifers may result from a decline in the concentration of binding sites for estradiol at the hypothalamus and/or pituitary. In this study density of receptors for estradiol in the anterior hypothalamus, medial basal hypothalamus and anterior pituitary significantly declined as puberty approached, while receptors for GnRH in the anterior pituitary did not change prior to puberty. It is thought that the declining concentration of these estradiol receptors 50 d prior to puberty indicated fewer number of sites at which estradiol can bind, thereby the negative feedback effects of LH secretion can be overridden and LH can increase in pulsatile release. They suggested that the frequency of pulsatile release of LH might be a better indicator of time of puberty than examining mean LH concentrations, and the increased pulse frequency may stimulate

follicular growth and steroid hormone synthesis in the ovaries. Additionally the prepubertal increase in LH may result from a decrease in the ability of estradiol to inhibit secretion of GnRH from the hypothalamus, and from a decrease in a negative feedback of estradiol directly at the pituitary so that the responsiveness of the pituitary to GnRH is enhanced during the peripubertal period. Therefore, when the negative feedback of estradiol is overridden by increasing LH, follicles increase production of estradiol which will ultimately cause the first pre-ovulatory surge of LH (ovulation) and puberty will occur.

Administering estradiol at different dosages and routes of administration has been shown to elicit a response in secretion of LH in prepubertal heifers (Swanson et al., 1978), prepubertal ovariectomized heifers (Rodrigues et al., 2002; Day et al., 1984; Dyer et al., 1990 and Schillo et al., 1982) and ovariectomized mature cows (Stumpf et al., 1989). Day et al. (1984) demonstrated the negative feedback of estradiol on LH secretion decreases as heifers approach puberty, and this decreased feedback is important for endocrine regulation of puberty. This study consisted of two experiments, the difference between being dietary intake manipulation (experiment 1-dietary manipulation, experiment 2-natural occurring puberty). Heifers either received no additional treatments (CONT), ovariectomized (OVX) or were ovariectomized and subcutaneously implanted with estradiol-17ß (OVX-E_2). LH secretion increased rapidly following ovariectomy in all ovariectomized heifers (OVX). In experiment 1, OVX- E_2 heifers had a maintained low level of LH secretion until a synchronous rapid increase was noted coincidental with puberty in the CONT heifer; whereas, LH secretion increased gradually in OVX heifers and attained castrate levels coincidental with puberty in CONT heifers. Estradiol negative feedback decreased in an abrupt manner when reproductive state was synchronized by dietary manipulation; whereas, changes were more gradual in heifers that attained puberty spontaneously. Exogenous estradiol completely inhibited the postcastration increase in LH secretion in ovariectomized prepubertal heifers in both experiments. This seems logical because ovariectomy would eliminate the natural source of estrogen in the heifer, thereby removing the negative feedback and inducing an LH surge. However exogenous estradiol would only inhibit the LH release due to artificial estradiol negative feedback. Increased age to puberty would decrease this negative

feedback on LH secretion, as well as, administration of estradiol during the prepubertal period (Day et al., 1984). These findings coincide with those by Dyer et al (1990) that compared intact prepubertal heifers that received no treatment (INT), or two estradiol implants between d 16 and 30 (INT+E) verses ovariectomized heifers that received no treatment (OVX), a single estradiol implant on d 0 (OVXE), or a single implant on d 0 followed by two additional implants between d 16 and 30 of the study (OVXE+E). Mean LH levels and LH pulse frequency increased after ovariectomy in the OVX heifers and remained high throughout the experiment. The single implant of estradiol on d 0 following ovariectomy prevented the increase in mean concentration and pulse frequency of LH for OVXE and OVXE+E heifers. LH mean concentration and pulse frequency increased for these heifers as pubertal age approached; however, age at puberty was not significantly reduced due to exposure to estradiol. Maximal inhibition was attained with a single implant of estradiol that remained in place throughout the study. The +E treatments did not further suppress LH levels for OVXE+E heifers, due to the fact that mean concentration of LH and pulse frequency increased more rapidly in OVXE+E and INT+E than in OVXE and INT heifers. As OVXE and OVXE+E heifers approached pubertal age, exogenous estradiol enabled them to elicit increased LH responses, despite the fact that the natural occurrence of estradiol did not exist. They suggested that the effects of short-term exposure to estradiol on hypothalamo-pituitary function could not be expressed until the prepubertal decline in estradiol negative feedback was initiated. These findings are in agreement with results from Rodrigues et al. (2002) where ovariectomized heifers experienced a significant increase of frequency of LH pulses, and exogenous estradiol suppressed this response of LH secretion until pubertal age in age-matched control heifers.

Gonadotropin Secretion in Prepubertal Heifers

Secretion of GnRH by the hypothalamus stimulates secretion of LH and FSH from the anterior pituitary. Over the past decades, various studies have shown the effects of administering GnRH to prepubertal heifers (Nakada et al., 2002; McLeod et al., 1985; Barnes et al., 1980 and Kaltenbach et al., 1974) beef cows (Riley et al., 1981) and ewes (McLeod et al., 1983; McLeod et al., 1982a; McLeod et al., 1982b and Crighton et al.,

1975) at different dosages and at different physiological states (prepubertal, post-partum, anestrus). In the seasonally anestrus ewe, the absence of ovulation is due to an inadequate pattern of LH secretion (McLeod et al., 1982a) as well as in the post-partum acyclic cow (Riley et al., 1981), and that continuous administration of GnRH is sufficient to induce ovulation and stimulate follicular development (McLeod et al., 1983). Specific endocrine responses (LH, FSH) in prepubertal heifers have been used to explain how the hypothalamic-pituitary-gonadal feedback system functions and at what age the development of the capacity of LH release in response to GnRH in the pituitary gland is initiated. Barnes et al. (1980) tested the effects of GnRH on plasma LH, FSH and estradiol-17ß in Holstein heifers at 3, 6, or 9 mo of age. Blood was collected for 2 h, at hourly intervals, before injection of GnRH. Blood was then collected every 20 min for 4 h and then at hourly intervals for the last 2 h. Levels of LH and FSH increased within 20 min of injection. For the 3 and 9 mo old heifers, peak values of LH and FSH occurred 20 min post injection, but for the 6 mo old heifers peak values were not reached until 2 h post injection. Despite considerable variability in individual plasma gonadotropin response to GnRH stimulation, patterns of LH and FSH release were very similar among animals and age groups. They reported the pituitary of the prepubertal heifer is capable of secreting high levels of both LH and FSH in response to GnRH stimulation by 3 mo of age and that the magnitude of this response did not change when a similar treatment dose was administered at either 6 or 9 mo of age. Kaltenbach et al. (1974) reported similar results (among 6 mo heifers) of a 2 h duration from injection of GnRH to peak plasma LH response. However, mean peak levels of LH were higher due to larger doses of GnRH (250? g I.M. as effective as 1mg I.V.). Therefore, it can be concluded that larger doses of GnRH would elicit a higher response in peak levels of LH.

Recent research examining the effects of GnRH administration on prepubertal heifers show the development of the capacity to secrete LH prior to puberty occurs at a younger age than previously reported (Barnes et al., 1980). Nakada et al. (2002) used 50 prepubertal Holstein-Friesian heifers treated with GnRH at 1, 2, 4, 6, and 8 mo of age. Plasma samples were collected at 0 to 360 min after GnRH treatment. For all age groups time of administration to peak of LH and FSH were similar and was prolonged with increasing age. Overall, the peak concentration of LH induced by GnRH treatment

gradually increased with age in all heifers, and the opposite effect was shown when examining FSH levels. The authors concluded that this rise in LH 30 min after GnRH treatment corresponds with an increase of LH accumulation in the pituitary gland. The peak concentration of LH rise and the areas under the curve (AUC) at 360 min after GnRH administration also increased with age. Therefore, it can be concluded that LH accumulation in the pituitary gland and the responsiveness to GnRH increases with age in prepubertal heifers. The peak concentration of FSH and the AUC showed a decreased responsiveness with increasing age. These results regarding FSH combined with results from previous research (Nakada et al., 2000; Evans et al. 1994a) reported decreased levels of FSH from birth to puberty, and this may be due in part to stronger inhibiting effects that increase with age. Evans et al. (1994a) reported no increases of FSH secretion as puberty approached in prepubertal Hereford heifers and it appeared that secretion of FSH during sexual maturation was not the limiting factor preventing first ovulation. However, an increase in LH secretion was needed, and that it was only during periods of increasing LH pulse frequency, during the follicular phase of cycles or the late prepubertal period, that follicles increase estradiol production (Evans et al., 1994a).

Long-term periodic administration of GnRH decreases gonadotropin secretion, resulting in decreased responsiveness to further stimulation with GnRH, also known as pituitary refractoriness and depletion (Jinnah and Conn, 1985). Administration of GnRH that would lead to this condition would be for an extended period of time (3 to 12 h), and at varying doses. Evidence suggests that the mechanisms of refractoriness involved in terminating the LH surge following ovulation can be tested by: 1) desensitization of GnRH receptors where the pituitary gland because insensitive to continued GnRH stimulation and 2) depletion of releasable stores of LH in the pituitary (Jinnah and Conn, 1985, Nett et al., 2002). McLeod et al. (1985) determined that GnRH induced LH episodes were of greater magnitude than naturally occurring episodes when 5 mo old Hereford X Friesian prepubertal heifers were on an extended period of treatment of low doses of GnRH (2 ? g). Each administration of GnRH resulted in an episode-like response in LH, but not FSH. In some of the heifers, the maximum LH concentration decreased with successive administrations of GnRH. There was a significant negative correlation between maximum LH concentration in response to each GnRH administration, and time

from start of treatment. This could be interpreted as evidence of pituitary refractoriness, in some animals; however there were alternating periods of increasing and decreasing sensitivity to GnRH administrations. This was likely to be the result of steroid modulation of pituitary responsiveness rather than a progressive desensitization. Either way, it was unclear whether the results indicated preovulatory LH/FSH surges were produced as a result of GnRH, or the positive feedback effects of estradiol from follicles induced to grow by the GnRH-induced increase in LH episode frequency. Research conducted on anestrous beef cows showed that frequent administration of exogenous GnRH can affect the amount of receptors in the pituitary gland. The pulsatile release of GnRH did not affect the number of GnRH-R or GnRH-R mRNA in the pituitary gland, yet continuous infusion decreased these concentrations of unoccupied receptors (Vizcarra et al., 1997). Therefore, the mode of administration of GnRH, and not just amount, can have a negative affect on the pituitary gland. However, Lamming and McLeod (1988) reported that continuous infusion of low doses of GnRH can stimulate gonadotropin release without incurring long-term pituitary down-regulation. This experiment directly conflicts with previous research (Heber and Swerdloff, 1981; Jinnah and Conn, 1985; Crowder et al, 1986) Lamming and McLeod (1988) hypothesized that these different views on the effects of periodic administration of GnRH could be related to species; different responses could be due to differences in the natural hormone patterns and different estrus cycle lengths which control reproductive cycles in domestic animals and primates. Further research is needed to determine the response to long-term continuous infusion of GnRH in prepubertal dairy cattle.

Photoperiod and season of birth

Two additional factors associated with the onset of puberty are photoperiod and season of birth. Photoperiod and season of birth are highly related due to increasing day length (spring) verses decreasing day length (autumn) and seasonal temperatures. Studies have indicated a seasonal influence on reproduction and the onset of puberty in cattle (Schillo et al., 1983; Grass et al., 1982). Ringuet et al. (1994) found that Holstein heifers under 16 h light to 8 h dark ratio verses 8 h light to 16 h dark ratio had a reduced age and weight at puberty. These findings are similar to those by Petitclerc et al. (1983).

There was an interaction between photoperiod and day length that indicated that heifers under short day length had an earlier increase in estradiol. Studies have indicated stimulated change in variations of day lengths (16 h light to 8 h dark ratio verses 8 h light to 16 dark ratio) advances the age at puberty, regardless of season of birth. Schillo et al. (1983) reported that effects of seasons are more pronounced during the second 6 mo of life. Twenty-eight Angus X Holstein heifers were reared under natural conditions until 6 mo of age. After 6 mo, heifers 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, verses those exposed to Fall to Spring conditions. Season of the first 6 mo of age did not appear to influence body weight. The environmental chambers possibly affected age at puberty by affecting the maturation of the estradiol-negative feedback system, but not by altering growth. The findings of Honamarooz et al. (1999) using prepubertal beef heifers contrast previous findings by Schillo et al. (1983). The age and weight at puberty did not differ between spring-born and autumn-born heifers; however, the range in age and weight at puberty was wider in the autumn-born heifers. Heifers were studied in natural conditions, not under simulated photoperiods or seasons. There are many inconsistent findings on effect of season on gonadotropin secretion in prepubertal heifers. This may be in part to breed, climates, and differences in management practices (Honamarooz et al., 1999). Grass et al. (1982) conducted a study using crossbred heifers (Holstein dams sired by Angus, Hereford, Simmental or Chianina bulls) and concluded that these dairy crossbred heifers attained puberty at younger ages than some of the beef breeds. In this study season affected the onset of puberty, but breed differences in age at puberty were not affected by varying levels of nutrition.

Many studies have reported that season of birth and photoperiod can alter levels of LH, FSH, estradiol, prolactin, and growth hormone (Honamarooz et al., 1999; Ringuet et al. 1994; Stumpf et al., 1988; Critser et al., 1987 and Schillo et al., 1983). Honamarooz et al. (1999) reported the pattern of LH secretion was different between spring-born and autumn-born heifers. Spring-born calves exhibited an early rise in secretion of LH, while in autumn-born heifers the levels of LH decreased. However, Schillo et al. (1983) found that mean levels of LH in September-born heifers were

greater than for March-born heifers from 26 to 29 wk of age, and September-born heifers generally had larger ovaries at this time. Treatment after 6 mo of age did not significantly influence LH concentration. Heifers with greater levels of LH between 26 and 29 wk of age reached puberty earlier. FSH levels in this study seemed to decrease with age in all groups. These findings coincide with Hansen et al. (1983) who determined that levels of LH for prepubertal heifers under varying degrees of photoperiod were not affected by photoperiod or photoperiod by age interactions between 22 and 36 wk of age; however, the sampling regimen was insufficient to assess pulsatile patterns of release. Therefore it is reasonable to hypothesize that seasonal effects on season development are somehow mediated by changes in LH patterns, but support for this hypothesis remains elusive (Schillo 2003).

