

ABSTRACT

SWEETMAN, ANNA ELIZABETH. The Effects of Supplemental GnRH on Reproductive Performance in Lactating Holstein Dairy Cows during the Summer and Winter. (Under the direction of Dr. C.S. Whisnant.)

During the summer trial lactating dairy cows were randomly assigned to four treatment groups. These groups included a control group that received no hormonal supplement, and three groups that received supplemental gonadotropin releasing hormone (GnRH) on either day 5, day 11, or both days 5 and 11 post insemination. The cows during the winter trial were randomly assigned to three treatment groups, a control, and cows receiving hormonal supplements on day 5, or days 5 and 11 post insemination. Blood samples and rectal temperatures were taken 9 and 2 days prior to insemination and every other day thereafter beginning on day 5 post insemination and ending around day 30. Pregnancy was checked on day 30 and then again between days 45 and 60 by ultrasonography. Daily maximum and minimum temperatures and humidity values were recorded in order to calculate the temperature-humidity index and determine the level of heat stress the cows were experiencing. Serum progesterone (P_4) and cortisol concentrations from all samples were analyzed using RIA. Environmental information for the summer indicated that the cows experienced an overall mild heat stress by an average THI of 76, whereas the winter environmental data showed an average THI of 43, within the thermo-neutral range. However, on individual days during the summer the THI reached values between 79 and 85, indicating that cows experienced medium heat stress. Serum P_4 concentrations were data was analyzed between days 11 through 17 post insemination, the expected luteal phase, during the summer. Treatment groups were compared and indicated that the serum P_4 concentration was greater in GnRH-D11 treated cows than control cows ($P < 0.05$) and than

GnRH-D5 treated cows ($P < 0.05$). Concentrations of P_4 between groups during the winter were also analyzed between days 11 and 22 post insemination, however the results were different. GnRH-D5 and GnRH-D5+11 were both greater than control concentrations, 6.87 ± 0.69 ($P < 0.09$) and 7.10 ± 0.56 ($P < 0.05$), respectively. Winter progesterone concentrations were greater than those of summer between treatment groups ($P < 0.05$), indicating that there was an effect of environment on hormonal levels. Analysis of cortisol concentrations during the summer indicated no significant difference between treatment groups, as was the same for winter groups, and there was no difference between seasons. Administration of GnRH appeared to have a beneficial effect on pregnancy rates during the summer between days 45 and 60. This was determined when all groups receiving the hormone supplement were combined ($P < 0.05$). In conclusion, the administration of GnRH to heat stressed cows increased P_4 concentrations and appeared to have a positive effect on pregnancy rates.

**THE EFFECTS OF SUPPLEMENTAL GNRH ON REPRODUCTIVE
PERFORMANCE IN LACTATING HOLSTEIN DAIRY COWS DURING THE
SUMMER AND WINTER**

by
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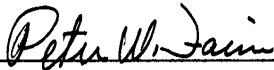
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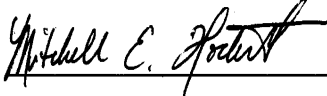
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BIOGRAPHY

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INTRODUCTION

Heat stress is an ever increasing concern in the dairy industry because of the negative effects that it exerts on fertility and other reproductive functions including decreased intensity of estrus, reduced blood flow from the viscera and the impairment of embryo development. The two main reproductive effects are reduced expression of estrus and decreased fertility. Over the past 50 years fertility has become an increasingly important concern for dairy farmers because of the negative correlation associated with increased milk production. The additional effect of heat stress causes a further reduction in pregnancy rates, which can reach as low as 20% and sometimes lower (Willard et al 2003), thus resulting in major economic losses.

Research has implied that increasing serum progesterone (P_4) concentrations during the early estrous cycle improves pregnancy rates. This can be achieved by administering Gonadotropin Releasing Hormone (GnRH). GnRH causes the release of luteinizing hormone (LH), which subsequently causes an increase in the amount of serum P_4 (Mee et al, 1993). Peters and colleagues (2000) performed a meta-analysis of studies where GnRH was administered to cows between day 11 and day 14 post-insemination. Although the effects of GnRH administration were not consistent, due to variation between studies, their overall conclusion was that administration of GnRH increased pregnancy rates.

Willard and colleagues (2003) studied the effects of administration of GnRH on day 5 and day 11 post insemination. Day 5 coincides with the 1st wave preovulatory dominant follicle so that when GnRH is administered ovulation of the follicle occurs. An accessory corpus luteum (CL) forms thus increasing the levels of P_4 . GnRH administered on day 11 of

the estrous cycle (GnRH-D11) increases P_4 during maternal recognition of pregnancy (days 14 to 16), similar to that seen following administration of GnRH on day 5. Enhancing P_4 around day 11 of the estrous cycle allows the embryo to develop further, which could prevent the luteolytic mechanism from occurring (Willard et al., 2003).

The purpose of this study was to determine if supplemental administration of the hormone GnRH on specific days post insemination, could improve reproductive performance of lactating dairy cattle during periods of heat stress. A similar study was performed during periods of cooler temperatures in order to compare reproductive performance between seasons.

REVIEW OF LITERATURE

It is thought that manipulation of the estrous cycle, through the use of hormones may improve reproductive performance in dairy cattle. Two specific times include 5 days after estrus, and/or 11 days after estrus. At both times ovulation will result when GnRH is administered inducing an accessory CL which increases P₄ concentrations. Day 11 coincides with maternal recognition of pregnancy which occurs between days 14 to 16. The increased P₄ concentrations are thought to assist the embryo in developing further to promote the recognition.

Estrous cycle

The estrous cycle is the period of time from one estrus to the next. Estrus is defined as the period of sexual receptivity in female cattle. The average number of days for estrous in cows ranges between 17 and 24 days and consists of two distinct phases, the follicular phase and the luteal phase (Senger, 1999). The follicular phase is comprised of two periods, proestrus and estrus. These periods can be categorized by increased amounts of estrogen secretion, whereas progesterone is the primary hormone for the luteal phase, which also has two periods, metestrus and diestrus. Proestrus begins after luteolysis, and a decline in progesterone. Estrogen levels begin to increase as the preovulatory follicle enlarges and the female reproductive tract is prepared for mating. Estrus is characterized by a peak in the secretion of estrogen, sexual receptivity and subsequently ovulation of the dominant follicle. Estrus is the most recognized period of the estrous cycle due to standing estrus behavior exhibited by the cow. The period after this behavior is when most artificial inseminations

occur; the visual behavioral changes make it easier to detect estrus and to breed the cows. Metestrus is the period of time between ovulation and the formation of the corpus luteum and the dominant hormone switches from estrogen to progesterone. The corpus luteum (CL) forms as a consequence of the cellular transformations that occurred in the ovulated follicle. The final period of the estrous cycle is diestrus. The CL is fully functional and progesterone is secreted at its maximum. This period ends when luteolysis occurs, destroying the CL, allowing the cycle to repeat (Senger, 1999). If the cow is pregnant, the CL is maintained by secretion of interferon- τ by the embryo (Mann et al., 1999). Trout and colleagues (1998) studied the effect of heat stress on length of the estrous cycle using environmental chambers. However, they found no difference in length between heat stressed cows and control cows.

Estrus Synchronization

The use of hormones, such as gonadotropin releasing hormone (GnRH) and prostaglandin F2 alpha (PGF2a), has enabled the synchronization of estrus and ovulation. Synchronization, using progesterone (P4) and luteolytic agents, such as PGF2a, is based on the premise of controlling the life span of the CL (Thatcher et al., 2001). Follicular growth and development also need to be addressed when cycles are synchronized, as varying sizes of follicles determine the period from CL regression until estrus and may affect embryo survival. For the purpose of this discussion pregnancy rate is defined as the number of cows that reach the end of gestation and give birth to healthy cows and conception rate is defined as the number of cows that become pregnant but abort before the end of gestation.

Administration of drugs to lactating cows is strictly monitored, and for many years PGF_{2a} and its analogs were the only drugs permitted for use (Whisnant et al., 1999).

Breeding protocols were designed to use PGF_{2a} to synchronize estrus and hopefully improve pregnancy rates. Studies comparing the effects of two injections of PGF_{2a}, 11 or 14 days apart have been performed. Rosenberg and colleagues (1990) found that when the second injection of PGF_{2a} was administered 11 days after the first, estrus occurred earlier. However, they also reported that when cows were given PGF_{2a} 14 days apart the percentage of cows detected to be in estrus were similar to that observed for 11. Folman and colleagues (1990) also reported that cows had greater pregnancy rates when they were given PGF_{2a} 14 days apart compared to 11 days apart. Not all cows respond to PGF_{2a} treatment, this can occur when there is no functional CL present to be regressed or early after formation when the CL does not have PGF_{2a} receptors (Whisnant et al., 1999). Stevenson and Pursley (1995) measured milk progesterone to determine when cows would respond to an injection of PGF_{2a}; they then compared the reproductive performance of these cows to those that were presented for AI based on twice-daily estrus detection. They determined that days to first AI, calving interval and cost of treatment per pregnancy were all decreased; however, it was still more expensive than administration of PGF_{2a} given without knowledge of responsiveness to the drug. The use of PGF_{2a} in timed insemination programs, has repeatedly shown a reduction in conception rates, but used in addition with GnRH there does appear to be an improvement in conception rates (Stevenson et al., 1987; Lucy et al., 1986).