In a study using ovariectomized-estradiol treated heifers (Critser et al., 1987), serum levels of FSH and LH were greater when heifers were exposed to a short photoperiod (fall to spring) than a long photoperiod (extra light stimulating spring to fall). Differences in LH and FSH concentrations throughout the study possibly due to different photoperiod treatments may indicate that the mechanisms regulating FSH may be more photosensitive than for LH. Prolactin levels were greater in heifers that were receiving extra light. Prolactin pulse frequency was affected by photoperiod, but not amplitude or duration. These findings contrast those by Hansen et al. (1983) where prolactin levels were not affected by photoperiod using intact prepubertal heifers under long or short day photoperiods. The results of Critser et al. (1987) coincide with findings on ovariectomized cows treated with or without estradiol where the highest amplitude of pulses of LH occurred during the spring equinox, and the lowest occurred during the fall equinox. Mean frequency of pulses did not vary between seasons (Stumpf et al., 1988).

Schillo et al. (1992) reported that photoperiod may influence the timing of the prepubertal increase in LH pulse frequency via a neuro-hormonal pathway similar to the ones identified in sheep and other seasonal breeders. This mechanism involves the use of photoperiod for release of melatonin from the pineal gland. Melatonin is found in plasma and cerebrospinal fluid, and has numerous effects including alteration of concentrations of LH, FSH and prolactin by acting on the hypothalamus and midbrain

(Hedlund et al., 1977). Melatonin influences the hypothalamus and GnRH receptors to influence the pulsatile release of LH from the anterior pituitary thus influencing the onset of puberty. However, Tortonese and Inskeep (1992) reported that the effect of melatonin on puberty was not mediated by either growth or nutritional factors. Levels of melatonin have been shown to accurately reflect the duration of darkness where the highest concentrations occur during darkness and drop off by daylight. In other words a diurnal pattern of melatonin release from the pineal gland is exhibited. In a study using 9 mo old Guernsey heifers (Hedlund et al., 1977), melatonin was reported to increase 17-fold in cerebrospinal fluid (38 $\pm$ 8 picograms/ml during the day verses 637 $\pm$ 133 picograms/ml during night) and 6-fold in plasma (19 $\pm$ 4 picograms/ml during the day verses 121 $\pm$ 24 picograms/ml during night). Therefore melatonin may also play an important role in the regulation of the onset of puberty.

The objectives of this study were to determine age at onset of puberty in purebred and crossbred Holstein and Jersey heifers. The first experiment used progesterone profiles to determine indication of first ovulation, and therefore puberty. The second experiment used anterior pituitary GnRH challenges to determine changes in the development of the capacity of LH release from the anterior pituitary gland in response to exogenous GnRH with increasing age to puberty in purebred and crossbred dairy heifers.

MATERIALS AND METHODS

EXPERIMENT 1

Animals and Treatments

Prepubertal heifers (n = 50) of different sire - dam breed classifications, 18 purebred Holstein (HH), 11 purebred Jersey (JJ), 10 Jersey sire - Holstein dam (JH), 11 Holstein sire - Jersey dam (HJ) from the Virginia Tech Dairy Center (Blacksburg, VA) were used from May 2003 to May 2005. The heifers were maintained in hutches until weaning (8 wk) and then moved into a paddock barn and kept until 12 mo of age. After weaning heifers were fed a total mixed ration, orchard grass hay and water ad libitum.

Blood Collection

Blood samples were collected weekly beginning at 4 mo of age and continuing until 12 mo to determine initial progesterone (P_4) rise as an indicator of the onset of puberty. Blood was collected in duplicate from the jugular vein in 7 ml vacutainer tubes (K_3 EDTA), and immediately placed on ice. Blood samples were centrifuged for 15 min at 3000 RPM at 0ºC. Plasma was harvested and stored at -20ºC until P_4 was measured. The onset of puberty was defined as the date when P_4 levels exceeded 1 ng/ml for two consecutive samples (taken at 7 d intervals) or exceeded 2 ng/ml for one sample.

Hormone Concentration Analysis

Concentrations of P_4 were determined using procedures similar to those used by Fajersson et al. (1999). P_4 concentrations were quantified by a Coat-a-Count solid-phase RIA (Diagnostic Products, Los Angeles, CA). The standard curve consisted of seven points (0, .1, .5, 2, 10, 20, 40 ng/ml) in duplicate. The sensitivity of this assay was 0.1 ng/ml. In the first step 100 µl of sample plasma was pipetted into the prepared coated tubes (TPG1- Progesterone Ab-Coated tubes). In step two, 1.0 ml of ^{125}I-Progesterone (TPG2) was added to the tubes. The samples were incubated for 3 h at 25ºC. After incubation, the supernatant was decanted and tubes dried for at least 1 h. The antibody-bound fraction of ^{125}I-Progesterone was quantified in a gamma counter (1 min). All samples were assayed in duplicate and the interassay CVs were between 5.2 and 7.5%.

Statistical Analysis

Progesterone data were analyzed using the General Linear Model (GLM) procedure of Statistical Analysis Systems software program (SAS Version 9.1 for Windows; SAS® Institute, Cary, NC) with a fixed model. In experiment 1, age at puberty, weight at puberty, wither height at puberty, and average daily weight gain from birth to 6 mo of age (ADWG) were analyzed. Effects tested for significance were breed, season of birth, measures of early growth rate, and all two-way and three-way interactions. In the initial model, growth parameters (weight and wither height at puberty, ADWG) were used to determine their effect on the onset of puberty. These variables did not influence the onset of puberty. Season of puberty was confounded with season of birth, and was therefore omitted from the model. The final model contained significant effects of breed and season of birth. The model used to evaluate age, weight and wither height at puberty and ADWG was:

$$Y_{ij} = \mu + B_i + S_j + E_{ij} \quad [1]$$

where:

Y_{ij} = Age, weight and wither height at puberty, ADWG

μ = mean (Y_{ij})

B_i = the effect of the i^{th} breed group (HH, HJ, JH, JJ)

S_j = the effect of the j^{th} season of birth (Fall, Winter, Spring, Summer)

E_{ij} = residual

Tukey-Kramer Multiple Comparison tests were used to compare least squares means for age at puberty, weight at puberty, wither height at puberty and ADWG. Four different seasons of birth were categorized as follows: Fall = October, November, December; Winter = January, February, March; Spring = April, May, June; Summer = July, August, September.

In the second model, breed effects were separated into additive, maternal and heterosis components. Coefficients for additive, maternal and heterosis covariates are shown in Table 1. Age at puberty, weight at puberty, wither height at puberty and ADWG were analyzed using the PROC GLM procedure. Effects that were tested for significance were season of birth and additive, maternal and heterosis effects. Season of puberty was confounded with season of birth, and was therefore omitted from the

Table 1. Coefficients for estimates of additive, maternal and heterosis genetic effects.

	Sire - Dam Combination[a]			
Effect	HH	HJ	JH	JJ
Additive[b]	1.0	0.5	0.5	0.0
Maternal[c]	1.0	0.0	1.0	0.0
Heterosis[d]	0.0	1.0	1.0	0.0

[a] H = Holstein, J = Jersey

[b] Estimates for additive effects represent purebred Holsteins minus purebred Jerseys.

[c] Estimates for maternal effects represent Holstein dams minus Jersey dams.

[d] Estimates for heterosis effects represent crossbreds minus purebreds.

model. The four seasons of birth used were the same as those analyzed in the first model. The final model contained significant effects of season of birth. The model used to evaluate age at puberty, weight at puberty, wither height at puberty and ADWG was:

$$Y_{ij} = a + S_i + b_1 (A_j) + b_2 (M_j) + b_3 (H_j) + b_{i+3} (SA)_{ij} + b_{i+7} (SM)_{ij} + b_{i+11} (SH)_{ij} + E_{ij} \quad [2]$$

where:

Y_{ij} = Age, weight and wither height at puberty, ADWG

a = intercept

S_i = the effect of the i^{th} season of birth (Fall, Winter, Spring, Summer)

A_j = the fraction of Holstein genes in the cross for heifer j (0, 0.5, 1) (Additive)

b_1 = the regression of Y_{ij} on A_j

M_j = the fraction of Holstein genes in the dam for heifer j (0, 1) (Maternal)

b_2 = the regression of Y_{ij} on M_j

H_j = the fraction of gene pairs from different breeds for heifer j (0, 1) (Heterosis)

b_3 = the regression of Y_{ij} on H_j

b_{i+n} = additional regression of Y_{ij} within each S_i (interaction of season of birth with A, M or H)

$(SA)_{ij}$ = interaction of i^{th} season of birth and additive genetic effect

$(SM)_{ij}$ = interaction of i^{th} season of birth and maternal genetic effect

$(SH)_{ij}$ = interaction of i^{th} season of birth and heterotic genetic effect

E_{ij} = residual

The interactions of season of birth by additive, maternal and heterotic genetic effects were not significant, and therefore removed from the model. The final model used to evaluate age, weight, and wither height at puberty and ADWG was:

$$Y_{ij} = a + S_i + b_1 (A_j) + b_2 (M_j) + b_3 (H_j) + E_{ij} \quad [3]$$

EXPERIMENT 2

Animals and Treatments

Prepubertal heifers (n = 16) of different sire - dam breed classifications (4 purebred Holstein (HH), 4 purebred Jersey (JJ), 4 Holstein sire - Jersey dam (HJ) and 4 Jersey sire - Holstein dam (JH) from the Virginia Tech Dairy Center (Blacksburg, VA) were used at 3, 6, 9, and 12 mo of age. The heifers were maintained as previously described in Experiment 1.

Blood Collection

Collection periods occurred at 3, 6, 9 and 12 mo (mo $\pm$ 1 wk) of age for each heifer (n=16). Blood samples were collected at 1 h prior and at time of injection (I.M.) of 200µg synthetic GnRH (Cystorelin®, Merial Limited, Iselin, NJ) or physiological saline (n = 3 at 6, 9 and 12 mo). These samples were baseline LH values for each heifer. Due to expected low LH frequency, heifers at 3 mo of age were excluded from the saline treatments. Blood was collected at 0.5 h intervals for 4.5 h after injection, and then 2 samples were collected at hourly intervals. Heifers were only restrained in a cattle chute during collection times, afterwards returning to their pen and given access to hay and water ad libitum. Plasma was obtained as previously described in Experiment 1.

Hormone Concentration Analysis

Luteinizing hormone (LH) concentrations were determined using procedures similar to those used by Ahmadzadeh et al. (1998). Concentrations of LH were quantified by a double-antibody radioimmunoassay. The primary antibody was rabbit anti-bovine LH provided by the USDA (USDA-309-684P) at a dilution of 1:100,000. The second antibody was sheep anti-rabbit gammaglobulin and used at a dilution of 1:10 in buffer (EDTA-PBS). The standard curve consisted of eight points (.05, .1, .2, .4, .8,

1.6, 3.2, 6.4 ng/tube) in quadruplicate. The sensitivity of this assay was 0.625 ng/ml. On day one of the assay, 200 µl of sample plasma was diluted with 300 µl buffer (0.5% BSA). Two hundred µl of primary antibody was added to all tubes. Samples were gently mixed and then incubated for 24 h at 25ºC. On day two, 100 µl of ^{125}I-bLH was added to the tubes. Samples were gently mixed and then incubated for 48 h at 25ºC. On day four, 200 µl of second antibody was added to all tubes, gently mixed and then incubated for 48 h at 4ºC. On day 6, samples were diluted with 1 ml DDPBS and then centrifuged at 3000 RPM for 30 min at 4ºC. After centrifugation, the supernatant was decanted and tubes dried for at least 2 h. The pellets in the tube (bound LH) were counted on a gamma counter for 1 min. All samples were assayed in duplicate, and inter- and intra- assay CV were 9.3% and 10.2%, respectively.

Statistical Analysis

Luteinizing hormone data were analyzed using the PROC MIXED procedure of Statistical Analysis Systems software program (SAS Version 9.1 for Windows; SAS® Institute, Cary, NC) with a mixed model. In experiment 2, baseline LH, peak LH, time to peak LH, return to baseline and area under the curve (AUC) values were analyzed. Saline treated heifers at 6, 9 and 12 mo of age did not exhibit a GnRH-induced LH release, and data on these heifers was therefore not analyzed.

Area under the curve (AUC) was calculated by measuring the area under the LH curve due to the GnRH challenge for each heifer and at every age at challenge. The first two blood samples from each heifer were averaged and multiplied by 6.5 h (length of the challenge) to determine the baseline LH area. The area for LH values between two consecutive points during the challenge was calculated by averaging the two points and then multiplying this number by 0.5. Points represented LH concentrations in blood samples taken at 0.5 h intervals. For the last two values of the GnRH challenge, blood samples were taken at 1 h intervals, and therefore the average measurement was multiplied by 1 h instead of 0.5 h. If average LH points were less than the heifer's baseline, then the baseline value was used. All areas for a heifer were summed, and baseline LH area was subtracted from the total area to give a final LH area attributable

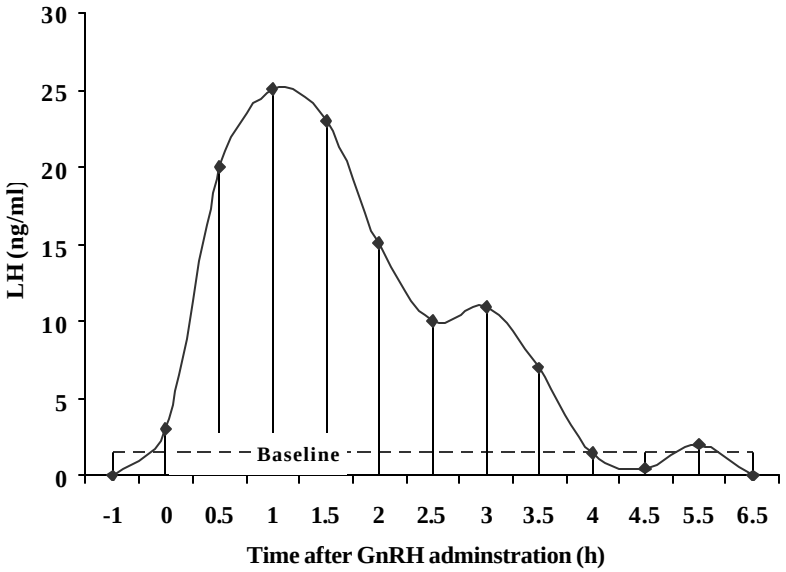

Figure 2. Area under LH curve (amount of LH measured in 6.5 h minus baseline).

to the GnRH challenge (Figure 2). Effects tested for significance were breed, age at challenge and two-way interactions. Season of birth was highly confounded with age at challenge, and was therefore omitted from the model. The final model contained significant effects of breed for time to peak LH values. The model used to evaluate baseline LH, peak LH, time to LH peak, return to baseline and AUC was:

$$Y_{ijk} = \mu + B_i + A_{(i)j} + C_k + BC_{ik} + E_{ijk} \quad [4]$$

where:

Y_{ijk} = baseline LH, peak LH, time to peak LH, return to baseline or AUC

μ = mean (Y_{ijk})

B_i = the effect of the i^{th} breed group (HH, HJ, JH, JJ)

$A_{(i)j}$ = the random effect of j^{th} heifer within the i^{th} breed

C_k = the effect of the k^{th} age at challenge (3, 6, 9, 12)

BC_{ik} = the effect of the i^{th} breed group and k^{th} age at challenge

E_{ijk} = residual

Linear, quadratic and cubic orthogonal contrasts, overall and by breed were used to determine if the response to the LH-induced response to exogenous GnRH was linear, quadratic or cubic as heifers aged from 3 mo to 12 mo. Tukey-Kramer Multiple

Comparison tests were used to compare the four sire-dam least squares means for baseline LH, peak LH, time to LH peak, return to baseline and AUC.

In the second model, breed effects were separated into additive, maternal and heterosis components. Cofficients for additive, maternal and heterosis covariates are the same as used in Experiment 1 (Table 1). Baseline LH, peak LH, time to LH peak, return to baseline and AUC were analyzed using the PROC MIXED procedure. Effects that were used in the model were age at challenge, additive, maternal and heterosis effects as well as interactions between age at challenge and each genetic effect (additive, maternal, heterosis). Season of birth was highly confounded with age at challenge, and was therefore omitted from the model. The model used to evaluate baseline LH, peak LH, time to LH peak, return to baseline and AUC was:

$$Y_{ij} = a + C_i + b_1 (A_j) + b_2 (M_j) + b_3 (H_j) + b_{i+3} (CA)_{ij} + b_{i+7} (CM)_{ij} + b_{i+11} (CH)_{ij} + E_{ij} \quad [5]$$

where:

Y_{ij} = baseline LH, peak LH, time to peak LH, return to baseline or AUC

a = intercept

C_i = the effect of the i^{th} age at challenge (3, 6, 9, 12)

b_1 = the regression of Y_{ij} on A_j

A_j = the fraction of Holstein genes in the cross for heifer j (0, 0.5, 1) (Additive)

b_2 = the regression of Y_{ij} on M_j

M_j = the fraction of Holstein genes in the dam for heifer j (0, 1) (Maternal)

b_3 = the regression of Y_{ij} on H_j

H_j = the fraction of gene pairs from different breeds for heifer j (0, 1) (Heterosis)

b_{i+n} = additional regression of Y_{ij} within each C_i (interaction of age at challenge with A, M or H)

$(CA)_{ij}$ = interaction of i^{th} age at challenge and additive genetic effect

$(CM)_{ij}$ = interaction of i^{th} age at challenge and maternal genetic effect

$(CH)_{ij}$ = interaction of i^{th} age at challenge and heterotic genetic effect

E_{ij} = residual

The interactions of age at challenge by additive, maternal and heterotic genetic effects were not significant, and therefore removed from the model. The final model

used to evaluate baseline LH, peak LH, time to LH peak, return to baseline and AUC was:

$$Y_{ij} = a + C_i + b_1 (A_j) + b_2 (M_j) + b_3 (H_j) + E_{ij} \quad [6]$$

Linear, quadratic and cubic orthogonal contrasts by breed were used to determine if the response to the LH-induced response to exogenous GnRH was linear, quadratic or cubic as heifers aged from 3 mo to 12mo. Tukey-Kramer Multiple Comparison tests were used to compare the four sire-dam least squares means for time to LH peak, which was the only response variable significantly affected by breed.

RESULTS

EXPERIMENT 1

Breed was found to significantly affect age, weight, wither height at puberty and ADWG. Least squares means for age, weight, and wither height at puberty and ADWG are shown in Table 2. Age at puberty for the HH classification was different from the HJ, JH and JJ classifications. Purebred Holstein heifers attain puberty at an older age ($P < 0.05$) than the HJ, JH or purebred Jersey heifers. Crossbred heifers attain puberty at an intermediate age between the purebred Holstein and Jersey heifers, although the differences in means between the crossbred heifers (HJ and JH) were not significant. Weight at puberty for the HH classification was 50 to 100 kg heavier than the other three sire - dam classifications. Purebred Holsteins were the heaviest in weight at puberty and were different ($P < 0.05$) from the other three sire - dam classifications. Purebred Jersey heifers were the lightest in weight at puberty, and the crossbred heifers were intermediate in weight between the purebred heifers. Reciprocal crosses did not differ in weight at puberty (Table 2). Wither height at puberty was similar to weight at puberty. The purebred Holstein heifers were the tallest in height at puberty and different ($P < 0.05$) from the other three sire - dam classifications (Table 2). The purebred Jersey heifers were the shortest in height at puberty and the crossbred heifers were intermediate for wither height between the purebred heifers. However, wither height for the crossbred heifers (HJ and JH) were not different from each other (Table 2). ADWG was measured from birth to 6 mo of age. The purebred Holstein heifers and HJ heifers had higher growth rates from birth to 6 month of age than the purebred Jersey heifers.

Table 2. Effect of breed of sire and dam on age, weight and wither height at puberty and ADWG for four different sire - dam classifications[a].

Sire	Dam	n	Age at puberty (wks)	Weight at puberty (kg)	Wither ht. at puberty (cm)	ADWG (kg/day)
Holstein	Holstein	18	48.3 ± 1.0[b]	300.9 ± 5.8[b]	122.5 ± 0.9[b]	0.72 ± 0.01[b]
Holstein	Jersey	11	43.4 ± 1.2[c]	253.9 ± 7.4[c]	115.7 ± 1.2[c]	0.69 ± 0.02[b]
Jersey	Holstein	10	42.5 ± 1.3[c]	247.2 ± 7.8[c]	114.7 ± 1.2[c]	0.66 ± 0.02[bc]
Jersey	Jersey	11	39.9 ± 1.3[c]	192.9 ± 7.6[d]	106.4 ± 1.2[d]	0.58 ± 0.02[c]

[a] Least squares means ± standard error.
[b, c, d] Means within the same column with different superscripts differ (*P< 0.05)*

However, differences were significant for the purebred Jersey heifers compared to the purebred Holstein and HJ classification. Therefore, ADWG was different (P < 0.05) between the purebred Jerseys and Holstein sired heifers. Three purebred Holstein heifers (HH) had not attained puberty by the last sampling date (wk 36). Assumed puberty was designated as the next sample after the last sampling date (wk 37) so as not to skew the data by using date at first estrus detection (15-16 mo).

Least squares means for age at puberty and weight at puberty by season of birth are shown in Table 3. Season of birth affected age and weight at puberty (P < 0.05), but not ADWG. Season of birth did not influence growth rates for the first 6 mo of age. Heifers attained puberty at an older age if born in the spring, and at a younger age if born in the other three seasons. However, the only significant differences between means

Table 3. Effect of season of birth on age and weight at puberty for four different seasons[a].

Season	Age at puberty (wk)	Weight at puberty (kg)
Fall	41.8 ± 1.2[b]	236.8 ± 7.4[b]
Spring	46.6 ± 1.3[c]	275.8 ± 7.8[c]
Summer	42.9 ± 1.1[bc]	240.3 ± 6.6[b]
Winter	42.7 ± 1.1[bc]	242.0± 6.6[b]

[a] Least squares means ± standard error.
[b, c] Means within the same column with different superscripts differ (*P< 0.05)*

existed between the fall and spring. Weight at puberty in the spring was different (P< 0.05) from the other three seasons, indicating that heifers were heavier in weight when attaining puberty if born in the spring. There were no differences in weight at puberty for heifers born in the fall, summer, or winter.

Distribution of the onset of puberty for the four sire - dam classifications by month is depicted in Figure 3. The HJ and JH classifications had even distribution from < than 9 mo of age to > than 12 mo of age (Figure 3). The purebred heifers (HH and JJ) were unevenly distributed regarding the onset of puberty. The majority of purebred Jersey heifers (6 of 11 heifers) attained puberty at 9 mo of age. The majority of purebred Holstein heifers (12 of 18 heifers) attained puberty from 11 to 12 mo of age. This uneven distribution is evident when considering the differences in age at puberty for the four sire - dam classifications as seen in Table 1.

The distribution of the season of birth for the four sire - dam classifications by season is depicted in Figure 4. Season of birth was evenly distributed among the four

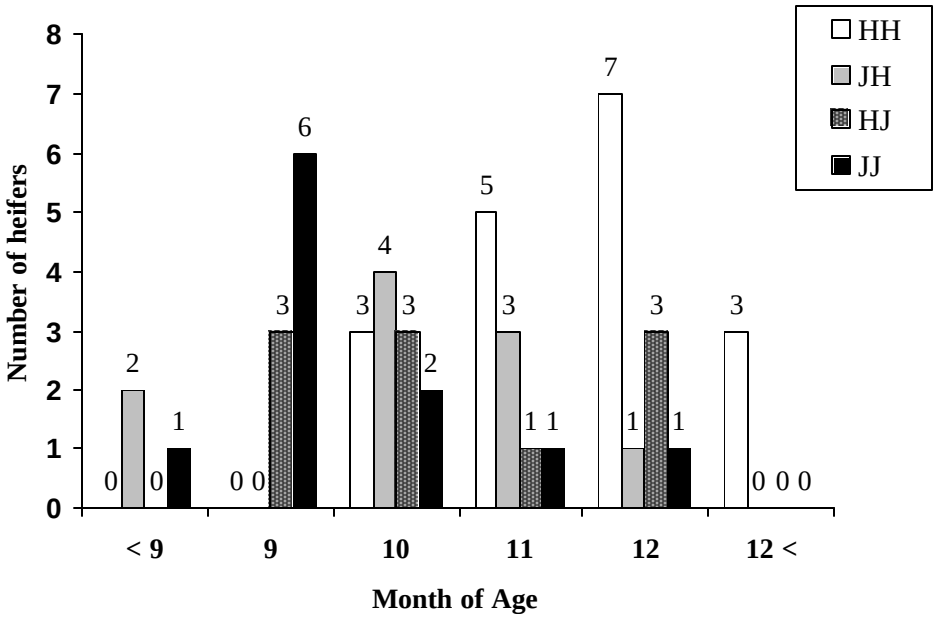

Figure 3. Distribution of the onset of puberty for the four sire - dam classifications by month of age.

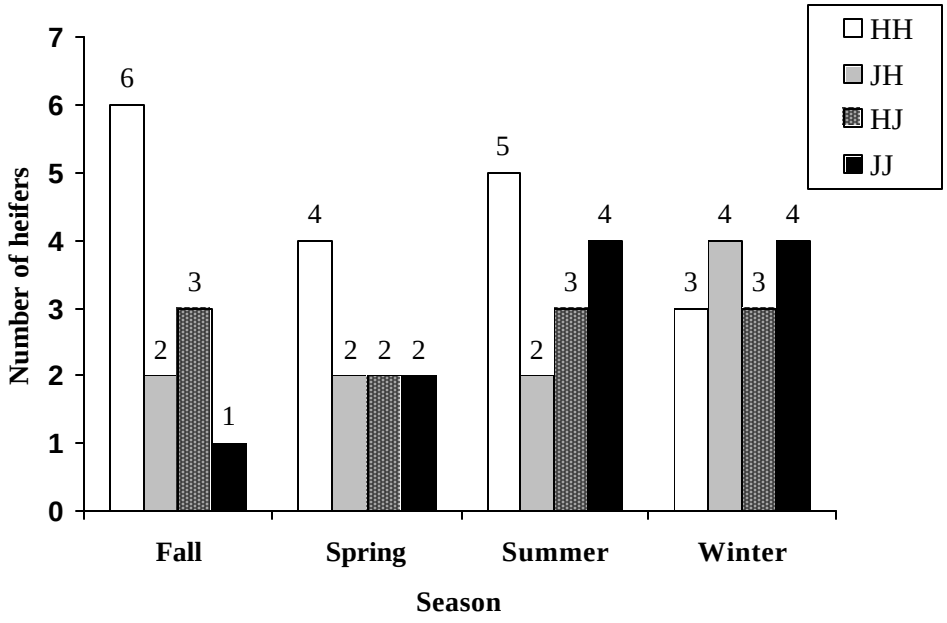

Figure 4. Distribution of the season of birth for the four sire - dam classifications by season.

sire - dam classifications, with an exception of the fall season. In this experiment, there was no control over breeding date of the dam and therefore season of birth of the heifer.

Evaluation of additive, maternal and heterosis components are shown in Table 4. The additive estimates were significant for age, weight and wither height at puberty and ADWG while the maternal and heterosis parameters were not significant (Table 4). Additive estimates for age at puberty (Table 4) indicate that purebred Jersey heifers attain puberty 9.4 wk earlier than purebred Holstein heifers. Additive estimates for weight at puberty indicate that purebred Jersey heifers attain puberty at a weight that is 114.5 kg lighter than purebred Holsteins, for wither height at puberty are 17.1 cm shorter than purebred Holstein heifers, and for ADWG gain 0.17 kg/day less than purebred Holstein heifers before 6 mo of age. Therefore, significant additive estimates indicated that crossbred heifers are intermediate between the parent breeds and genetic control is primarily additive. While not significant, maternal estimates indicated a reduced age at puberty and reduced growth parameters (weight, wither height, ADWG) from the purebred Holsteins. Heterosis estimates, also not significant, indicated a reduced age at puberty for the crossbred heifers, and increased growth parameters in comparison to the purebred Holstein and Jersey heifers.

Table 4. Prediction equations for additive, maternal, and heterosis components for age, weight and wither height at puberty and ADWG for all four sire - dam classifications.