An estrus synchronization program has been developed in which the growth of the preovulatory follicle and the regression of the CL are controlled. The program works by administering a GnRH agonist to cause turnover of a dominant follicle and thus recruit a new wave of follicles (Macmillan and Thatcher 1991; Wolfenson et al., 1994) followed seven days later by an injection of PGF_{2a}, thus resulting in regression of the corpus luteum

(Badinga et al., 1994; Schmitt et al., 1996; Thatcher et al., 1989; Wolfenson et al., 1994). Ovulation of the follicle can then be induced, and the need for estrus detection eliminated, by administering a second injection of the GnRH agonist 48 hours after the injection of PGF2a (Burke et al., 1996; Pursley et al., 1997; Schmitt et al., 1996; Stevenson et al., 1996). This program is now commonly referred to as the Ovsynch protocol or Timed AI. Heat stress has an adverse effect on estrus detection in that both the length and intensity are reduced (Abilay et al., 1975; Gangwar et al., 1965). These changes reduce the ability of dairy personnel to successfully detect heat, thus further reducing the number of cows becoming pregnant (Hansen and Arechiga, 1999). One proposed explanation for the reduction of estrus expression is the physical lethargy produced by heat stress, which is probably an adaptive response that limits heat production (Hansen and Arechiga, 1999). The Ovsynch protocol allows cows to be bred at an induced ovulation without the producer spending valuable time watching for estrus that may not be detected.

De la Sota and colleagues (1998) compared the Ovsynch program with that of insemination at detected estrus under heat stress conditions. The percentage of cows inseminated at detected estrus was 18.1% whereas in the timed insemination group it was 100%. However, the conception rate for the cows inseminated at detected estrus was 22.9% but the timed insemination group was only 13.2%. At 120 days postpartum the timed insemination group had a higher pregnancy rate at 27% as opposed to the detected inseminated cows at 16.5%. They concluded that the Ovsynch protocol resulted in a significant increase in the percentage of pregnant cows, and administration of the second GnRH agonist injection eliminated the need for estrus detection. However, as indicated by

the lower conception rate, there was no benefit for the embryo and the heat stress induced embryonic death could not be prevented.

Heat stress

Detection

Heat stress has been defined as the external forces acting upon the cow to displace body temperature from the resting state (Hansen and Arechiga, 1999), but “How is heat stress determined in cattle?” Parameters need to be set to determine when the cow is experiencing heat stress. One of the simplest and most practical measures to determine heat stress is that of the temperature-humidity index (THI) (Ingraham et al., 1974). Heat stress was defined as a combination of the air temperature and the relative humidity. The THI made it possible to determine the degree of heat stress the cow is experiencing for any given temperature at a given relative humidity. The degree of heat stress increases as the relative humidity increases for any given temperature, and is calculated by the equation $THI = T(^{\circ}F) - (.55 - .55RH)(T(^{\circ}F) - 58^{\circ}F)$ (Ingraham et al., 1974).

Ingraham and colleagues (1974) studied the relationship between the THI and the breeding efficiency in Holstein cattle located in a subtropical environment (Mexico). They determined that there was a negative correlation between conception rates and the daily maximum and minimum temperature and humidity values. They also analyzed the conception rate versus the THI response curve for days surrounding breeding, in order to minimize the effect of season as much as possible. The results indicated that the THI 2 days prior to breeding significantly influenced the conception rate regardless of season. When the average THI of the 2 days prior to breeding were greater than the THI on the day of breeding

there was a decrease in the conception rate, compared to when the average THI of the 2 days prior was less than on the day of breeding. It was hypothesized that there are numerous hormonal changes occurring within the cow, which may be altered by heat stress.

Suppression of estrogen may change the environment within the follicle thus affecting the maturation of the egg. They concluded that an average daily THI above 70 on days prior to breeding negatively affected conception rates. Ingraham and colleagues (1976) performed the same experiment in Hawaii, as they had previously done in Mexico (Ingraham et al., 1974). They wanted to determine if the relationship between the THI and conception rate was the same for an environment that was mild and relatively unvaried. Over a three year period the year with the highest THI value, 74.6, had the lowest conception rate, 46%, and was the only year in which the relationship was significant. They found that an increased THI value 2 days prior to breeding decreased the conception rate. A THI value below 70 is classed as normal, and the cattle are in a thermo-neutral environment, when values exceed 70 the cows become heat stressed.

Table 1. Temperature Humidity index chart

		Relative Humidity Intervals (Percent)									
		10	20	30	40	50	60	70	80	90	100
Dry bulb temp(F)	78			70	71	72	74	75	75	77	78
	80		70	72	73	74	75	77	78	79	80
	82	70	71	73	74	75	76	78	79	81	82
	84	71	72	74	75	77	78	80	81	83	84
	86	72	74	75	77	78	80	81	83	84	86
	88	73	75	76	78	80	81	83	85	87	88
	90	74	76	78	79	81	83	85	87	88	90
	92	75	77	79	81	83	84	86	88	90	
	94	76	78	80	82	84	86	88	90		
	96	77	79	81	84	86	88	90			
	98	78	80	82	85	87	89				
100	79	82	84	86	88	91					

Adapted from "Notes on Temperature-Humidity Index", NOAA National Weather Service
EMERGENCY STRESS - Index of 84 or Greater
DANGER STRESS - Index of 79 to 83
NO STRESS - Index of 78 or less

Badinga and colleagues (1985) studied the effects of environmental and management factors, on breeding efficiency in a subtropical environment (Monticello, FL). They observed both heifers and lactating cows for the period of a year and found that heifers were consistently more fertile during the year than were the cows. Lactating cows showed a significant decrease in fertility during the months of June through August, and did not recover until November. When the maximum daily temperature exceeded 30°C, conception rates of the lactating cows decreased whereas conception rates of heifers did not decrease until the temperature reached 35°C. The difference is likely due to elevated internal temperatures in the cow associated with lactation. Cavestany and colleagues (1985) reported that pregnancy rates in lactating dairy cows began to decrease at 30°C, and as the temperature increased to, and above, 35°C there was a rapid decline in the percentage of pregnancies.

Overall effects of heat stress on reproduction

Reproduction in lactating dairy cows is substantially affected by heat stress. The effects range from decreased duration and intensity of estrus (Her et al., 1988; Imtiaz-Hussain et al., 1992), impaired embryonic development (Hansen and Arechiga, 1999), reduced uterine blood flow (Roman-Ponce et al., 1978), hormonal relationships (Alvarez and Johnson, 1971; Abilay et al., 1975; Gilad et al., 1993; Wolfenson et al., 1995; Ronchi et al., 2001), and decreased conception rates (Badinga et al., 1985; Wolfenson et al., 1988). Heat stress contributes to serious economic losses and affects about 60% of the world dairy cattle population (Wolfenson et al., 2000). Hyperthermia directly affects tissues of the reproductive system by altering and impairing cellular functions. Indirect responses such as redistribution of blood flow among body organs and a reduction in food intake also influence the functions of the reproductive system (Wolfenson et al., 2000). Although the impact of the indirect and direct effects of heat stress on reproductive functions have not been fully separated and measured, it is currently thought that hyperthermia is main cause of impairment on cellular functions (Wolfenson et al 2000). One main limiting factor is the inability of the high producing dairy cow to maintain normothermia because of the concurrent rise in metabolic heat production (Wolfenson et al., 2000).

Effects on ovarian follicles

Heat stress alters the follicular development pattern in cattle. It can lead to a reduction in the size of the dominant follicles of the first and second follicular waves of the estrous cycle (Badinga et al., 1993; Wilson et al., 1989a, b). Conception rates of lactating cows decreased from about 50% in cooler months to 20% in hotter months. Also, during the

transition period, from hot months to cool months, there is still reduced fertility, around 30%, even though the ambient temperatures decreased and the cows are no longer exposed to thermal stress (Ron et al., 1984; Cavestany et al., 1985). The possibility of a delayed effect of heat stress on follicular dynamics is supported by the fact that small antral follicles take about 40-50 days to develop into large preovulatory follicles (Lussier et al., 1987). Exposure to heat stress during the early stages of follicular development in the summer may subsequently impair preovulatory follicular function in the autumn.

Roth and colleagues (2000) studied the effect of heat stress on follicular growth during a complete non-synchronized estrous cycle. They included the possibility of a delayed effect of heat stress on follicular development, and characterized changes in plasma FSH and inhibin concentrations, and their involvement in the alteration of follicular dynamics. Two groups of cows were included in the study in which rectal temperatures and blood samples were taken. One of the groups was exposed to direct solar radiation for 7 hours per day, whereas the other group received cooling (Sprinklers and ventilation) for 12 hours for the first estrous cycle. During this treated estrous cycle the heat stressed cows experienced hyperthermia, whereas the cooled cows experienced normothermia. Both treatment groups were cooled during the following estrous cycle, and both showed normothermia. The second follicular wave of the second estrous cycle occurred earlier in the heat stressed cattle, which was most likely due to an inability of the first wave dominant follicle to produce sufficient amounts of inhibin to suppress FSH secretion and the onset of another follicular wave. This suggests a carry over effect of heat stress to the subsequent estrous cycle.

Two studies, one conducted by Wolfenson and colleagues (1995) the other by Roth (1998), observed a 50% increase in the number of large follicles (>10mm) during the first follicular wave of heat stressed cattle. The dominant follicle was not capable of suppressing smaller follicles from developing due to the insufficient amount of estrogen and inhibin produced. The reduction in follicular dominance may also be affected by differences in energy balance and/ or the stage of lactation of the cows (Wolfenson et al., 1995). It appears that follicular dynamics and production of inhibin were altered; therefore, the dominant follicle could not function effectively to suppress other growing follicles. However, the overall pattern of follicular development was not suppressed in heat stressed cattle (Wolfenson et al., 2000).

Supplementation of hormones may be used to effectively alter follicular dynamics. Diaz and colleagues (1998) administered hCG resulting in ovulation of the first wave dominant follicle as well as recruitment and earlier emergence of the second wave dominant follicle. Badinga and colleagues (1992) reported that when the ovary bearing the dominant follicle was removed there was an increase in FSH concentration, which lead to the same results as seen in Diaz and colleagues (1998) study. Diaz and colleagues (1992) also noted that the second wave follicle emerged earlier in heifers treated with hCG, but the size was reduced and it maintained a shorter life span. This, they reported, was due to elevated concentrations of progesterone. Kinder and colleagues (1996) observed that elevated concentrations of progesterone reduced growth of the dominant follicle. Turnover of the follicle was induced by reducing LH secretion which supported the findings from the previous experiment.