Effect	Estimate	Standard Error	Pr > F
	Age at puberty (wk)		
Additive[a]	9.38	2.41	0.004
Maternal[b]	-0.92	1.78	NS
Heterosis[c]	-1.16	1.20	NS
	Weight at puberty (kg)		
Additive	114.75	14.54	0.001
Maternal	-6.76	10.73	NS
Heterosis	3.64	7.14	NS
	Wither height at puberty (cm)		
Additive	17.05	2.27	0.001
Maternal	-0.94	1.67	NS
Heterosis	0.77	1.11	NS
	ADWG (kg/d)		
Additive	0.17	0.04	0.002
Maternal	-0.03	0.03	NS
Heterosis	0.02	0.02	NS

[a]Estimates for additive effects represent purebred Holsteins minus purebred Jerseys.
[b]Estimates for maternal effects represent Holstein dams minus Jersey dams.
[c]Estimates for heterosis effects represent crossbreds minus purebreds.

.

EXPERIMENT 2

The effects of breed, age at challenge, and their interaction did not influence baseline LH, peak LH, time to LH peak, return to baseline and AUC. However, breed did affect time to LH peak ($P < 0.05$).

Least squares means for the characteristics in changes in LH variables by age at challenge, although not significant, are shown in Table 5. Least squares means for the effect of breed on baseline LH, peak LH, time to LH peak, return to baseline and AUC are shown in Table 6. The effect of breed was significant for time to LH peak. Tukey-Kramer multiple comparison tests revealed that HJ and the JJ classifications were the only two different from each other. There may be an effect of the sire to cause significant differences of time to LH peak.

Table 5. Characteristics of changes in LH levels in response to GnRH treatment in prepubertal heifers of four different sire-dam classifications at different ages[a].

Breed[b] of Sire	Dam	n	Baseline LH (ng/ml)	Time to LH peak (h)	Peak LH (ng/ml)	AUC[c]	Return to baseline (h)
Age: 3 mo							
H	H	4	0.00 ± 0.17	1.5 ± 0.2	19.7 ± 4.0	38.2 ± 7.9	3.8 ± 0.5
H	J	4	0.42 ± 0.17	1.8 ± 0.2	15.2 ± 4.0	29.7 ± 7.9	3.0 ± 0.5
J	H	4	0.08 ± 0.17	2.0 ± 0.2	23.1 ± 4.0	44.9 ± 7.9	3.5 ± 0.5
J	J	4	0.00 ± 0.17	1.1 ± 0.2	21.4 ± 4.0	43.2 ± 7.9	4.0 ± 0.5
Age: 6 mo							
H	H	3	0.05 ± 0.04	2.1 ± 0.4	19.8 ± 4.1	48.1 ± 13.0	3.5 ± 0.7
H	J	3	0.00 ± 0.04	1.3 ± 0.4	16.5 ± 4.1	35.5 ± 13.0	4.5 ± 0.7
J	H	3	0.05 ± 0.04	1.2 ± 0.4	30.6 ± 4.1	67.3 ± 13.0	4.7 ± 0.7
J	J	3	0.00 ± 0.04	1.0 ± 0.4	22.1 ± 4.0	49.5 ± 13.1	4.9 ± 0.7
Age: 9 mo							
H	H	3	0.14 ± 0.06	1.3 ± 0.4	25.4 ± 7.1	63.9 ± 18.9	4.0 ± 0.6
H	J	3	0.00 ± 0.06	2.1 ± 0.4	33.5 ± 7.0	72.4 ± 19.0	3.8 ± 0.6
J	H	3	0.05 ± 0.06	1.8 ± 0.4	28.8 ± 7.0	68.6 ± 19.0	4.4 ± 0.6
J	J	3	0.17 ± 0.06	1.5 ± 0.4	29.3 ± 7.0	70.5 ± 19.1	4.6 ± 0.6
Age: 12 mo							
H	H	3	0.32 ± 0.22	1.6 ± 0.5	23.4 ± 10.5	40.9 ± 19.2	3.3 ± 0.6
H	J	3	0.13 ± 0.22	2.5 ± 0.5	38.0 ± 10.4	67.3 ± 19.3	3.6 ± 0.6
J	H	3	0.43 ± 0.22	1.3 ± 0.5	36.6 ± 10.4	58.6 ± 19.3	4.4 ± 0.6
J	J	3	0.09 ± 0.22	1.1 ± 0.5	24.3 ± 10.5	48.1 ± 19.2	4.0 ± 0.6

[a] Least squares means ± standard error.

[b] H = Holstein, J = Jersey

[c] AUC = Area under the curve of LH release in response to GnRH challenge

Linear and quadratic orthogonal contrasts by breed are graphically shown in Figures 5-8. Baseline LH increased quadratically ($P = 0.08$) for the HJ classification only (Figure 5). Peak LH increased in a linear fashion ($P < 0.05$) with age for the HJ heifers (Figure 6). Time to peak LH increased linearly ($P = 0.08$) with age for the HJ heifers (Figure 7). There were no significant relationships when examining return to baseline values for any of the four sire - dam classifications. For AUC, there was a significant linear relationship ($P < 0.05$) for the HJ classification (Figure 8). The AUC increased linearly with age for the HJ heifers. The results of the linear, quadratic and cubic orthogonal contrasts for age at challenge across all breeds are graphically shown in Figures 9-11. Baseline LH means were not different across all ages challenged (3, 6, 9,

Table 6. Characteristics of changes in LH levels in response to GnRH treatment in prepubertal heifers of four different sire - dam classifications over all ages[a].

Breed[e] of Sire	Dam	n	Baseline LH (ng/ml)	Time to LH peak (h)	Peak LH (ng/ml)	AUC[f]	Return to baseline (h)
H	H	13	0.13 ± 0.07	1.6 ± 0.2[bc]	22.1 ± 3.6	47.8 ± 9.2	3.7 ± 0.4
H	J	13	0.14 ± 0.07	1.9 ± 0.2[b]	25.8 ± 3.7	51.2 ± 9.5	3.7 ± 0.4
J	H	13	0.15 ± 0.07	1.6 ± 0.2[bc]	29.8 ± 3.7	59.9 ± 9.5	4.3 ± 0.4
J	J	13	0.06 ± 0.07	1.2 ± 0.2[c]	24.3 ± 3.7	52.8 ± 9.4	4.4 ± 0.4

[a] Least squares means ± standard error.
[b, c, d] Means within the same column with different superscripts differ (*P < 0.05*).
[e] H = Holstein, J = Jersey
[f] AUC = Area under the curve of LH release in response to GnRH challenge

and 12 mo of age). Peak LH increased linearly (P < 0.05) with increasing age for all heifers (Figure 9). There were no differences in time to LH peak across ages. Time to returning to baseline levels of LH showed a significant quadratic effect (P < 0.05) when examined over all ages at the challenge (Figure 10). The AUC for LH response increased quadratically (P = 0.07) with age for all sire - dam classifications (Figure 11).

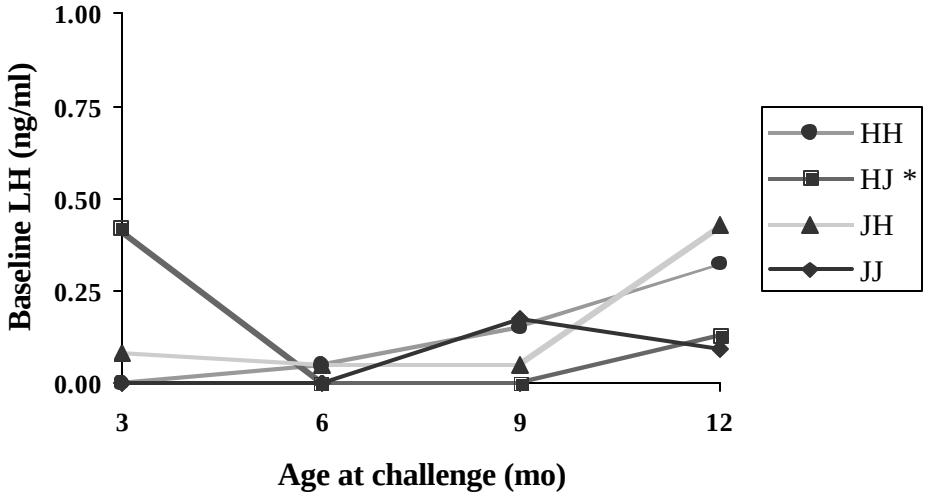

Figure 5. Baseline LH for the four sire - dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge. * Trend (P = 0.08) toward a quadratic effect for the HJ sire - dam classification.

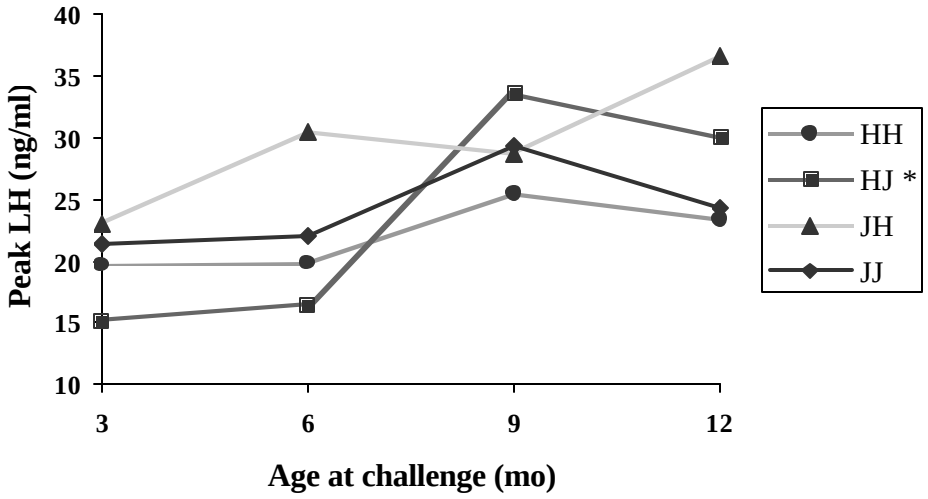

Figure 6. Peak LH for the four sire - dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge. * Linear increasing effect ($P < 0.05$) for the HJ sire - dam classification.

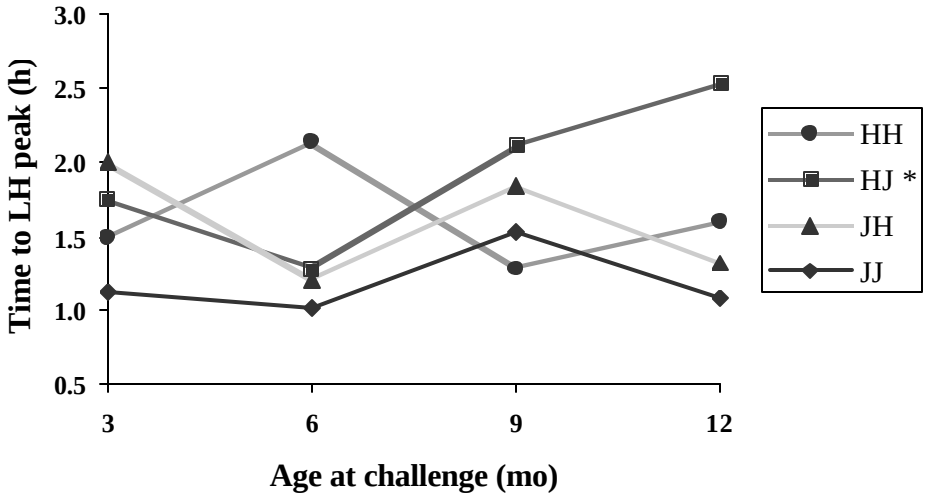

Figure 7. Time to LH peak for the four sire - dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge. * Trend of a linear effect ($P = 0.08$) for the HJ sire - dam classification.

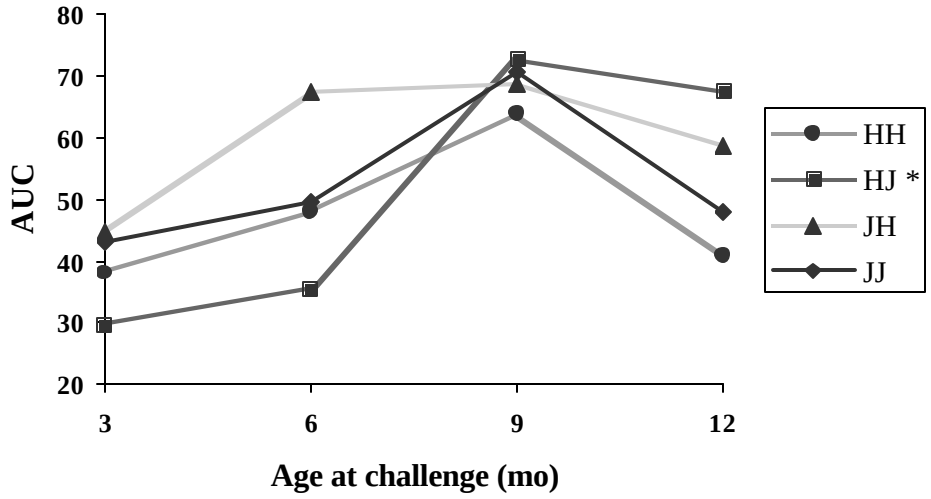

Figure 8. AUC for the four sire – dam classifications (breed, Holstein (H), Jersey (J)) by age at challenge. * Linear effect ($P < 0.05$) for the HJ sire - dam classification.

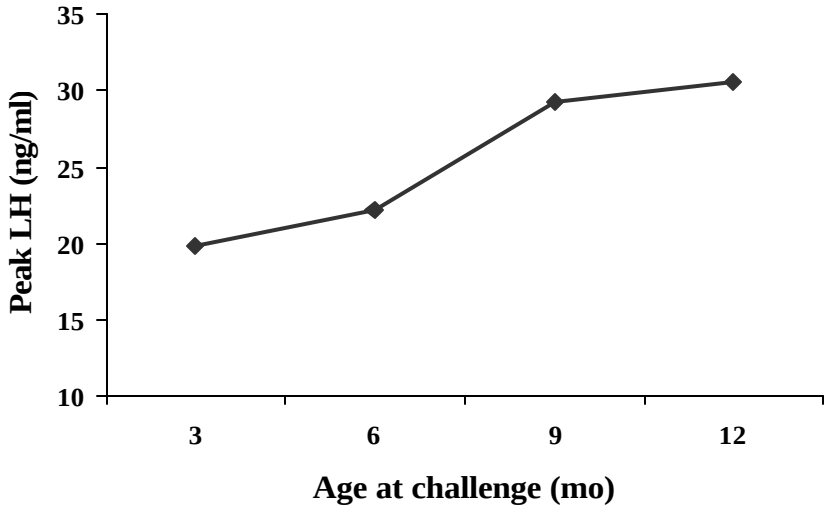

Figure 9. Peak LH for all four sire - dam classifications at different age at challenge. * Significant linear effect ($P < 0.05$).