Effects on embryos

Heat stress also has an impact on embryogenesis. Early embryonic development was compromised due to actions directly on the embryo or on the oviduct or uterine environment (Hansen and Arechiga, 1999). Ryan and colleagues (1993) observed the effects of heat stress on development of the embryo. The majority of early embryonic mortality during elevated temperatures occurred between days 6 and 14 of pregnancy when rectal temperatures were 39°C. Putney and colleagues (1988b) also reported early embryonic death on day 7 after estrus when rectal temperatures were 41°C. Biggers and colleagues (1987) measured the conceptus weight after the dam was exposed to heat stress for 17 days. It was reported that wet weights of the conceptuses from cows undergoing heat stress were much lower than those from control cows. This hypothesis was that increased temperatures within the uterus could cause an increase in the metabolic rate of the conceptus. This change potentially results in impaired development due to alterations in nutrient uptake and conceptus growth.

Hansen and Arechiga (1999) hypothesized that the process of embryonic death may be due to the severity of heat stress, and improvement of fertility may depend on climatic conditions. It appears that embryos in the very earlier stages of development are more susceptible to heat stress than those in later stages. Embryo transfer may be used to alleviate some of the effects of heat stress in cows. It is likely that an embryo transferred from a non-heat stressed cow at day 7 of development into a heat stressed cow at day 7 after estrus would have a better chance of survival than for inseminated cows (Hansen et al., 2001). Ryan and colleagues (1993) studied the effect of elevated temperatures on cultured cattle embryos. Incubator temperatures of 38.6°C or 40°C were used for the cultures and it was determined that increased temperature adversely effected embryo development. In vivo experiments

have shown that between days 1 through 3 there is a reduction in the survival of the embryo and pregnancy rates (Dunlap et al., 1971; Ealy et al., 1993;) Ealy and colleagues (1993) exposed cows to heat stress with no shade. There was reduced viability and development on embryos on day 8 after estrus if the superovulated cows were exposed to heat stress on day 1 post estrus. However there was no effect on embryos when cows were exposed to heat stress on days 3, 5, or 7 after estrus. This implies that as embryos age heat tolerance is acquired.

Effects on corpora lutea and progesterone concentrations

The effects of heat stress on the corpus luteum may be determined by measuring plasma or serum concentrations of progesterone (Wolfenson et al., 2000). The uterus needs progesterone from the CL to maintain pregnancy. Plasma progesterone is affected by numerous factors in the body. During heat stress concentrations are affected by adrenal release of progesterone, metabolism in the liver, hemodilution, or hemoconcentration, the degree of hyperthermia, the type of heat exposure (acute/chronic), the age of the cow, stage of lactation and the type of feeding (Jonsson et al., 1997; Trout et al., 1998). Howell and colleagues (1994,) suggested that serum progesterone concentrations decreased in cows subjected to long periods of elevated temperature conditions, such as those seen during summer conditions. They measured the concentration of progesterone over the entire estrous cycle in both spring and summer. They did not find a significant difference between season until the data from day 0 to 5, and greater than day 18 were excluded. They were then able to report that the level of progesterone between days 6 and 18 was lower in the summer than in the spring.

Wolfenson and colleagues (1995) studied the production of progesterone secreted in vitro from luteal cells collected during the summer and the winter. The cells collected during the summer showed a marked decrease in progesterone levels after a 2-day incubation period at 38°C. The luteal cells collected from the winter were incubated also for two days but at 40°C, and showed a 30% decrease in progesterone levels compared to similar cells that were incubated at 38°C. The decrease in the amount of progesterone can have adverse effects both before and after insemination (Wolfenson et al., 2000). Prior to insemination the low concentration of P₄ can cause aberrant follicular development, leading the oocyte to mature abnormally in the ovulatory follicle and ultimately early embryonic death (Ahmad et al., 1995). A low progesterone level following artificial insemination affects steroidogenesis not only in the dominant follicle, but also in the corpus luteum that forms from it. Adverse effects on endometrial morphology and function were also observed in the following estrous cycle (Shaham-Albalancy et al., 1996a).

Effects on hormones

Cortisol

Cortisol is considered to be the hormone of stress. When a cow becomes stressed the hypothalamic-pituitary adrenal axis is activated and the amount of cortisol secreted increases. During acute heat stress levels increase initially and then decrease to normal levels. In the first 20 minutes plasma cortisol concentrations increase rapidly. Hormone concentrations plateau between 2 and 4 hours after onset of heat stress, and then begin to fall. Chronic heat stress has the same initial increase as seen in acute heat stress. However after the plateau phase there is a further reduction in concentration to below normal. This can be attributed to

adjustments in the turnover rate and plasma concentration of the hormone (Christison and Johnson, 1972). Dairy cows are exposed to a variety of different stressors on the farm including, unfamiliar occurrences, different management techniques, and environmental factors including elevated temperatures. Ronchi and colleagues (2001) measured the mean levels of plasma cortisol in heat stressed heifers. The heifers were exposed to 32°C and 70% relative humidity (THI=84) for 24 hours a day beginning 4 days before the expected second estrus and ending when all heifers ovulated in the third estrus. There was no significant difference between the heat stressed heifers and those that were not heat stressed. Other studies have shown that plasma cortisol concentrations decrease in cattle after extended periods of exposure to elevated temperatures compared to acute periods of heat stress (Christison and Johnson, 1972). When cows are exposed to chronic heat stress there is an observed decrease in adrenocortical activity (Ronchi et al., 2001) This precludes an increase in metabolic heat production due to a thermoregulatory protective action, because cortisol is a thermogenic hormone (Ronchi et al., 2001). Alvarez and Johnson (1971) found that in non-lactating dairy cows glucocorticoid concentrations were elevated during the first hour of exposure to heat stress conditions. However, the following three and a half hours revealed that levels decreased to lower values than those observed in control cows. In a similar study, Christison and Johnson (1972) measured plasma cortisol levels in cows exposed to acute heat stress, 35°C, 50% relative humidity (RH) 4hrs a day with the remaining 20hrs at 18°C, and 50% RH. Cows exposed to chronic heat stress were maintained in 35°C, 30% RH conditions for 10 weeks. These studies indicated that there was an immediate response by the cow to heat stress, which was proven by the initial increase in cortisol levels. Cortisol remained elevated for up to 12hrs after exposure, and then returned to normal after 1 to 2 days. After a

few days of chronic heat stress the plasma cortisol levels returned to pre-exposure levels as the cows adjusted to the environment, thus reducing heat production. Wise and colleagues (1988) also reported an increase in cortisol levels but speculated that this increase could have been due to milk production maintenance by increasing blood glucose levels. There was no significant difference in milk production between heat-stressed cows, and cooled cows, so the rise in cortisol levels may have helped to maintain high milk production levels. Abilay and colleagues (1975) used heifers in their study and reported that there was a significant increase in cortisol concentrations on the first day of exposure. Cortisol levels decreased with prolonged exposure, which they hypothesized to be a protective mechanism to decrease metabolic heat. There may be several factors that affect cortisol levels, these could include the stage of lactation and age of the cow, but also the response may depend on the severity of the heat stress.

Gonadotropins

There are limited data on both LH and FSH under heat stress conditions. Collection directly from the pituitary would provide the most accurate data for LH and FSH. However, due to the difficulty of this procedure, peripheral concentrations were measured. Wise and colleagues (1988) studied the effects of heat stress on LH during the early and midluteal phases of the estrous cycle. They reported that on day 5 of the cycle there was a significant decrease in the pulsatile secretion of LH between heat stressed cows and those in the thermo-neutral group. However, on day 12 there was no difference between the mean release, or in the amplitude, of the hormone. Low levels of LH during this period may have negative effects, and compromise the development of the corpus luteum. During early

diestrus LH alters steroidogenic cell numbers of the corpus luteum so as to enhance its development. The effect of heat stress on plasma LH is controversial due to different studies reporting increases (Roman-Ponce et al., 1981), decreases (Madan and Johnson, 1973) or unchanged concentrations of the hormone (Gwazdauskas et al., 1981; Gauthier, 1986). It was hypothesized that these discrepancies were due to the frequency of sampling, and the type of heat stress (acute or chronic) that the cows were subject to (Gilad et al., 1993). Seasonal differences could be due to photoperiod as well as heat stress.

The effect of heat stress on FSH has not been well documented. Gilad and colleagues (1993) administered GnRH to heifers and studied its effects on FSH surges during two consecutive summers. For both summers they determined that there was no significant difference between the control heifers or the treated heifers under conditions of chronic heat stress. However, during the second summer treated heifers that exhibited lower concentrations of estradiol had mean concentrations of FSH reduced to 81% of the control group. There was also a reduction in the GnRH-induced FSH surge peak to 75% of that of the controls. Ronchi and colleagues (2001) also studied the effects of heat stress on FSH secretion in heifers; however they found no differences in control or treated groups. The heifers had only short-term exposure to elevated temperatures, and there was no difference in estradiol concentrations thus contributing to the lack of response. Feed restriction has been shown to also play a role in the release of FSH. Loope and colleagues (1996) indicated an increase in FSH concentrations after short-term feed restrictions and a decrease after long-term feed restriction in ovariectomized cows.

Estradiol

Follicular growth and development is affected by heat stress, which in turn affects estrogen production by the follicles. The effect of heat stress on estradiol is controversial as some studies have reported no difference in the estradiol levels (Roman-Ponce et al., 1981; Ronchi et al., 2001), while other studies have reported greater concentrations of estradiol following heat stress (Rosenberg et al., 1982; Wise et al., 1988). It has been hypothesized that the discrepancies between these studies were related to the physiological status of the cow, stage of lactation, or the intensity of the heat stress (Wilson et al., 1998).

Therefore, it is unclear whether there is an increase, decrease or no change in concentration of estrogen under heat stress conditions. Wilson and colleagues (1998) reported that when lactating dairy cows were exposed to heat stress in experimental chambers, there was a decrease in the serum concentration of estrogen compared to those cows that were confined to normothermic conditions. This study was conducted during the development of the preovulatory follicle, but Gwazdauskas and colleagues (1981) reported that under similar conditions, during the proestrus phase there was a rise in estradiol, then a decrease when the first wave dominant follicle was establishing dominance. Further studies need to be performed to more accurately determine the response of estradiol to heat stress.

Hormone Supplementation

The proper hormonal environment is essential for pregnancy to occur and be maintained. Several studies suggest that hormonal supplementation can improve pregnancy rates as summarized by Thatcher and colleagues (2001). There are many events of pregnancy that can be manipulated by administration of hormones to help improve pregnancy

rate and survival of the embryo. These hormonal manipulations include managing ovarian follicles and CL functions to synchronize ovulation by use of a timed insemination, and manipulating growth and development of the embryo.

Follicular growth can be synchronized with such hormones as progesterone, a combination of progesterone and estradiol, and GnRH and its agonists (GnRHa). Many steroid treatments in dairy cattle are prohibited in the USA so GnRH is widely used in synchronization protocols (Thatcher et al., 2001). GnRH administration results in the induction of an LH surge and FSH surge (Chenault et al., 1990). This LH surge results in ovulation or luteinization of the dominant follicle present at the time of administration of GnRH (Macmillian and Thatcher, 1991), where as the FSH induced surge, along with the endogenous FSH surge, allows for the recruitment of a new follicular wave.

Estrus is most commonly synchronized using gonadotropin releasing hormone agonists (GnRHa) and prostaglandin F_{2a} (PGF_{2a}) hormones. Thatcher and colleagues (2001) summarized several studies that compared different combinations of hormones to synchronize estrus. The effects of using a single injection of PGF_{2a} were compared to an injection of GnRHa followed 7 days later by an injection of PGF_{2a}. The combination of hormones proved to be more effective in synchronizing estrus, which occurred 2-3 days after the administration of PGF_{2a}, than the single injection of PGF_{2a}. Pursley and colleagues (1995) advanced the protocol further by injecting a second dose of GnRHa 48 hours after the injection of PGF_{2a}. This induced a timed ovulation that occurred approximately 30 hours after the final injection. This protocol is known as the Ovsynch program or Timed AI due to the synchronizing of ovulation and allowing for a timed insemination.

Progesterone effects

The concentration of progesterone has been shown to be associated with embryo development and its ability to secrete interferon- τ , the antiluteolytic hormone involved in maternal recognition of pregnancy (Mann et al., 1999). Endometrial function, embryo development and the secretion of interferon- τ are stimulated by progesterone produced by the dam (Mann et al., 1999; Mann and Lamming., 2001). Mann and Lamming (2001) studied the relationship between the maternal hormonal environment and the survival of the embryo in beef cattle. At day 16 after the first insemination the cows were slaughtered and the embryos collected for analysis. The comparison between the poorly developed embryos and those that were developing normally showed a significant difference in the production of interferon- τ and the concentration of progesterone. In cows with poorly developed embryos there was little or no production of interferon- τ . After ovulation the increase in progesterone was delayed and the plateau of the luteal phase was lower than in the cows possessing well-developed embryos. The smaller increase in progesterone was probably due to poor luteinization or inadequate LH support (Garverick et al., 1992). Garrett and colleagues (1988) supplemented progesterone to pregnant beef cows to study the effects of embryo development and uterine secretions. Cows treated with progesterone on days 1-4 after mating had elevated plasma progesterone levels on days 2-5, whereas the control group did not reach the same levels until day 5. Administration of progesterone during that 4-day period appeared to affect the function of the uterus as seen in changes in uterine secretion. There was a difference in polypeptide release from day 5 to 14 of pregnancy in the progesterone treated cows compared to the controls suggesting that the uterus is undergoing changes following the administration of progesterone. The conceptus length was greater in

the treated cows, and there was increased variation, which could have been due to individual uterine sensitivity to progesterone. It was concluded that uterine secretions change during those 5 days of elevated progesterone levels, thus affecting the development of the conceptus. On day 14 of pregnancy the length of the conceptuses were longer for progesterone treated cows than those of the control group. The final conclusions were that supplementing progesterone during the first four days after insemination induced an earlier activation of the conceptus by changing uterine secretions such as ovarian steroids.

Progesterone is needed for embryo survival, but the relationship between the systemic or milk concentrations of progesterone and pregnancy rate remains unclear (Chagas et al., 2002). Chagas and colleagues (2002) observed that heifers and cows determined to be pregnant on days 45-60 had higher concentrations of progesterone on day 21 after estrus than cows and heifers that were assumed to be pregnant but later identified as open. In both cows and heifers embryo death may have occurred after day 21 due to lower secretions of interferon- τ before day 21. Also embryo death could have occurred before day 21 with progesterone levels already decreasing at this time. Heifers had a higher pregnancy rate than cows, but more frozen-thawed transfers were performed in them as opposed to the cows, which could have affected the statistical analysis of pregnancy rates. On day 7 after estrus, recipient cows had significantly lower plasma progesterone levels than heifers, which were related to lower pregnancy rates in cows, but not in heifers. There was a positive correlation between plasma progesterone levels on day 6 and 7 and pregnancy rates in cows, but not in heifers. The conclusions were that in lactating cows low plasma progesterone levels on day 7 may negatively affect embryo survival, whereas in heifers there was no apparent effect. Also, lactating cows were more likely to experience embryo loss than heifers. However,

some evidence suggest that higher progesterone is associated with better embryo development (Mann et al., 1999).

GnRH supplementation

The administration of GnRH causes the release of LH, and subsequently causes a significant increase in the amount of serum progesterone (Mee et al., 1993). Ullah and colleagues (1996) observed the effects of administering GnRH during estrus on corpus luteum function and fertility during the summer. The cows that were treated with GnRH showed a significant increase in the amount of serum progesterone during the luteal phase compared to controls. They concluded that the GnRH had a stimulatory effect on the corpus luteum thus resulting in the increased levels of progesterone. There also seemed to be an effect on fertility of these cows. At day 45 the conception rates were 28.6 and 17.9% for the treated and control groups, respectively. On a subsequent palpation, after day 45, the pregnancy rates were 28.6% and 14.3% for treated and control groups, respectively. This indicated that all of the cows treated with GnRH and determined to be pregnant maintained their pregnant state. They showed that the rectal temperatures between the control group and the treated group did not differ and thus concluded that the suppression of corpora lutea function is a factor that compromises embryo survival in heat stress conditions.

Schmitt and colleagues (1996) determined that an injection of a GnRH agonist on day 5 of the estrous cycle ovulated and formed an accessory corpus luteum in non-lactating cows. They also showed that there was an increase in the amount of plasma progesterone between days 11 and 16 when compared with the control group. It was not shown however, that the

induction of an accessory corpus luteum and the increased levels of progesterone improved conception rates in the cows.

GnRH can be used on the day of insemination to help improve pregnancy rates in dairy cows that may need to be inseminated more than once (Stevenson et al., 1990; Peters, 1996). Its use is to ensure that ovulation and induction of a corpus luteum occur in accordance with insemination (Peters et al., 2000). Stevenson and colleagues (1990) studied the effects on pregnancy rates in lactating dairy cows when GnRH was administered to cows that were identified as being repeat breeders. They also compared the pregnancy rates between cows receiving double AI in the same estrus period to those cows identified as repeat breeders that were given GnRH treatment. They determined that following AI, an injection of GnRH for cows receiving a third or fourth service, improved pregnancy rates. When cows were inseminated twice during the same estrus period the results indicated that there was no improvement in pregnancy rate compared to the cows that received a single AI followed by an injection of GnRH. They hypothesized that the GnRH treatment coincided with the timing of ovulation and progesterone secretion by the CL. Conflicting results have been reported on the effect of GnRH to improve pregnancy rates, but several factors will affect its success. These could include the type and dose. Different types of hormones may have slightly different effects on the cows and the amount administered may also change the response. Timing of GnRH injection in relation to estrus and AI will invoke different responses. GnRH can result in ovulation, luteinization, or both depending on when it is administered. The study reported that GnRH improved the pregnancy rate for cows that were identified as repeat breeders and thus proved to be of some benefit. However, there was no advantage to a double AI.

Administration of GnRH between days 11 and 14 is believed to improve the survival rate of embryos by suppressing the luteolytic mechanism that ensues if maternal recognition of pregnancy does not occur (Peters, 1996). Peters and colleagues (2000) conducted a meta-analysis of 19 studies that examined the administration of GnRH between days 11 and 14 to determine the effects that were caused on pregnancy rates and the survival of the embryo. The timing of the administration is important as it is around this time that maternal recognition of pregnancy occurs. They concluded that the effect of the treatment post insemination was not consistent between the studies and that there were variations in protocols, timing and method of pregnancy diagnosis. However, under certain conditions and circumstances it appeared that GnRH produced significant benefits.