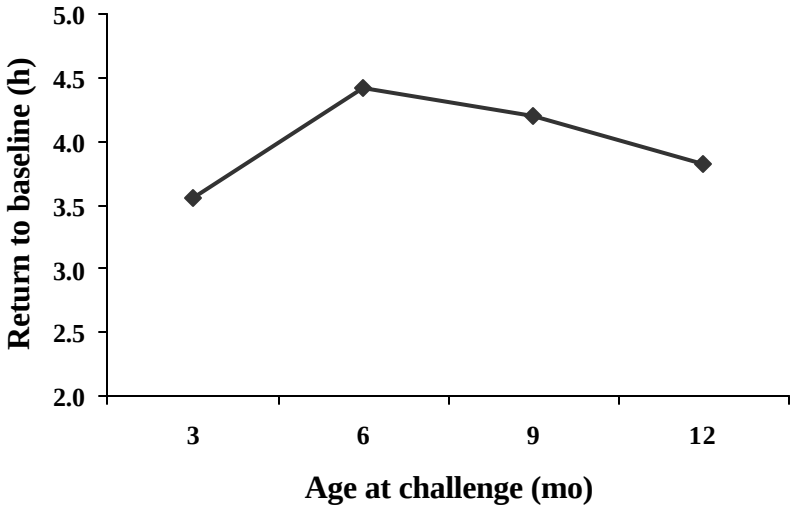

Figure 10. Return to baseline for all four sire - dam classifications at different age at challenge. * Significant quadratic effect ($P < 0.05$).

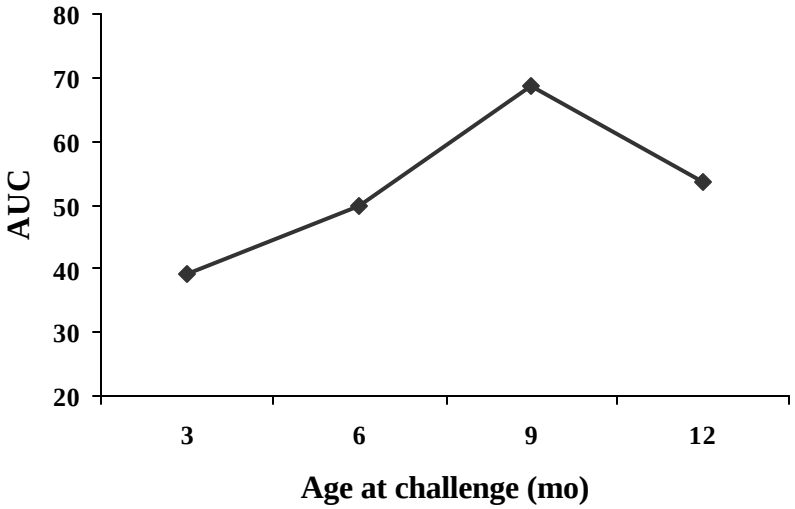

Figure 11. AUC for all four sire - dam classifications at different age at challenge. * Trend of a quadratic effect ($P = 0.07$).

Table 7. Prediction equations for additive, maternal, and heterosis components for baseline LH, peak LH, time to LH peak, return to baseline and AUC for all sire - dam classifications and age at challenge.

Effect	Estimate	Standard Error	Pr > F
	Baseline LH (ng/ml)		
Additive[a]	0.00	0.06	NS
Maternal[b]	0.04	0.05	NS
Heterosis[c]	-0.02	0.03	NS
	Peak LH (ng/ml)		
Additive	-10.70	6.16	NS
Maternal	8.57	4.35	0.07
Heterosis	1.65	3.08	NS
	Time to LH peak (h)		
Additive	1.73	1.07	0.03
Maternal	-1.19	0.76	NS
Heterosis	0.58	0.54	0.05
	Return to baseline (h)		
Additive	-1.10	0.76	NS
Maternal	0.57	0.54	NS
Heterosis	-0.19	0.38	NS
	AUC[d]		
Additive	-19.06	15.16	NS
Maternal	14.26	10.74	NS
Heterosis	0.18	7.58	NS

[a]Estimates for additive effects represent purebred Holsteins minus purebred Jerseys.
[b]Estimates for maternal effects represent Holstein dams minus Jersey dams.
[c]Estimates for heterosis effects represent crossbreds minus purebreds.
[d] AUC = Area under the curve of LH release in response to GnRH challenge

Evaluation of additive, maternal and heterosis components are depicted in Table 7. These genetic components were not significant for baseline LH, return to baseline and AUC values (Table 7). Additive estimates for time to peak LH ($P < 0.05$) indicate that purebred Jersey heifers reach peak LH (after GnRH administration) 1.73 h earlier than purebred Holsteins. Heterosis estimates for time to peak LH ($P < 0.05$) indicate that the crossbred heifers (HJ and JH) exceeded the purebred Holstein and Jersey heifers by reaching peak LH 0.58 h sooner (Table 7). There was a trend for peak LH values ($P = 0.07$) for maternal genetic components. These maternal estimates indicate that peak LH

may have an advantage from Holstein dams, and that purebred Holstein heifers and JH heifers have an LH peak that is 8.57 ng/ml greater than heifers with a Jersey dam (purebred Jersey heifers and HJ).

Means for LH concentrations for each blood sample for all ages at challenge and sire - dam classifications, although not statistically analyzed, are shown in Figures 12-15.

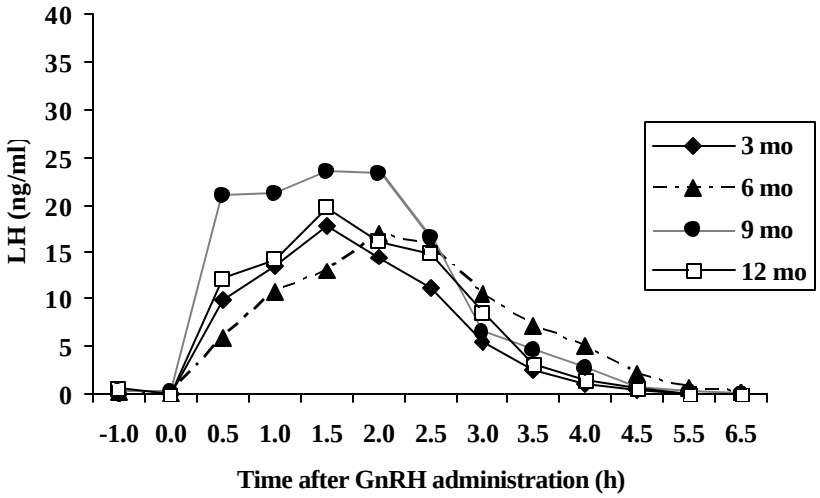

Figure 12. Changes in LH leves in response to exogenous GnRH at all ages at challenge for the HH sire - dam classification.

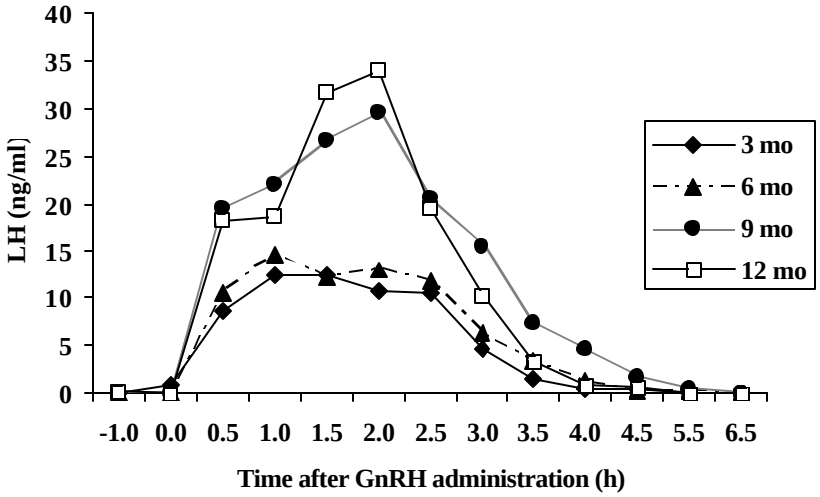

Figure 13. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the HJ sire - dam classification.

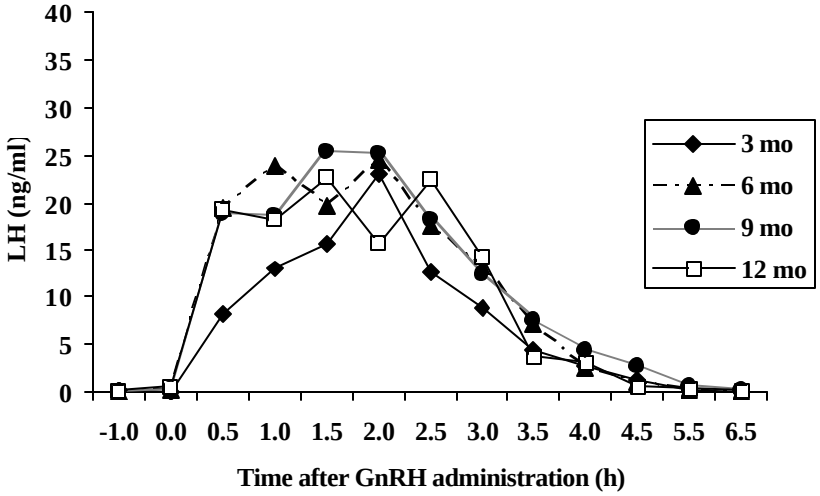

Figure 14. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the JH sire - dam classification.

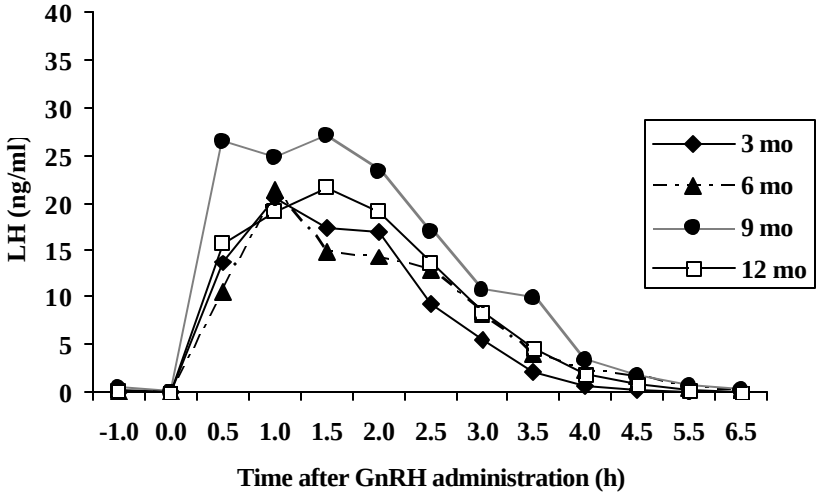

Figure 15. Changes in LH levels in response to exogenous GnRH at all ages at challenge for the JJ sire - dam classification.

DISCUSSION

Differences in age at onset of puberty and growth parameters among different breeds have been well documented in beef heifers (Wiltbank et al., 1966; Laster et al., 1976, 1979) and to a lesser extent in dairy heifers (Stelwagen et al., 1990; Sejrsen et al., 1996). In this study, breed was found to have a significant effect on age at puberty. Age at puberty was significantly different between the purebred Holsteins and the other three sire - dam classifications. There was no difference between the other three sire -dam classifications, therefore purebred Jersey heifers were not different from the crossbred heifer groups (HJ and JH), and the crossbred heifer groups were not different from each other regarding age at puberty. Contrary to our findings, Nelsen et al. (1982) reported that age at puberty was not different between purebred Holstein and Jersey heifers. Research on beef heifers (Laster et al., 1976, 1979) has shown differences at ages at puberty in reciprocal crosses of Angus and Hereford breeds. There were differences in age at puberty of 35 $\pm$ 4 days ($P < 0.01$) in heifers from Angus than Hereford dams, and a difference of 32 $\pm$ 6 days for heifers born from Hereford sires and Angus dams verses the reciprocal mating (Angus sires and Hereford dams). They also used beef X dairy crosses and concluded that it appeared that heifers of dairy breeds with a higher genetic ability for milk production reach puberty at younger ages than heifers of other breeds. These studies (Laster et al., 1976, 1979) incorporated Brown Swiss and Jersey into the experiments for comparison and noted that they were the youngest at puberty compared to the other beef breeds. Spelman et al. (2004) reported in records of New Zealand crossbred Holstein X Jersey cattle that age at puberty (through blood samples and estrus detection) had a large phenotypic variation from 155 d to 470 d. Age at puberty in the present study showed an intermediate effect of age at puberty between the purebred heifers; however, these differences were not significant. Similar results by Nelsen et al. (1982) showed an intermediate effect of age at puberty for Holstein X Jersey heifers verses purebred heifers, however differences between sire - dam classifications were not evaluated to determine significance. Previous studies (Laster et al., 1976, 1979) worked with a larger number of heifers (reciprocal crosses n = 89). This study only had 50 heifers, and the purebred Holsteins represented the largest percentage of this number (n = 18). A smaller sample size may have masked significant differences.

Breed was also found to have a significant effect on growth parameters such as weight at puberty, wither height at puberty and ADWG. These growth parameters show that purebred Holstein and Jersey heifers are significantly different when examining weight at puberty and wither height at puberty, which is similar to results by Nelsen et al. (1982). Weight at puberty and wither height at puberty were not statistically different among the crossbred heifers, which was the same effect as seen with age at puberty. The ADWG was significantly different between purebred Jerseys (0.58 $\pm$ 0.02 kg/d) and the Holstein sired heifers (0.72 $\pm$ 0.01 kg/d (HH) and 0.69 $\pm$ 0.02 kg/d (HJ)). Laster et al. (1979) reported differences in growth rates between breeds, and larger later maturing breeds grew faster as age increased than breeds that are more intermediate, which would explain why the growth parameters of all four sire - dam classifications were different. From this information, one would assume the larger maturing breed to be purebred Holstein and the smaller to be purebred Jersey. Matthews et al. (1975) examined external and internal anatomy (body weight, external dimensions, weights of organs including heart, intestines, stomach, endocrine glands, udder) of Holsteins and Jerseys and reported that Jerseys matured 1 to 2% faster than Holsteins. Further research on purebred Jersey heifers and crossbred Holstein and Jersey heifers needs to be conducted to determine relationships of growth parameters that facilitate the onset of puberty.