Willard and colleagues (2003) studied the effects of supplemental GnRH post insemination on serum concentrations of progesterone and pregnancy rates in lactating dairy cows under heat stressed conditions. They administered GnRH on day 5, which induced ovulation of the 1st wave dominant follicle and formed an accessory CL, and also on day 11 which was timed with maternal recognition of pregnancy. They concluded that administration of GnRH resulted in increased serum concentrations of progesterone and improved pregnancy rates. It was hypothesized that GnRH may indirectly be anti-luteolytic. It reduces follicular pools, which results in decreased estradiol, or reduced release of endogenous PGF_{2a}.

hCG supplementation

Human chorionic gonadotropin (hCG) has also been shown to increase plasma progesterone levels, and to have an effect on luteal phase follicles. Schmitt and colleagues

(1996) performed several experiments using both heifers and cows to determine the effects of supplemental hCG on day 5 of the estrous cycle. They hypothesized that inducing an accessory corpus luteum during the luteal phase of the estrous cycle, by way of a gonadotropin challenge, may increase concentrations of progesterone in plasma during the luteal phase. They injected 3000 IU of hCG on day 5 of the estrous cycle in heifers and lactating cows. Ovulation of the first wave dominant follicle was induced and in all of the heifers an accessory CL was formed. However, the control group did not ovulate the first wave dominant follicle. Combined, 91% of cows and heifers receiving hCG supplements formed an accessory corpus luteum. Similar results were reported by Santos and colleagues (2001), where 86.2% of cows treated on day 5 with hCG had an accessory CL, compared to 23.2% observed in the controls. Both studies also reported that the injection of hCG also increased the amount of plasma concentrations of progesterone, which was hypothesized to be caused by combined effects of the original, and induced CL. Santos and colleagues (2001) also noted that among dairy cows with an accessory CL those treated with hCG had higher serum progesterone concentrations than controls. Fricke and colleagues (1993) reported that all of the beef cows that received an injection of hCG on day 6, and who had a follicle present on day 6, ovulated resulting in the formation of a corpus luteum. Price and Webb (1989) also reported that large luteal phase follicles were capable of responding to hCG by the production of a luteinized structure.

Early studies have reported that treatment with hCG had no effect on stimulating conception rates. Diaz and colleagues (1998) noted that when heifers were treated with hCG the progesterone concentrations during the second follicular wave were no different than those of the control group. The results show that subsequent follicular development and

growth are not affected or altered, and that CL development and function in that cycle is not influenced by administration of hCG. Breuel and colleagues (1989) also reported that upon administration of hCG to beef heifers, CL's from subsequent cycles were not affected. The timing of the injection also appears to be important. The administration of hCG on day 1 or day 17 of the estrous cycle has not shown to have an effect on the concentration of progesterone. Levels remained the same as those observed before the injection of the hormone (Breuel et al., 1989). However, when an injection of hCG is administered on day 5 there is an increase in the concentration of progesterone (Diaz et al., 1992; Schmitt et al., 1996; Santos et al., 2001). Rajamanhendran and Sianangama (1992) administered hCG on day 7 after estrus and also reported that there was a significant increase in the plasma progesterone levels.

It has been shown that repeated administration of hCG in cattle induced the formation of antibodies. This results in the neutralization of the hormone and causes a reduced number of molecules to bind to the receptors in bulls (Sundby and Torjesen, 1978). Breuel and colleagues (1989) also noted that hCG may have resulted in the formation of antibodies in heifers preventing increases in P₄ concentrations. In order to increase progesterone concentrations, alternate hormones need to be administered to avoid those problems seen with hCG. GnRH, being of similar amino acid structure in all mammals, should not cause an anti-immune response which can occur after frequent use of hCG.

MATERIALS AND METHODS

Cattle

Mature lactating dairy cows were used to study the response of supplemental GnRH administration on day 5, 11 or both days 5 and 11 after insemination on reproductive performance during periods of heat stress (June to August) and periods of cooler temperatures (December to January). The summer trial was conducted at two farms in North Carolina, the Dairy Educational Unit (DEU), North Carolina State University (n=18), and North Carolina Department of Agriculture (NCDA) Piedmont Research Station, Salisbury, where two summer trials were performed, (Jun-Jul n=17), (Jul-Aug n=15). Only cows at DEU-NCSU, were used during the winter trial (n=15). The average days in milk were relatively balanced between treatment groups, as was range of parity for both seasons. Production was not available for cattle in the winter trial but the average milk production during the summer indicated that the GnRH-D5 group was higher than the other groups but GnRH-D11 was lower. Serum progesterone concentrations, cortisol concentrations and pregnancy were determined for both the summer and winter trials. The cows were milked twice daily, 5am and 5pm, at both NCSU DEU facility and NCDA Piedmont Research Station, had unlimited access to water and were fed a total mixed ration diet.

Table 2 Cow information: Summer trial

	Control	GnRH-D5	GnRH-D5+11	GnRH-D11
Production	55.5	84.3	52.3	36
DIM	151	137	189	175
Parity range	1-5	1-4	1-5	1-3

Table 3 Cow information: Winter trial

	Control	GnRH-D5	GnRH-D5+11
DIM	118	86	98
Parity range	1-5	1-2	1-3

Housing

The cows at NCSU DEU were housed in an open sided concrete floor barn, with self-locking stanchions adjacent to a free stall area with sand bedding, and access to pastures. Cows located the NCDA Piedmont research station were also housed in a free stall area with sand bedding, but were guided through a cattle chute for blood samples, rectal temperatures and to administer injections.

Treatment Groups

Ovulation was synchronized in all of the cows using the OvSynch protocol. On day -9, (before timed AI), the cows were given GnRH (Fetagyl®, 100ug i.m.; Intervet INC, Millsboro, DE, USA). Followed 7 days later, on day -2, with a 5ml i.m. injection of PGF2a (Lutalyse, 25 mg i.m.; Pharmacia & Upjohn, Peapack, NJ, USA). On day 0 a second injection of GnRH (100ug i.m.) was given to induce ovulation. This allowed for a timed insemination 16 hours later. At each location there were three treatment groups, however they differed slightly on the days of hormonal administration. At the DEU location the cows were divided into the control group, which did not receive an injection of supplemental GnRH. On day 5 post insemination a second group of cows received a 2ml (100ug) i.m. injection of GnRH. Finally, Group 3 was administered 2ml i.m. injections of GnRH on days 5 and 11 post insemination. At the NCDA Piedmont Research Station the three groups included the control group (No GnRH supplementation), cows receiving 2ml i.m. injections of GnRH on days 5 and 11 post insemination, and a third group of cows received a 2ml i.m.

injection of GnRH on day 11 post insemination. When the data from the two locations were pooled together there were a total of four treatment groups, control, GnRH-D5, GnRH-D11 and GnRH-D5+11.

Sampling

On each of the days when the cows received injections, rectal temperatures were taken from each cow using a digital thermometer (GLA M525 Digital Thermometer, San Luis Obispo, CA USA) and blood samples were taken from the coccygeal vein for analysis of progesterone and cortisol concentrations. Blood samples and rectal temperatures were taken from each cow 5 days post insemination and continued every other day for 30 days at the same time of day, around 10 o'clock in the morning, during the afternoon feeding. Daily high and low temperatures and maximum and minimum humidity values were measured throughout the duration of the study from the WRAL news weather station located at Raleigh-Durham airport. Using this data the temperature-humidity index (THI) was calculated to determine the degree of heat stress the cows were experiencing. The formula used to calculate the THI: $THI = ^\circ F - (0.55 - 0.55(RH)(^\circ F - 58))$ (Ingraham et al., 1974).

Pregnancy was determined approximately 30 days post insemination and again between days 45 and 60, using real time ultrasound. Blood samples were centrifuged on the day of collection to obtain serum, and were then stored at -20°C until analysis of the progesterone and cortisol concentrations.

Progesterone Assay

Serum progesterone was measured using commercial kits (Coat-A-Count, TKPG Diagnostic Product Corporation, Los Angeles, CA, USA) by a validated solid phase RIA assay, without extraction. Assays were conducted over 9 days. The intra-assay coefficient of variance for each of the assays were 5.7%, 5.38%, 5.59%, 4.01%, 5.09%, 2.03%, 5.28%, 5.11%, and 3.6%. The inter-assay coefficient of variance was 4.64%.

Cortisol Assay

Serum cortisol was measured also using commercial kits (TKC Coat-A-Count, Diagnostic Product Corporation, Los Angeles, CA, USA) by a validated solid phase RIA assay. The procedure was modified slightly for bovine serum cortisol levels. The suggested volume of serum to be assayed was 25ul. However, after a pre-trial assay, cortisol concentrations were non-detectable. The sensitivity of the assay was 0.14ng/ml. The amount of serum was increased to 100ul, which allowed for measurable levels of the hormone. Assays were conducted over 2 days, the intra-assay coefficient of variance for each assay were, 1.1%, and 6.5%. The inter-assay coefficient of variance was 3.8%

Statistical Analysis

The data was analyzed using the MIXED procedure of SAS for both progesterone and cortisol. The model statement used for the comparison of progesterone concentrations within treatment groups was “progesterone = day trt day*trt.” For the comparison of progesterone concentrations between seasons within treatment groups the model statement was “progesterone = day trt season season*trt.” Treatment groups were also compared within

season, and between seasons using the least square means.^z The model statements used for the comparison of cortisol concentrations within treatment groups was “cortisol = day trt day*trt.” For cortisol concentrations between seasons within treatment groups the model was “cortisol = day trt season season*trt.” Pregnancy rates were analyzed using the Chi square option of PROC FREQ of SAS.

RESULTS

Summer Progesterone

The overall environmental data indicate that cows at both locations were subjected to mild heat stress with average THI values of 75.8 at DEU and 76.2 at the NCDA Piedmont Research Station over the duration of the trial. On several individual days the cows were exposed to medium heat stress indicated by the THI reaching values between 79 and 85. The average temperature for both farms was 26.1°C. Humidity values were 68% and 66% for the DEU and NCDA Piedmont Research Station respectively. The average rectal temperature for the cows at DEU was 39.5°C, and 38.7°C at the NCDA Station.

DEU

Cows were synchronized to ovulate using the Ovsynch protocol. The concentration of serum progesterone was measured on days -9 and -2, before the synchronized ovulation, and every other day thereafter beginning on day 5. No treatment by day interaction was noted, but as expected there were differences between days ($P < 0.0001$), and between treatments ($P < 0.001$). For the overall estrous cycle the cows receiving supplemental GnRH-D5 (2.88 ± 0.22 , $P < 0.001$) and GnRH-D5+11 (3.89 ± 0.25 , $P < 0.05$) had higher progesterone concentrations than the control group (2.63 ± 0.23). The GnRH-D5+11 group also had a higher P_4 concentration than cows receiving GnRH-D5 ($P < 0.05$). When serum progesterone levels were analyzed between days 11 and 17, the expected luteal phase and time of maternal recognition of pregnancy, there was no longer a significant day effect ($P = 0.609$). Again, there was also no interaction between day and treatment ($P = 0.395$), but

treatment was still significant ($P < 0.001$) (Table 2). Analysis of treatment groups indicated that P_4 levels of the GnRH-D5+11 treated group remained higher than the control group ($P < 0.05$). GnRH-D5+11 continued to have higher levels of P_4 than the GnRH-D5 group ($P < 0.09$), and GnRH-D5 cows had higher levels than those of the control cows ($P < 0.05$) (Table 3).

Piedmont trial 1

Between days 5 and 32 of the experiment there was a significant effect of day ($P < 0.0001$), and treatment ($P < 0.05$). However, the day by treatment interaction ($P < 0.1$) tended to be significant (Table 2). The cows in the GnRH-D11 group had higher P_4 levels than those of the control group ($P < 0.05$), and also GnRH-D11 had higher levels than those of the GnRH-D5+11 ($P < 0.05$) (Table 4). Serum progesterone levels were again analyzed between days 11-18, within the expected luteal phase of the estrous cycle. There was a significant day effect ($P < 0.05$), and a significant treatment effect ($P < 0.05$), but no significant treatment by day effect (Table 2). The GnRH-D5+11 group had higher levels of P_4 than the control group ($P < 0.05$), and also, GnRH-D5+11 cows had higher levels of P_4 than GnRH-D11 cows ($P < 0.05$). This differs to the results found from the experimental period (Table 4).

Piedmont trial 2

Between days 5 and 33 of the experiment there was a significant effect of day ($P < 0.05$), and treatment ($P < 0.0001$), however, there was no significant effect of the day by treatment interaction (Table 2). In this trial the control group had higher P_4 levels than those

of the GnRH-D5+11 group ($P < 0.0001$), but the GnRH-D11 group had higher levels than the control group ($P < 0.001$). Also GnRH-D11 treated cows had greater levels than those in the GnRH-D5+11 group ($P < 0.0001$)(Table 5). Analysis of the data between days 12-19 indicated that there was a significant treatment effect ($P < 0.05$)(Table 2), and that P₄ levels from the GnRH-D11 group remained greater than the GnRH-D5+11 group ($P < 0.05$)(Table 5).

Pooled Locations

There were no interactions in serum progesterone concentrations for location and treatment. Therefore, the data from both farms were pooled together to determine if there were any significant effects. The results indicated a significant day effect ($P < 0.0001$) and treatment effect ($P < 0.0001$), but there was no difference from the day by treatment interaction (Table 2). Upon analysis of treatment groups the GnRH-D11 group had higher levels of P₄ than the control group ($P < 0.0001$), as was the case when GnRH-D11 was compared to the GnRH-D5+11 group ($P < 0.0001$) and the GnRH-D5 group ($P < 0.0001$)(Table 6). When days 12-18 were analyzed only the treatment effect tended to be significant ($P < 0.06$)(Table 2). When treatment groups were compared, the GnRH-D11 treated cows had higher P₄ levels than both the control cows ($P < 0.05$) and the GnRH-D5 cows ($P < 0.05$)(Table 6).

Table 4. Significance of day, treatment, and day by treatment effects on P₄ concentrations during the summer

Trial location	Days of Cycle	Day Effect	Tx Effect	Day*Tx Effect
DEU	5 thru 27	<i>P</i> < 0.0001	<i>P</i> < 0.001	<i>P</i> > 0.10
	11 thru 17	<i>P</i> > 0.10	<i>P</i> < 0.001	<i>P</i> > 0.10
Piedmont Trial 1	5 thru 32	<i>P</i> < 0.0001	<i>P</i> < 0.05	<i>P</i> < 0.10
	11 thru 18	<i>P</i> < 0.05	<i>P</i> < 0.05	<i>P</i> > 0.10
Piedmont Trial 2	5 thru 33	<i>P</i> < 0.05	<i>P</i> < 0.0001	<i>P</i> > 0.10
	12 thru 19	<i>P</i> < 0.10	<i>P</i> < 0.05	<i>P</i> > 0.10
Pooled locations	5 thru 27	<i>P</i> < 0.0001	<i>P</i> < 0.0001	<i>P</i> > 0.10
	11 thru 17	<i>P</i> < 0.10	<i>P</i> < 0.05	<i>P</i> > 0.10

Table 5. Serum P₄ (ng/ml ± SEM) concentrations during the summer at DEU

Tx	Day of cycle	
	5 thru 27	11 thru 17
Control	2.63 ± 0.23 ^a	3.29 ± 0.37 ^a
GnRH-5+11	3.89 ± 0.25 ^b	5.56 ± 0.42 ^b
GnRH-5	2.88 ± 0.22 ^a	4.58 ± 0.36 ^c

Values lacking a common superscript within column differ, *P* < 0.05

Table 6. Serum P₄ (ng/ml ± SEM) concentrations during the summer at NCDA Piedmont during trial 1

Tx	Day of cycle	
	5 thru 32	11 thru 18
Control	3.66 ± 0.29 ^a	5.13 ± 0.39 ^a
GnRH-5+11	3.97 ± 0.26 ^a	6.86 ± 0.36 ^b
GnRH-11	4.86 ± 0.28 ^b	5.7 ± 0.39 ^a

Values lacking a common superscript within column differ, *P* < 0.05

Table 7. Serum P₄ (ng/ml ± SEM) concentrations during the summer at Piedmont during trial 2

	Day of cycle	
	5 thru 33	12 thru 19
Control	4.17 ± 0.29 ^a	4.75 ± 0.67 ^{ab}
GnRH-5+11	1.85 ± 0.29 ^a	3.21 ± 0.67 ^b
GnRH-11	6.89 ± 0.58 ^c	7.17 ± 1.3 ^a

Values lacking a common superscript within column differ, $P < 0.05$

Table 8. Serum P₄ (ng/ml ± SEM) concentrations during the summer at all locations

	Day of cycle	
	5 thru 27	11 thru 17
Control	3.47 ± 0.17 ^a	4.64 ± 0.33 ^a
GnRH-5+11	3.32 ± 0.17 ^a	5.31 ± 0.33 ^{ab}
GnRH-11	5.20 ± 0.26 ^a	6.18 ± 0.5b
GnRH-5	2.96 ± 0.28 ^b	4.58 ± 0.55 ^a

Values lacking a common superscript within column differ, $P < 0.05$

Winter Progesterone

Only one trial was conducted during the winter, which took place at the DEU. Only three treatment groups were involved in this trial. Environmental considerations indicated that the cows did not experience heat stress. The average THI for the cooler months was 43.8, and ranged from 32 to 56.

Analysis of winter progesterone levels between days 5 and 27 of the experiment, indicated a significant day effect ($P < 0.05$), but there was no overall treatment effect ($P < 0.1$). Like the pooled data from the summer there was no day by treatment interaction (Table 6). When specific treatment groups were compared between days 5 and 27 of the trial the GnRH-D5+11 group had greater P₄ levels than the control group ($P < 0.05$)(Table 7).

Samples from days 11 through 22 of the estrous cycle were analyzed and the results showed

there were significant effects from day ($P < 0.05$); treatment ($P < 0.08$) and day by treatment interaction ($P < 0.08$) tended to be significantly different (Table 6). Comparison of the treatment groups the GnRH-D5 group had higher P_4 levels than the control group ($P < 0.09$). GnRH-D5+11 cows also had higher P_4 levels compared to the controls ($P < 0.05$)(Table 7).

Table 9. Day, treatment, and day by treatment effects on progesterone concentrations during the winter

Day of Cycle	Day effect	Tx effect	Day*Tx effect
5 thru 27	$P < 0.05$	$P > 0.10$	$P > 0.10$
11 thru 22	$P < 0.05$	$P < 0.08$	$P < 0.08$

Table 10. Serum P_4 (ng/ml \pm SEM) concentrations during the winter

Tx	Day of cycle	
	5 thru 27	11 thru 22
Control	4.42 ± 0.46^a	5.27 ± 0.59^a
GnRH-5+11	5.78 ± 0.45^b	7.10 ± 0.56^b
GnRH-5	5.10 ± 0.53^{ab}	6.87 ± 0.69^{ab}

Values lacking a common superscript within column differ, $P < 0.05$

Summer vs. Winter

A post hoc comparison of progesterone between seasons revealed significant differences between day ($P < 0.0001$), treatment ($P < 0.0001$), and season ($P < 0.0001$), but there was no treatment by season interaction over the complete period. Analysis of P_4 concentrations from days 9 through 17 of the cycle indicated both day ($P < 0.01$) and season effects ($P < 0.01$), but no treatment or treatment by season effects (Table 8). Each treatment group was compared between seasons over the entire estrous cycle and the expected luteal phase. The mean serum progesterone concentrations were noticeably lower during the summer than in the winter (Figures 1.1, 1.2, 1.3). Control progesterone concentrations from

the summer were compared to those of the winter from which it was determined that the winter had greater P_4 levels ($P < 0.05$). This was also true when the GnRH-D5+11 ($p < 0.0001$) and GnRH-D5 ($P = 0.0032$) groups from each season were compared (Table 9).