Age at puberty was not influenced by weight at puberty, wither height at puberty and ADWG. These results were unexpected because of the extensive literature that links onset of puberty to different growth parameters (Menge et al., 1960; Wiltbank et al., 1966; Valentine et al., 1987; Stelwagen et al., 1990; Sejrsen et al., 1996). Stelwagen et al. (1990) reported that age at puberty declined linearly with increasing average daily gain in Holstein heifers. These heifers gained 0.61, 0.74 and 0.90 kg/d and attained puberty at 365, 313 and 305 d, respectively. Despite the high association with pre-pubertal growth and age at puberty (Laster et al., 1979), and despite the idea that weight can be one of the limiting factors in determining age at puberty (Wiltbank et al., 1966), there are a few potential reasons as to why growth parameters had no significant effect on age at puberty in this study. Menge et al. (1960) reported that there are significant amounts of variation in the age at which an animal attains puberty. These have been ascribed both to genetic and to certain identifiable environmental factors. Therefore it is

important to understand both the genetic factors that regulate age and body weight at onset of puberty varies widely within as well as between breeds (Sejrsen et al., 1996). Foldager et al. (1988) reported in a study of Friesian and Danish Red heifers that onset of puberty ranged from 5 to 6 up to 18 to 20 mo of age. The variation in weight at puberty was 150 to 400 kg and growth rate ranged from 450-800 g/d. Therefore, while growth parameters do have an effect on age at puberty, the presence of variation within each of the four sire - dam classifications might have reduced significance of these growth parameters in relation to age at puberty. Wiltbank et al. (1966) reported that after a certain critical weight is reached, variation in average daily gain has little or no effect on age at puberty; this critical weight can vary between heifers within the same breed and same management practices (Frisch and Revelle, 1970). Other factors that regulate the onset of puberty, such as breed and season of birth in this study, may play more of a role in determining differences in age at puberty. Another potential reason as to why growth parameters had no significant effect on age at puberty in this study may be the sampling method. Weights were collected monthly and could have decreased the accuracy of ADWG; weekly measures of weight may have given a more accurate measure of ADWG and possible differences in age at puberty.

Season of birth had a significant effect on age and weight at puberty, but not on wither height at puberty or ADWG. Age at puberty for heifers born in the spring was significantly different from age at puberty for heifers born in the fall; 46.6 $\pm$ 1.3 verses 41.8 $\pm$ 1.2 wk, respectively. However, differences between other seasons were not significant. These results agree with previous work done by Schillo et al. (1983) on twenty-eight Angus X Holstein crosses. Heifers born in September (autumnal equinox) reached puberty at a younger age than those born in March (spring/vernal equinox). Age at puberty for the summer and winter were not different from the spring or fall seasons. Sexual development in heifers occurs over a few seasons; therefore season of birth is confounded with season at subsequent stages of development, or in this study, season of puberty (Hansen 1985). Heifers with the genetic ability to attain puberty at early ages may be affected by season of birth differently from those that attain puberty at older ages (Hansen 1985). Favorable environmental conditions will also hasten the onset of puberty, to where exposure to spring - summer conditions of increasing day length and

temperature results in early onset of puberty verses autumn winter conditions of decreasing day length and temperature (Schillo et al., 1983).

Weight at puberty for heifers born in the spring was significantly different (275 $\pm$ 7.8 kg) from weight at puberty for heifers born in the other three seasons (236.8 $\pm$ 7.4 kg (fall), 240.3 $\pm$ 6.6 kg (summer), 242.0 $\pm$ 6.6 kg (winter)). This was expected because heifers that were born in the spring were older at puberty, and animals which are older at puberty are usually heavier at puberty (Frisch and Revelle, 1970).

Genetic estimates indicated an additive effect of age, weight and wither height at puberty and ADWG. Genetic variation is separated into additive and nonadditive components (Fuerst and Sölkner, 1994). In terms of production traits such as milk yield and milk fat percentage, heterosis and additive effects are significant and cause genetic variation within and between breeds, with heterosis being the primary factor (Ahlborn-Breier and Hohenboken, 1991). In terms of production traits regarding onset of puberty, additive effects found in the study contrast previous studies that found significant effects of heterosis on age at puberty, weight at puberty and wither height at puberty (Martin et al., 1992; Nelsen et al., 1982; Laster et al., 1976). Heterosis would most likely cause crossbred heifers to reach puberty at younger ages and heavier weights than the average of their straight bred counterparts (Martin et al., 1992). In this study, crossbred heifers were intermediate for age and weight at puberty between their straightbred counterparts. However, even previous literature has discrepancies regarding genetic variation. Laster et al. (1976) reported significant heterosis effects in age at puberty in Hereford-Angus cross heifers, but not for weight or wither height at puberty. On the other hand, Nelsen et al. (1982) reported significant heterosis effects for weight and wither height at puberty, but not for age at puberty in Angus, Brahman, Hereford, Holstein and Jersey breeds and all possible two-breed crosses. While there was no single cross which exhibited significant heterosis for age, weight and wither height at puberty, crossbred heifers in this study tended to be younger, heavier, and taller at puberty than their straightbred counterparts. Similar results were found by Gregory et al. (1978).

Discrepancies that might have changed the outcomes of the present study, and the studies by Laster et al., (1976) and Nelsen et al. (1982) were sample size, and the method of detection of onset of puberty. There were fewer crossbreds in the present study (21

heifers) and in Laster et al. (1976) (28 heifers), where in Nelsen et al. (1982) there were 400 crossbred heifers divided into 10 different crossbred groups of different sizes. Larger heifer numbers in the present study, and a larger frequency of weight and wither height measurements might have identified significant differences between the crossbreds and significant effects of heterosis. The other discrepancies between the present study, Laster et al. (1976), and Nelsen et al. (1982) were the methods of detection of onset of puberty. Laster et al. (1976) defined puberty as first observed standing estrus. Nelsen et al. (1982) defined puberty as the presence of a corpus luteum (CL) by rectal palpation at 21-d intervals from 7 mo of age until puberty. The present study defined puberty as the presence of a corpus luteum by examining plasma P_4 levels by blood collection at 7-d intervals from 4 mo of age until 12 mo of age. Age at puberty in other studies may measure different traits (age at puberty, age at first behavioral estrus, and age at which behavioral estrus is first detected). This could affect how the significance of genetic components (additive, maternal, heterosis) was determined, and explain the differences found between the literature regarding genetic components of age, weight and wither height at puberty and ADWG.

The GnRH challenges in this study showed that prepubertal heifers as young as 3 mo of age have the ability to secrete LH in response to exogenous GnRH; this capacity has been shown to occur at younger ages in prepubertal heifers (Nakada et al., 2002). When examining baseline LH, peak LH, time to LH peak and AUC, these values were not significant for the effects of breed, age at challenge or their interaction. The HJ sire - dam classification had significant linear effects for peak LH, AUC, and trends of a linear effect for time to LH peak and quadratic for baseline LH; these effects were not seen in the other three sire - dam classifications. These findings were not expected. The crossbred heifers (HJ and JH) were not considered statistically different in terms of age, weight, wither height at puberty or ADWG; similar results would be expected from the GnRH challenges. From a biological view, interpretation of why HJ was the only sire - dam classification with significant linear and quadratic relationships is difficult.

When examining relationships using age at challenge over all four classifications, there was a significant linear effect for peak LH, a significant quadratic effect for return to baseline and a trend of a quadratic effect for AUC. Regarding this study, linear

relationships were expected for all variables that were examined as age at challenge increased. Nakada et al. (2002) examined the GnRH induced LH response at 1, 2, 4, 6 and 8 mo of age in 50 prepubertal Holstein heifers. Of all the variables that were examined (basal LH, time to LH peak, peak LH and AUC) all linearly increased with age. These results indicated that the capacity of LH release in response to GnRH in the anterior pituitary gland develops with age. LH levels increased 30 min after GnRH administration with age as corresponding with an increase in LH accumulation in the pituitary gland, indicating that the responsiveness for LH release to GnRH increases with age. Earlier research by Barnes et al. (1980) reported no differences in responses of GnRH induced LH release when examining heifers at 3, 6 and 9 mo of age. While peak LH increased with age, these differences were not significant, and AUC was not measured in this study. These researchers concluded that the prepubertal heifer is capable of secreting large amounts of LH in response to GnRH administration; however this response does not increase with age. Barnes et al. (1980) and the current study administered 200 µg GnRH at each age and did not dose according to BW for each heifer, whereas Nakada et al. (2002) dosed GnRH as one microgram per 1 kg BW in 5 ml saline. These differences in administration of GnRH might indicate a dilution effect of the GnRH explaining why there was no increase in response due to age in Barnes et al. (1980) as well as in the present study. Another factor that may have affected results of this study include route of administration of GnRH. Nakada et al. (2002) and Barnes et al. (1980) administered GnRH i.v. (intravenously) verses i.m. (intramuscularly) in the present study. Kaltenbach et al. (1974) reported that 250 µg of GnRH given i.m. was as effective as 1mg given intracarotidly in a study of Hereford, Angus and crossbred heifers weighing between 350 to 427 kg; however, these heifers were post-pubertal. Arimura et al. (1973) reported that in men, 100 µg GnRH given either subcutaneously or intravenously produced similar increases in serum LH, indicating that the route of administration may not be critical. Wenzel et al. (2002) reported an increase in plasma LH concentrations 15 min after i.m. injection of GnRH with peak LH ranging from 32.5 to 50.4 ng/ml occurring within 2 h after injection. By five h serum LH was declining toward pre-injection concentrations. These results are similar to those found in the

present study (Table 4). Therefore route of administration of GnRH (i.m. verses i.v.) was not likely a factor that caused any differences between the four sire - dam classifications.

Nakada et al. (2002) reported that the onset of puberty is thought to be caused by the development of a GnRH releasing pattern similar to sexually mature cattle. This would assume that prepubertal LH activity would not drastically change within the months directly after puberty. In the current study, over all heifers used, AUC increased from 3 to 9 mo of age, and then decreased at 12 mo of age. This trend of a quadratic effect was not expected; AUC values at 12 mo of age were expected to either be at similar levels at 9 mo of age or greater. Nakada et al. (2002) reported increasing AUC values in heifers from 1 to 8 mo of age. However in the current study, heifers were challenged with exogenous GnRH before and after puberty. McLeod et al. (1984) conducted a study using six 4 mo old and six 10 mo old heifers. Two animals of each age group were subjected to nine consecutive injections of 0.5, 2.0 or 5.0µg GnRH at 2 h intervals. The mean maximum LH concentration of episodes attained in heifers in this study in response to the 5 µg GnRH dose (7.01 $\pm$ 0.23 ng/ml, 36 episodes) was significantly greater ($P < 0.001$) than that reported for mature, acyclic post-partum cows (2.63 $\pm$ 0.16 ng/ml, 35 episodes) (Riley et al., 1981). There were differences in responses to exogenous GnRH on animals prior to and after puberty in this study and may be one explanation as to why AUC decreased at 12 mo of age in the present study. However, the studies by McLeod et al. (1984) and Riley et al. (1981) cannot be directly compared due to differences in physiological and nutritional states of the animals used.

Pituitary refractoriness and depletion was examined as a possible explanation for decreasing AUC values at 12 mo of age in all four sire - dam classifications. Pituitary refractoriness is caused by a desensitization where the pituitary gland becomes insensitive to further exogenous GnRH administrations. This is caused by down regulation of GnRH receptors, and long term continuous administration can cause depletion of releasable stores of LH in the pituitary (Nett et al., 2002). Crowder et al. (1986) reported a 50% decrease in the number of receptors for GnRH after a 24 h i.v. infusion of GnRH (2.5 micrograms/h) in ewes. The number of receptors for GnRH was restored to control values by 6 h after the infusion, and the amount of LH released in response to an i.v. injection of 100 micrograms GnRH was reduced by 82% at the end of

the infusion period. A decrease in both the amplitude and frequency of endogenous pulses of LH was observed from 0 to 12 h after the end of the infusion period. These researchers among others (Heber and Swerdloff, 1981; Zilberstein et al., 1983) indicate that continuous exposure to GnRH may inhibit the hypothalamic pulse generator as well as the pituitary response to the pulse generator. Other research (Riley et al., 1981; Lamming and McLeod, 1988) has shown contrasting evidence regarding pituitary refractoriness and depletion; however, this does not directly relate to the present study. Few studies have been published regarding pituitary refractoriness and depletion when a larger single GnRH dose is administered, as opposed to long term continuous administration. If research on the pituitary responsiveness to long term continuous GnRH administration is not fully understood among contrasting researchers, then it cannot be assumed that short-term administration would either cause or not cause pituitary refractoriness and depletion. Kaltenbach et al. (1974) reported a failure to stimulate pituitary release of LH and FSH when 250µg (i.m.) GnRH was given near the end of a non-induced ovulatory surge. This could be evidence of pituitary depletion in the heifers on the study. However, it cannot be assumed and is highly unlikely that all heifers in the present study at 12 mo of age had a non-induced ovulatory surge just prior to the GnRH challenge. This would explain the decrease in AUC; however, AUC values were still relatively high for evidence of pituitary refractoriness.

Ultimately from a statistical point of view, the lack of significance for breed, age at challenge and the interaction when examining baseline LH, peak LH, time to LH peak, return to baseline and AUC was likely due to low heifer numbers and due to the wide variation of response within and between sire - dam classifications. At 3 mo of age, there were 4 heifers per sire - dam classification, and when using saline treated heifers, this number decreased to 3 heifers per sire - dam classification at 6, 9 and 12 mo of age. Similarly, for Experiment 1, there were only 18 HH, 11 JJ, 10 JH and 11 HJ. While this is a total of 50 heifers, there were not enough heifers per sire - dam classification to determine differences regarding age at puberty between the two crossbred groups and purebred Jerseys; age at puberty was the primary interest in the first experiment and results only showed differences from the purebred Holstein heifers. McLeod et al. (1984) administered GnRH at varying dosages and at different ages to prepubertal

heifers. There were only 2 animals per dose group, and only two different ages. The researchers noted that with 2 heifers per GnRH dose, it was not possible to determine an age effect in the LH response, and this could be directly related to the present study.