Table 11. Summer vs. winter effects of day, treatment, season, and treatment by season

Day of Cycle	Day effect	Tx effect	Season effect	Tx*Season effect
5 thru 27	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P > 0.10$
9 thru 17	$P < 0.01$	$P > 0.10$	$P < 0.010$	$P > 0.10$

Table 12. P_4 (ng/ml \pm SEM) concentration comparisons between season and synonymous treatment groups

Tx	Day of Cycle	Summer	Winter	p-value
Control	5 thru 27	3.64 ± 0.17	4.65 ± 0.41	$P < 0.05$
	9 thru 17	4.65 ± 0.25	5.97 ± 0.68	$P < 0.07$
GnRH-5+11	5 thru 27	3.79 ± 0.17	6.05 ± 0.41	$P < 0.001$
	9 thru 17	5.24 ± 0.25	6.44 ± 0.66	$P < 0.09$
GnRH-5	5 thru 27	3.66 ± 0.26	5.26 ± 0.47	$P < 0.01$
	9 thru 17	4.78 ± 0.38	6.21 ± 0.75	$P < 0.09$

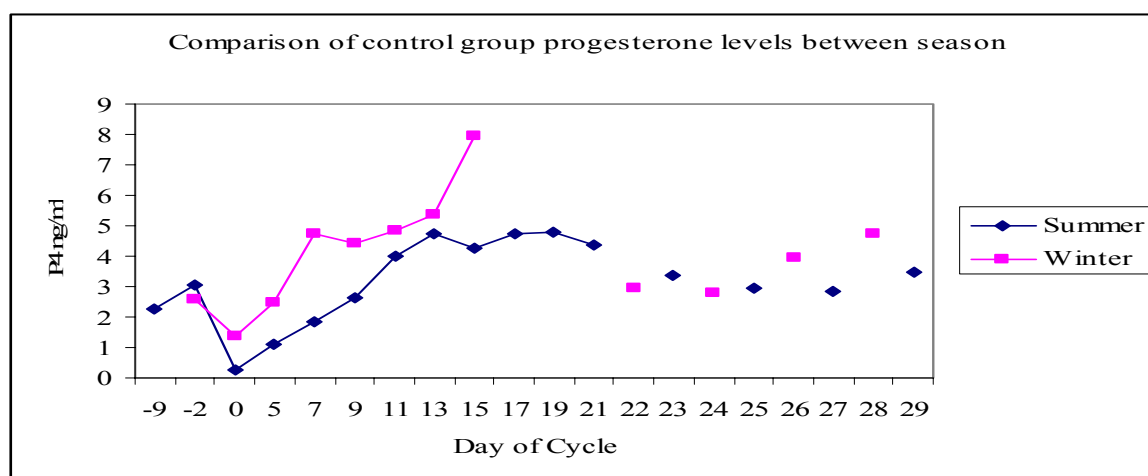


Figure 1.1

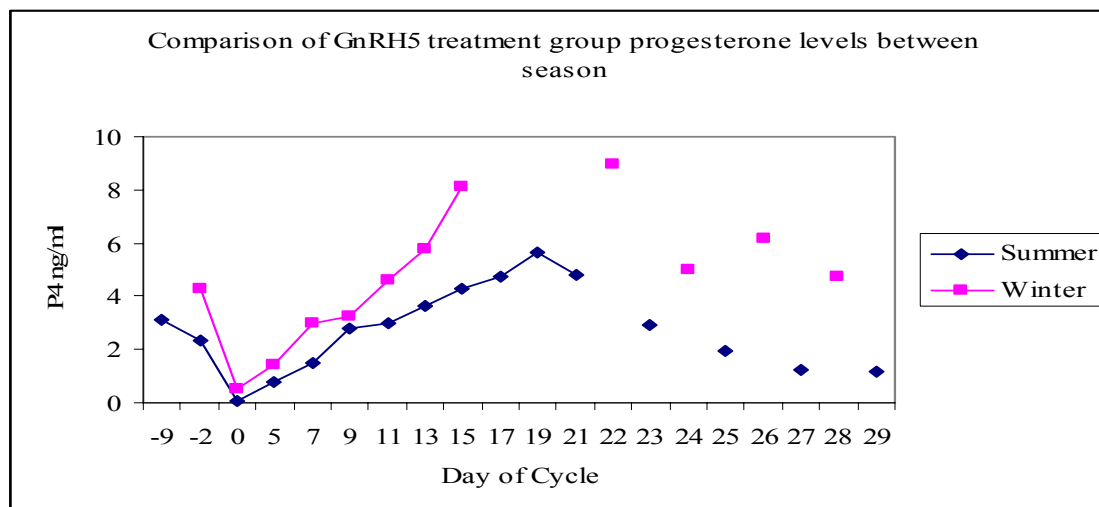


Figure 1.2

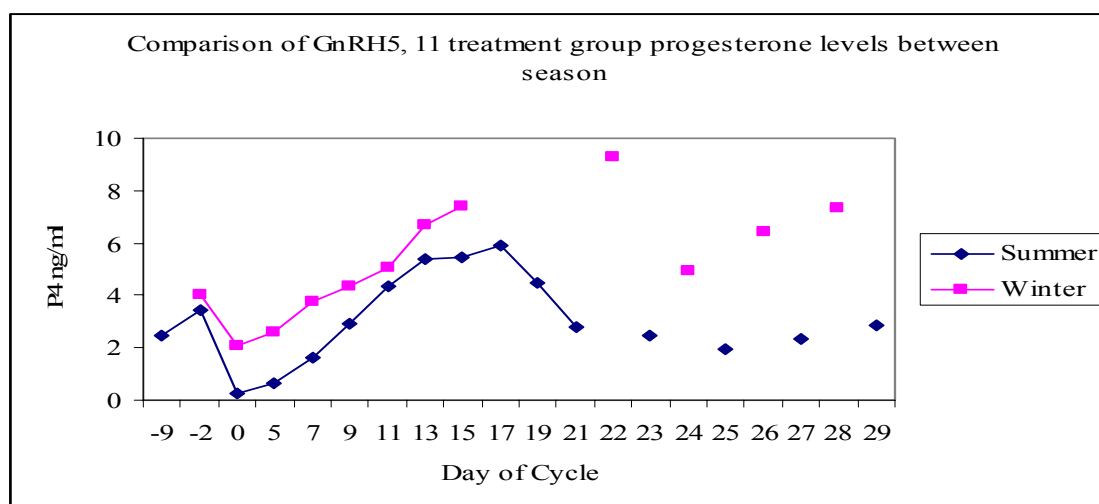


Figure 1.3

Summer Cortisol

Pooled locations

Serum cortisol concentrations, in each of the treatment groups from both locations, were compared. There was no treatment effect, or day by treatment effect, however the results showed there tended to be a day effect ($P < 0.08$)(Table 10). Upon comparison of

treatment groups there was only a noticeably higher concentration in the GnRH-D11 than in GnRH-D5 ($P < 0.05$)(Table 11).

Winter Cortisol

The winter results were similar to those of the summer in that only a day effect was significant ($P < 0.0009$), but there were no significant differences between treatment groups (Tables 10 and 11 respectively). A comparison between season was performed from which it was determined there was a significant effect of season ($P < 0.0001$)(Table 12). Summer and winter cortisol levels by treatment groups were also compared against one another. The control group in the summer had higher concentrations than those of the winter ($P < 0.0004$), as was true of the GnRH-D5 ($P < 0.0024$) and GnRH-D5+11 groups, ($P < 0.1209$)(Table 13). The average serum cortisol concentrations for each treatment during the summer were plotted against the day of cycle. Interestingly, there was an apparent increase in concentrations during the onset of estrus after which the concentrations slowly decreased (Figure 2).

Table 13. Significance effects of day, treatment, and day by treatment on cortisol concentrations

Pooled trials	Summer	Winter
	p-value	p-value
Day effect	$P < 0.08$	$P < 0.001$
Tx Effect	$P > 0.10$	$P > 0.10$
Day*Tx effect	$P > 0.10$	$P > 0.10$

Table 14. Comparison of cortisol concentrations (ug/dl ± SEM) within treatment group

Tx	Summer	Winter
Control	4.93 ± 0.35 ^{ab}	2.53 ± 0.42 ^a
GnRH-5+11	4.48 ± 0.35 ^a	2.28 ± 0.48 ^a
GnRH-5	3.93 ± 0.59 ^b	2.91 ± 0.42 ^a
GnRH-11	5.53 ± 0.54 ^{ab}	--

Values lacking a common superscript within column differ, $P < 0.05$

Table 15. Effects of day, treatment, season, and treatment by season interaction of cortisol

Summer vs. Winter Effect	p-value
Day effect	$P > 0.10$
Tx effect	$P > 0.10$
Season Effect	$P < 0.0001$
Tx*Season Effect	$P > 0.10$

Table 16. Comparison of cortisol (ug/dl ± SEM) concentrations between season

Tx	Summer	Winter	p-value
Control	4.98 ± 0.38	1.92 ± 0.74	$P < 0.001$
GnRH-5+11	4.54 ± 0.38	1.89 ± 0.75	$P < 0.010$
GnRH-5	3.98 ± 0.58	2.56 ± 0.67	$P > 0.10$