CONCLUSIONS

Purebred Holstein heifers attain puberty at a significantly older age than crossbred heifers (HJ and JH) and purebred Jersey heifers. However, differences in age at puberty between the crossbred heifers and purebred Jersey heifers were not significant. Season of birth had a significant effect on age and weight at puberty, with heifers born during long day photoperiods (spring) attaining puberty at older ages than heifers born during short day photoperiods (fall). Heifers born in the spring were also heavier when attaining puberty than heifers born in the other three seasons. Age, weight and wither height at puberty and ADWG are affected by additive genetic components, meaning that these effects in the crossbred heifers was the sum of the average effects of these individual estimates from the purebred Holstein and Jersey breeds. Previous work produced inconsistent results of the effects of heterosis on age, weight and wither height at puberty. However, differences in methods of detection of puberty may contribute to the variable results.

Prepubertal heifers demonstrated the ability to secrete LH in response to exogenous GnRH as young as 3 mo of age. Previous research has shown this response to be as early as 1 mo of age. When examining breed, age at challenge and the interaction of the two, there was no difference regarding baseline LH, peak LH, return to baseline and AUC. However, breed did affect time to LH peak. There were significant linear and quadratic effects of age on peak LH, baseline LH and AUC, meaning that with increasing age these measured variables either increased in a linear or quadratic fashion. The ability of the prepubertal heifer to secrete LH in response to exogenous GnRH increased with age from 3 mo to 9 mo due to LH accumulation in the anterior pituitary gland; however, this value decreased at 12 mo of age (after assumed puberty). There could be many factors that caused this response including a dilution effect of GnRH at different BW and the low number of heifers. Therefore, the mechanisms that regulate

the onset of puberty in regards to pituitary maturation at different ages and between different breeds has not be elucidated in this study. Further studies in the US need to be implemented in the future to determine the differences in age at puberty and maturation of crossbred Holstein X Jersey heifers in order to understand the potential benefits of incorporating crossbreeding into dairy herds.

LITERATURE CITED

Ahlborn-Breier, G., Hohenboken, W.D., 1991. Additive and nonadditive genetic effects on milk production in dairy cattle: evidence for major individual heterosis. J Dairy Sci. 74, 592-602.

Ahmadzadeh, A., Barnes M.A., Pearson, R.E., 1998. Effect of naloxone on serum luteinizing hormone concentration in anovulatory holstein cows during the early postpartum period. Domest. Anim. Endocrinol. 15, 177-181.

Alexander, G.I., Reason, G.K., Clark, C.H., 1984. The development of the australian friesian sahiwal – a tick-resistant dairy breed. World Anim. Rev. 51, 27-34.

Anderson, W.J., Forrest, D.W., Schulze A.L., Kraemer, D.C., Bowden, M.J., Harms, P.G., 1985. Ovarian inhibition of pulsatile luteinizing hormone secretion in prepuberal holstein heifers. Domest Anim Endocrinol. 2, 85-91

Andrews, W.W., Advis, J.P., Ojeda, S.R., 1981. The maturation of estradiol negative feedback in female rats: evidence that the resetting of the hypothalamic "gonadostat" does not precede the first pre-ovulatory surge of gonadotropins. Endocrinology. 109, 2022-2031.

Arimura, A., Saito, M., Yaoi, Y., Kumasaka, T., Sato, H., Koyama, T., Nishi, N., Kastin, A.J., Schally, A.V., 1973. Comparison of the effects of subcutaneous and intravenous injection of synthetic LH-releasing hormone (LH-RH). J Clin Endocrinol Metab. 36, 385.

Barnes, M.A., Bierley, S.T., Halman, R.D., Hendricks, D.M., 1980. Follicle stimulating hormone, luteinizing hormone and estradiol-17 beta response in GnRH treated prepubertal Holstein heifers. Biol Reprod. 22, 459-65.

Bossis, I., Wettemann, R.P., Welty, S.D., Vizcarra, J., Spicer, L.J., 2000. Nutritionally induced anovulation in beef heifers: Ovarian and endocrine function during realimentation and resumption of ovulation. Biol Reprod. 62, 1436-1444.

Crighton, D.B., Foster, J.P., Haresign, W., Scott, S.A., 1975. Plasma LH and progesterone levels after single or multiple injections of synthetic LH-RH in anoestrous ewes and comparison with levels during the oestrous cycle. J Reprod Fert. 44, 121-124.

Critser, J.K., Lindstrom, M.J., Hinshelwood, M.M., Hauser, E.R., 1987. Effect of photoperiod on LH, FSH and prolactin patterns in ovariectomized oestradiol-treated heifers. J Reprod Fert. 79, 599-608.

Crowder, M.E., Herring, R.D., Nett, T.M., 1986. Rapid recovery of gonadotroph function after down-regulation of receptors for GnRH in ewes. J Reprod Fert. 78, 577-585.

Day, M.L., Imakawa, K., Garcia-Winder, M., Zalesky, D.D., Schanbacher, B.D., Kittok, R.J., Kinder, J.E., 1984. Endocrine mechanisms of puberty in heifers: Estradiol negative feedback regulation of luteinizing hormone secretion. Biol Reprod. 31, 332-341.

Day, M.L., Imakawa, K., Zalesky, D.D., Kittok, R.J., Kinder, J.E., 1986. Effects of restriction of dietary energy intake during the prepubertal period on secretion of luteinizing hormone and responsiveness of the pituitary to luteinizing hormone-releasing hormone in heifers. J Anim Sci. 62, 1641-1648.

Day, M.L., Imakawa, K., Wolfe, P.L., Kittok, R.J., Kinder, J.E., 1987. Endocrine mechanisms of puberty in heifers. Role of hypothalamo-pituitary estradiol receptors in the negative feedback of estradiol on luteinizing hormone secretion. Biol Reprod. 37, 1054-1065.

DesJardins, C., Hafs, H.D., 1968. Levels of pituitary FSH and LH in heifers from birth to puberty. J Anim Sci. 27, 472-477.

Dodson, S.E., McLeod, B.J., Haresign, W., Peters, A.R., Lamming, G.E., 1988. Endocrine changes from birth to puberty in the heifer. J Reprod Fert, 82: 527-538.

Dyer, R.M., Bishop, M.D., Day, M.L., 1990. Exogenous estradiol reduces inhibition of luteinizing hormone by estradiol in prepubertal heifers. Biol Reprod. 42, 755-761.

Evans, A.C., Adams, G.P., Rawlings, N.C., 1994a. Endocrine and ovarian follicular changes leading up to the first ovulation in prepubertal heifers. J Reprod Fert. 100, 187-194.

Evans, A.C., Adams, G.P., Rawlings, N.C., 1994b. Follicular and hormonal development in prepubertal heifers from 2 to 36 weeks of age. J Reprod Fert. 102, 463-470.

Fajersson, P., Stanko, R.L., Williams, G.L., 1999. Distribution and repeatability of anterior pituitary responses to GnRH and relationship of response classification to the postpartum anovulatory interval of beef cows. J Anim Sci. 77, 3043-3049.

Foldager, J., Sejrsen, K., Sorensen, J.T., 1988. The effect of plane of nutrition on growth and feed utilization in RDM and SDM heifers-revision of energy requirements for growth. (In Danish with English summary and subtitles.) Report 648, natl. Inst. Anim. Sci., Foulum, Denmark.

Freitas, A.F., Wilcox, C.J., Costa, C.N., 1998. Breed group effects on milk production of brazilian crossbred dairy cows. J Dairy Sci. 81, 2306-2311.

Frisch, R.E., Revelle, R., 1970. Height and weight at menarche and a hypothesis of critical body weights and adolescent events. Science. 169, 397-399.

Fuerst, C., Sölkner, J., 1994. Additive and nonadditive genetic variances for milk yield, fertility, and lifetime performance traits of dairy cattle. J Dairy Sci. 77, 1114-1125.

Grass, J.A., Hansen, P.J., Rutledge, J.J., Hauser, E.R., 1982. Genotype X environmental interactions on reproductive traits of bovine females. I. Age at puberty as influenced by breed, breed of sire, dietary regimen and season. J Anim. Sci. 55, 1441-1457.

Gregory, K.E., Laster, D.B., Cundiff, L.V., Koch, R.M., Smith, G.M., 1978. Heterosis and breed maternal and transmitted effects in beef cattle. II. Growth rate and puberty in females. J Anim. Sci. 47, 1042-1053.

Hansen, P.J., Kamwanja, L.A., Hauser, E.R., 1983. Photoperiod influences age at puberty of heifers. J Anim Sci. 57, 985-992.

Hansen, P.J., 1985. Seasonal modulation of puberty and the postpartum anestrus in cattle: A review. Livest. Prod. Sci. 12, 309-327.

Harris, G.W., 1955. Neural control of the pituitary gland. Edward Arnold Publishers, LTD, London, UK.

Hedlund, L., Lischko, M.M., Rollag, M.D., Niswender, G.D., 1977. Melatonin: Daily cycle in plasma and cerebrospinal fluid of calves. Science (Washington, D.C.) 195, 686.

Herber, D., Swerdloff, R.S., 1981. Down-regulation of pituitary gonadotropin secretion in postmenopausal females by continuous gonadotropin-releasing hormone administration. J Clin Endocrinol Metab. 52, 171-172.

Hocking, P.M., McAllister, A.J., Wolynetz, M.S., Batra, T.R., Lee, A.J., Lin, C.Y., Roy, G.L., Vesely, J.A., Wauthy, J.M., Winter, K.A., 1988. Factors affecting herd life in purebred and crossbred dairy cattle. J Dairy Sci. 71, 1011-1024.

Hoffman, P.C., 1997. Optimum body size of holstein replacement heifers. J Anim Sci. 75, 836-845.

Honaramooz, A., Chandolia, R.K., Beard, A.P., Rawlings, N.C., 1999. Effects of season of birth on the prepubertal pattern of gonadotropin secretion and age at puberty in beef heifers. Theriogenology. 52, 67-79.

Jinnah, H.A., Conn, P.M., 1985. GnRH-stimulated LH release from rat anterior pituitary cells in culture: refractoriness and recovery. Am J Physiol Endocrinol Metabol. 249, E619-E625.

Kaltenbach, C.C., Dunn, T.G., Kiser, T.E., Corah, L.R., Akbar, A.M., Niswender, G.D., 1974. Release of FSH and LH in beef heifers by synthetic gonadotropin releasing hormone. J Anim Sci. 38, 357-362.

Kuenzel, W.J., 2000. Central nervous system regulation of gonadal development in the avian male. Poultry Science. 79, 1679-1688.

Lamming, G.E., McLeod, B.J., 1988. Continuous infusion of GnRH reduces the LH response to an intravenous GnRH injection but does not inhibit endogenous LH secretion in cows. J. Reprod. Fert. 82, 237-246.

Laster, D.B., Smith, G.M., Gregory, K.M., 1976. Characterization of biological types of cattle IV. Postweaning growth and puberty of heifers J Anim Sci. 43, 63-70.

Laster, D.B., Smith, G.M., Cundiff, L.V., Gregory, K.E., 1979. Characterization of biological types of cattle (cycle 11) II. Postweaning growth and puberty of heifers. J Anim Sci. 48, 500-508.

Looper, M.L., Bizcarra, J.A., Wettemann, R.P., Malayer, J.R., Braden, T.D., Geisert, R.D., Morgan, G.L., 2003. Influence of estradiol, progesterone, and nutrition on concentrations of gonadotropins and GnRH receptors, and abundance of mRNA for GnRH receptors and gonadotropin subunits in pituitary glands of beef cows. J Anim Sci. 81, 269-278.

Martin, L.C., Brinks, J.S., Bourdon, R.M., Cundiff, L.V., 1992. Genetic effects on beef heifer puberty and subsequent reproduction. J Anim. Sci. 70, 4006-4017.

McAllister, A.J., 2002. Is crossbreeding the answer to questions of dairy breed utilization? J Dairy Sci. 85, 2352-2357.

McDowell, R.E., 1985. Crossbreeding in tropical areas with emphasis on milk, health and fitness. J Dairy Sci. 68, 2418-2435.

McLeod, B.J., Haresign, W., Lamming, G.E., 1982a. The induction of ovulation and luteal function in seasonally anoestrus ewes treated with small-dose multiple injections of Gn-RH. J Reprod Fert. 65, 215-221.

McLeod. B.J., Haresign, W., Lamming, G.E., 1982b. Response of seasonally anoestrus ewes to small-dose multiple injections of Gn-RH with and without progesterone treatment. J Reprod Fert. 65, 223-230.

McLeod, B.J., Haresign, W., Lamming, G.E., 1983. Induction of ovulation in seasonally anoestrus ewes by continuous infusion of low doses of Gn-RH. J Reprod Fert. 68, 489-495.

McLeod, B.J., Haresign, W., Peters, A.R., Lamming, G.E., 1984. Plasma LH and FSH concentrations in prepubertal beef heifers before and in response to repeated injections of low doses of Gn-RH. J Reprod Fert. 70, 137-144.

McLeod, B.J., Peters, A.R., Haresign, W., Lamming, G.E., 1985. Plasma LH and FSH responses and ovarian activity in prepubertal heifers treated with repeated injections of low doses of Gn-RH for 72 h. J Reprod Fert. 74, 589-596.

Melvin, E.J., Lindsey, B.R., Quintal-Franco, J., Zanella, E., Fike, K.E., Van Tassell, C.P., Kinder, J.E., 1999. Circulating concentrations of estradiol, luteinizing hormone, and follicle-stimulating hormone during waves of ovarian follicular development in prepubertal cattle. Biol Reprod. 60, 405-412.

Menge, A.C., Mares, S.E., Tyler, W.J., Casida, L.E., 1960. Some factors affecting age at puberty and the first 90 days of lactation in holstein heifers. J Dairy Sci. 43, 1099-1107.

Nakada, K., Moriyoshi, M., Nakao, T., Watanabe, G., Taya, K., 2000. Changes in concentrations of plasma immunoreactive follicle-stimulating hormone, luteinizing hormone, estradiol 17-ß, testosterone, progesterone, and inhibin in heifers from birth to puberty. Domest Anim Endocrinol. 18, 57-69.

Nakada, K., Ishikawa, Y., Nakao, T., Sawamukai, Y., 2002. Changes in responses to GnRH on luteinizing hormone and follicle stimulating hormone secretion in prepubertal heifers. J Reprod Dev. 48, 545-551.

Nelsen, T.C., Long, C.R., Cartwright, T.C., 1982. Post inflection growth in straightbred and crossbred cattle II. Relationships among weight, height and pubertal characters. J Anim. Sci. 55, 293-304.

Nett, T.M., Turzillo, A.M., Baratta, M., Rispoli, L.A., 2002. Pituitary effects of steroid hormones on secretion of follicle-stimulating hormone and luteinizing hormone. Domest Anim Endocrinol. 23, 33-42.

Petersen, S.L., Ottem, E.N., Carpenter, C.D., 2003. Direct and indirect regulation of gonadotropin-releasing hormone neurons by estradiol. Biol Reprod. 69, 1771-1778.

Petitclerc, D., Chapin, L.T., Emery, R.S., Tucker, H.A., 1983. Body growth, growth hormone, prolactin and puberty response to photoperiod and plane of nutrition in holstein heifers. J Anim Sci. 57, 892-898.

Riley, G.M., Peters, A.R., Lamming, G.E., 1981. Induction of pulsatile LH release, FSH release and ovulation in post-partum acyclic beef cows by repeated small doses of GnRH. J Reprod Fert. 63, 559-565.

Ringuet, H., Pelletier, G., Brazeau, P., Gaudreau, P., Guilbault, L.A., Morisset, J., Couture, Y., Petitclerc, D., 1994. Long-term effects of human growth hormone-releasing hormone and photoperiod on hormone release and puberty in dairy heifers. J Anim Sci. 72, 2709-2717.

Rodrigues, H.D., Kinder, J.E., Fitzpatrick, L.A., 2002. Estradiol regulation of luteinizing hormone secretion in heifers of two breed types that reach puberty at different ages. Biol Reprod. 66, 603-609.

Rorie, R.W., Bilby, T.R., Lester, T.D., 2002. Application of electronic estrus detection technologies to reproductive management of cattle. Theriogenology 57, 137-148.

Schillo, K.K., Hansen, P.J., Kamwanja, L.A., Dierschke, D.J., Hauser, E.R., 1983. Influence of season on sexual development in heifers: Age at puberty as related to growth and serum concentrations of gonadotropins, prolactin, thyroxine and progesterone. Biol Reprod. 28, 329-341.

Schillo, K.K., Hall, J.B., Hileman, S.M., 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J Anim Sci. 70, 3994-4005.

Schillo, K.K. 'Oestrus Cycles', 2003. Encyclopedia of Dairy Sciences. San Diego, CA: Academic Press.

Sejrsen, K., Purup, S., 1996. Influence of prepubertal feeding levels on milk yield potential of dairy heifers: A review. J Anim Sci. 75, 828-835.

Senger, P.L., 1999. Pathways to Pregnancy and Parturition. 1st revised ed. Pullman, WA: Current Conceptions, Inc.

Skynner, M.J., Sim, J.A., Herbison, A.E., 1999. Detection of estrogen receptor alpha and beta messenger ribonucleic acids in adult gonadotropin-releasing hormone neurons. Endocrinology. 140, 5195-5201.

Smith, M.J., Jennes, L., 2001. Neural signals that regulate GnRH neurons directly during the oestrous cycle. Reproduction. 122, 1-10.

Spelman, R.J., Hooper, J.D., Stanley, G., Kayis, S.A., Harcourt, S., 2004. Friesian Jersey crossbred trial: Generating phenotypes for the discovery of quantative trait loci. Proceedings of the New Zealand Society of Animal Production. 64, 92-95.

Stelwagen, K., Grieve, D.G., 1990. Effect of plane of nutrition on growth and mammary gland development in Holstein heifers. J Dairy Sci. 73, 2333-2341.

Stumpf, T.T., Day, M.L., Wolfe, P.L., Wolfe, M.W., Clutter, A.C., Kittok, R.J., Kinder, J.E., 1988. Feedback of 17ß-estradiol on secretion of luteinizing hormone during different seasons of the year. J Anim Sci. 66, 447-451.

Terasawa, E., Fernandez, D.L., 2001. Neurobiological mechanisms of the onset of puberty. Endocrine Reviews. 22, 111-151.

Tortonese, D.J., Inskeep, E.K., 1992. Effects of melatonin treatment on the attainment of puberty in heifers. J Anim Sci. 70, 2822-2827.
Touchberry, R.W., 1992. Crossbreeding effects in dairy cattle: The Illinois experiment, 1949-1969. J Dairy Sci. 75, 640-667.

Valentine, S.C., Dobos, R.C., Lewis, P.A., Bartsch, B.D., Wickes, R.B., 1987. Effect of liveweight gain before or during pregnancy on mammary gland development and subsequent milk production of australian holstein-friesian heifers. Aust. J. Exp. Agric. 27, 195.

VanRaden, P.M., Sanders, A.H., 2003. Economic merit of crossbred and purebred US dairy cattle. J Dairy Sci. 86, 1036-1044.

Vecchio, R.P., Neuendorff, D.A., Stahinger, R.C., Randel, R.D., 1992. Concentrations of 13, 14-dihydro-15-keto-prostaglandin F2a, estradiol-17ß and progesterone during the peripubertal period in heifers. Theriogenology. 38, 419-429.

Vizcarra, J.A., Wettemann, R.P., Braden, T.D., Turzillo, A.M., Nett, T.M., 1997. Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows. Endocrinology. 138, 594-601.

Weigel, K.A., Barlass, K.A., 2003. Results of a producer survey regarding crossbreeding on US dairy farms. J Dairy Sci. 86, 4148-4157.

Wenzel, J.G.W., Sree Balaji, K.S., Koushik, K., Navarre, C., Duran, S.H., Rahe, C.H., Kompella, U.B., 2002. Pluronic® F127 gel formulations of deslorelin and GnRH reduce drug degradation and sustain drug release and effect in cattle. Journal of Controlled Release. 85, 51-59.

Wiltbank, J.N., Gregory, K.E., Swiger, L.A., Ingalls, J.E., Rothlisberger, J.A., Koch, R.M., 1966. Effects of heterosis on age and weight at puberty in beef heifers. J Anim Sci. 25, 744-751.

Wolfe, M.W., Stumpf, T.T., Roberson, M.S., Kittok, R.J., Kinder, J.E., 1991. Opioid and 17ß-estradiol regulation of LH and FSH secretion during sexual maturation in heifers. Domest Anim Endocrinol. 8, 491-498.

Zilberstein, M., Zakut, H., Naor, Z., 1983. Coincidence of down-regulation and desensitization in pituitary gonadotrophs stimulated by gonadotropin releasing hormone. Life Sci. 32, 663-669.

APPENDIX A. STATISTICAL ANALYSIS OF CHANGES IN LH VARIABLES BY AGE AT CHALLENGE AND BREED

Table 8. Analysis of Variance for Baseline LH; fixed effects from PROC MIXED.

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Breed	3	17.5	0.32	0.8125
Age at challenge	3	13.6	2.04	0.1564
Breed * Age at challenge	9	15.8	0.96	0.5057

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	16.9	1.03	0.3256
Quadratic Age at challenge	1	19.1	2.80	0.1105
Cubic Age at challenge	1	12.8	0.16	0.6960
Linear HH	1	17	1.66	0.2155
Quadratic HH	1	19.5	0.16	0.6936
Linear HJ	1	16.8	1.21	0.2877
Quadratic HJ	1	18.6	3.30	0.0852
Linear JH	1	16.8	1.67	0.2136
Quadratic JH	1	18.6	1.91	0.1834
Linear JJ	1	17	0.28	0.5996
Quadratic JJ	1	19.4	0.07	0.7933

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Age at challenge	3	18.7	2.13	0.1305
Additive	1	12.1	0.01	0.9255
Maternal	1	12.4	0.83	0.3795
Heterosis	1	12.2	0.37	0.5566

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	24.5	0.94	0.3415
Quadratic Age at challenge	1	27.5	2.81	0.1051
Cubic Age at challenge	1	21.1	0.18	0.6747

Table 9. Analysis of Variance for Peak LH; fixed effects from PROC MIXED.

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Breed	3	12.5	0.76	0.5336
Age at challenge	3	12.1	2.08	0.1566
Breed * Age at challenge	9	12.8	0.52	0.8343

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	12.5	4.75	0.0492
Quadratic Age at challenge	1	11.3	0.03	0.8755
Cubic Age at challenge	1	7.98	0.77	0.4056
Linear HH	1	12.3	0.22	0.6502
Quadratic HH	1	11.8	0.02	0.8777
Linear HJ	1	12.7	5.62	0.0343
Quadratic HJ	1	9.99	0.06	0.8054
Linear JH	1	12.7	1.14	0.3051
Quadratic JH	1	9.99	0.00	0.9785
Linear JJ	1	12.2	0.20	0.6660
Quadratic JJ	1	13.5	0.18	0.6799

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Age at challenge	3	17.5	2.24	0.1199
Additive	1	10.1	3.02	0.1127
Maternal	1	10.1	3.88	0.0767
Heterosis	1	10.1	0.29	0.6042

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	17.7	5.20	0.0352
Quadratic Age at challenge	1	17.4	0.04	0.8531
Cubic Age at challenge	1	12.6	0.75	0.4016

Table 10. Analysis of Variance for Time to LH peak; fixed effects from PROC MIXED.

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Breed	3	16	3.33	0.0461
Age at challenge	3	14.3	0.33	0.8068
Breed * Age at challenge	9	16.6	1.10	0.4163

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	10.9	0.22	0.6460
Quadratic Age at challenge	1	23.1	0.10	0.7578
Cubic Age at challenge	1	13.3	0.68	0.4230
Linear HH	1	11	0.10	0.7553
Quadratic HH	1	23.7	0.15	0.7030
Linear HJ	1	10.7	3.53	0.0877
Quadratic HJ	1	22.4	1.13	0.2996
Linear JH	1	10.7	0.67	0.4310
Quadratic JH	1	22.4	0.11	0.7246
Linear JJ	1	11.2	0.05	0.8343
Quadratic JJ	1	23.2	0.19	0.6708

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Age at challenge	3	14.5	0.34	0.7943
Additive	1	16.1	5.36	0.0342
Maternal	1	15.4	1.93	0.1840
Heterosis	1	16.1	4.35	0.0532

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	11.2	0.05	0.8343
Quadratic Age at challenge	1	23.2	0.19	0.6708
Cubic Age at challenge	1	12.8	0.66	0.4316

Table 11. Analysis of Variance for Return to baseline; fixed effects from PROC MIXED.

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Breed	3	12.4	0.91	0.4639
Age at challenge	3	14.1	2.09	0.1468
Breed * Age at challenge	9	15.4	0.47	0.8749

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	22.2	0.17	0.6801
Quadratic Age at challenge	1	21	5.38	0.0305
Cubic Age at challenge	1	11.1	0.68	0.4273
Linear HH	1	22.5	0.09	0.7695
Quadratic HH	1	20.8	0.18	0.6779
Linear HJ	1	21.9	0.13	0.7269
Quadratic HJ	1	19.4	2.87	0.1062
Linear JH	1	21.9	0.69	0.4140
Quadratic JH	1	19.4	1.57	0.2256
Linear JJ	1	22.2	0.01	0.9389
Quadratic JJ	1	23.6	1.66	0.2099

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Age at challenge	3	20.2	2.22	0.1167
Additive	1	16.2	2.11	0.1653
Maternal	1	15.8	1.10	0.3104
Heterosis	1	16.2	0.26	0.6141

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	29.4	0.21	0.6482
Quadratic Age at challenge	1	29.4	5.80	0.0225
Cubic Age at challenge	1	15.7	0.80	0.3855

Table 12. Analysis of Variance for AUC; fixed effects from PROC MIXED.

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Breed	3	13.1	0.29	0.8294
Age at challenge	3	12.7	2.87	0.0778
Breed * Age at challenge	9	13.7	0.34	0.9456

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	15	3.15	0.0964
Quadratic Age at challenge	1	18.3	3.55	0.0757
Cubic Age at challenge	1	10.3	2.04	0.1830
Linear HH	1	14.9	0.12	0.7378
Quadratic HH	1	18.1	1.36	0.2582
Linear HJ	1	15	4.31	0.0555
Quadratic HJ	1	16.7	0.17	0.6875
Linear JH	1	15	0.34	0.5660
Quadratic JH	1	16.7	1.46	0.2445
Linear JJ	1	14.7	0.26	0.6150
Quadratic JJ	1	21	0.94	0.3422

Fixed Effects

Effect	Num DF	Den DF	F value	Pr > F
Age at challenge	3	18	3.38	0.0411
Additive	1	14.1	1.58	0.2291
Maternal	1	14.1	1.76	0.2054
Heterosis	1	14.1	0.00	0.9813

Contrasts

Label	Num DF	Den DF	F value	Pr > F
Linear Age at challenge	1	20	3.48	0.0770
Quadratic Age at challenge	1	26	4.27	0.0488
Cubic Age at challenge	1	15.2	2.36	0.1448

VITA

KAREN GETZEWICH **MASON NECK, VIRGINIA**

EDUCATION:

HIGH SCHOOL:

Hayfield Secondary High School **1998**
Alexandria, Virginia

COLLEGE:

Virginia Polytechnic Institute and State University **1998-2002**
Blacksburg, Virginia
Bachelor of Science in Biology
Minor in Animal Science

Virginia Polytechnic Institute and State University **2003-2005**
Blacksburg, Virginia
Master of Science in Dairy Science

PUBLICATIONS:

Cassell, B.G., **Getzewich, K.E.**, Nebel, R.L., Pearson, R.E., Franklin, S.T., McAllister, A.J., 2004. Early results of Holstein-Jersey crossbreeding at Virginia Tech and Kentucky. J Dairy Sci. 87, Suppl.1 p.284.

Cassell, B.G, McAllister, A.J., Nebel, R.L., Franklin, S.T., **Getzewich, K.E.**, Ware, J., Cornwell, J.M., Pearson, R.E., 2005. Birth weights, mortality, and dystocia in Holsteins, Jerseys, and their reciprocal crosses in the Virginia Tech and Kentucky crossbreeding project. J Dairy Sci. 88, Suppl.1 p.92.