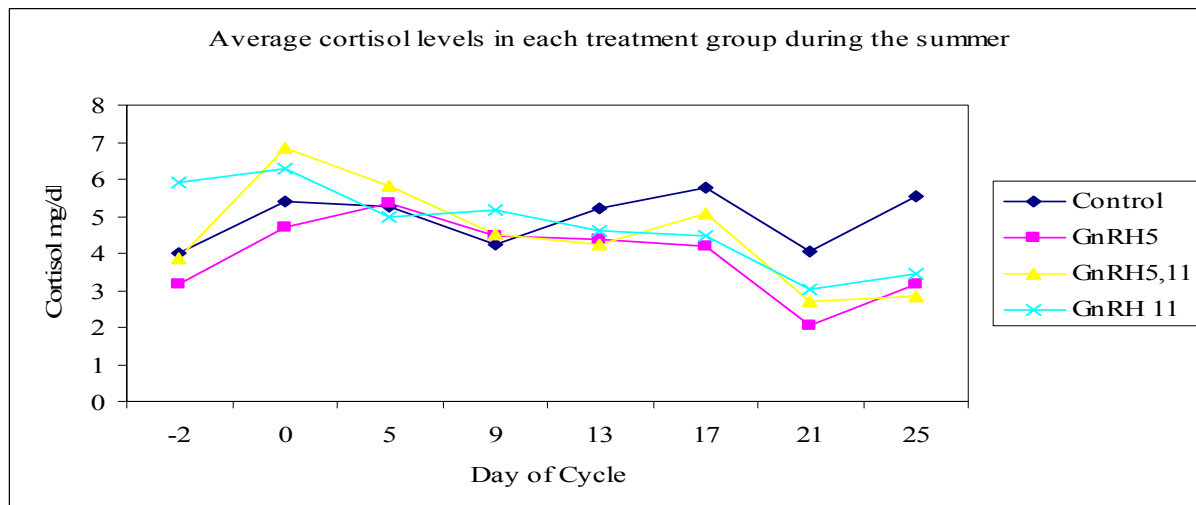


Figure 2

Pregnancy

Pregnancy was checked at day 30 using ultrasonography and again between days 45-60. No pregnancy losses were recorded from day 30 to day 45-60. Pregnancy rates from each trial indicate that there was an increased frequency of pregnant cows in the control group during the summer. Cows treated with GnRH-D11 during the first trial at the NCDA Piedmont Research Station had a higher incidence of pregnancies, but during the second trial, the pregnancy rate was split. Due to low sample numbers, care must be taken in interpreting these data. The results of the Chi Square analysis indicated that GnRH treated cows had a greater pregnancy rate than an expected pregnancy rate of 20% ($P < 0.05$).

Figures 3 and 4 show the frequency of cows that became pregnant, remained open, and missing data during both summer and winter trials. The winter values were too small to perform a chi square analysis.

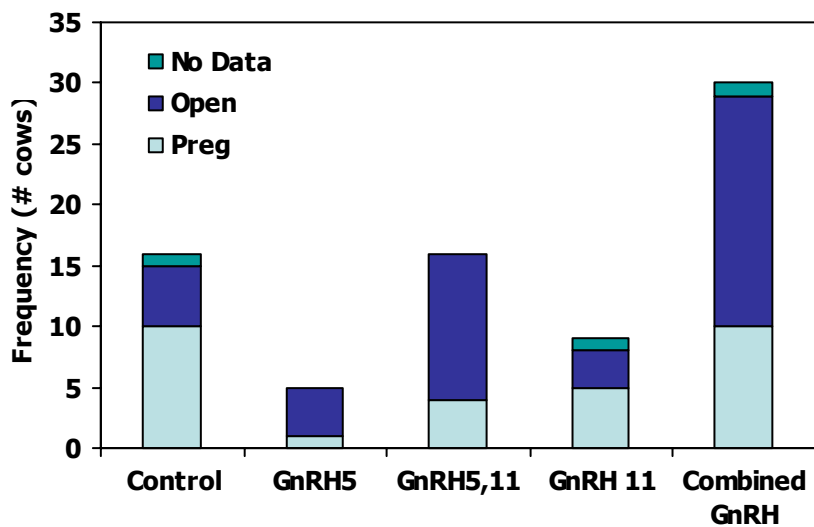


Figure 3 Frequency of cows pregnant between days 45-60 in each treatment group during the summer

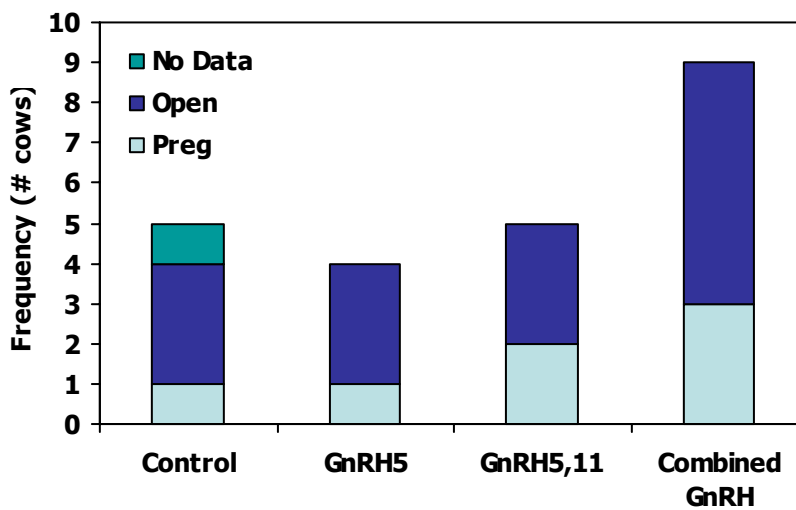


Figure 4 Frequency of cows pregnant between days 45-60 in each treatment group during the winter

DISCUSSION

The objective of this study was to determine if supplemental GnRH on selected days post insemination would improve reproductive performance in dairy cattle during periods of heat stress. The summer and winter studies were compared to observe any notable differences between seasons relating to serum progesterone levels and pregnancy rates. Many factors can result in decreased reproductive performance including disease, high milk production, and environmental factors. There is a negative correlation between high producing dairy cattle and reproductive performance, which is amplified when the cows experience heat stress. Heat stress is defined as the sum of external factors acting upon the cow to displace temperature from the normal resting state. When the THI is greater than 72, heat stress becomes a factor for decreasing reproductive performance (Du Preez et al., 1991). In the present study, the average daily THI values were 75.8 (DEU) and 76.2 (Piedmont), which are both classified as mild heat stress conditions. However, there were individual days when the THI reached values between 79 and 85 which are classified as moderate heat stress levels. The average rectal temperatures ranged from 38.6 to 39.5°C. However, individual cows experienced temperatures as high as 41.2°C and as low as 37.6°C during the trials. Interestingly, on days that were identified as inducing moderate heat stress, effects on rectal temperatures were not observed until the following day. Also, the range of rectal temperatures observed, have been associated with reduced fertility in cattle in previous studies (Ulberg and Burfening, 1967).

The rationale for administration of GnRH on day 5 post insemination is to induce ovulation of the next wave dominant follicle. However, ovulation will not occur when

progesterone levels are high therefore the follicle undergoes atresia. Administration of GnRH will induce ovulation of this follicle resulting in the formation of an accessory CL and, in turn, enhance the P₄ production early in the cycle (Willard et al, 2003). In a previous study it was found that GnRH treated cows had more luteal tissue than controls (Willard et al., 2003). The rationale for administering GnRH on day 11 of the cycle is similar to that of day 5 in that it enhances P₄ production (Willard et al., 2003). Also day 11 will produce elevated serum P₄ during the time of maternal recognition of pregnancy, which begins 3 to 4 days later and before the start of the luteolytic mechanism. It is hypothesized that this allows the embryo to develop further (Willard et al., 2003). Supplementing GnRH on both days 5 and 11 is rationalized by combining both effects observed on each of the days. It should enhance progesterone production and hopefully improve reproductive performance further. Increasing serum P₄ concentrations may be especially important in heat stress conditions since earlier studies have found lower serum P₄ in heat stressed cows (Ronchi et al., 2001; Howell et al., 1994). This earlier work agrees with data from the current study in which summer P₄ levels were lower than winter.

Peters and colleagues (2000) performed a meta-analysis on 19 studies that administered GnRH between days 11 to 14. The objective was to determine if a definitive response to the treatment could be established using previous data. However the analysis did not account for seasonal effects, which would have included heat stress. They determined that there were many different variables within each study, and that the pregnancy rates between each study varied considerably. These variations could be attributed to multiple factors including differing environmental factors, management, or breeds. Due to these variations they determined that a specific physiological effect had yet to be proven with the

GnRH treatment. A physiological effect that had been reported in previous studies was that GnRH elevated P₄ (Mee et al., 1993; Thatcher et al., 1989).

Peters and colleagues concluded that there were many inconsistencies with GnRH administration between the studies that could include protocol design and method of pregnancy diagnosis. However, they did determine that under certain conditions GnRH administration may be beneficial to improving reproductive performance. Overall, their conclusion from the meta-analysis was that GnRH administration on days 11-14 increased pregnancy rates.

Ullah and colleagues (1996) administered GnRH to Holstein dairy cows that were heat stressed at observed estrus. They reported that serum P₄ levels were elevated during the luteal phase in all cows given GnRH compared to those of the control group. Our study showed similar results when the expected days of the luteal phase were analyzed using the pooled data. GnRH-D11 treated cows had significantly greater P₄ concentrations compared to the control group ($P < 0.05$). However GnRH-D5 and GnRH-D5+11 were not different from the control group. The GnRH-D5 group had lower P₄ concentrations compared to the other treatment groups due to the difference in sample size number. When the values strictly from the DEU are analyzed they are greater than both the control group and the GnRH-5+11 group. However, when the data from the three locations were combined the sample sizes of the control group and GnRH-D5+11 group increase, resulting in a difference in values. GnRH treated groups resulted in elevated serum P₄ concentrations as compared to the controls in both the winter and under heat stress conditions. These results agree with data from a previous study performed under similar conditions (Willard et al., 2003). The

administration of GnRH to cows increased serum P₄ concentrations (Mee et al., 1993; Schmitt et al., 1996; Ullah et al., 1996).

A post hoc comparison revealed that control cows in the winter study tended to have higher serum P₄ than GnRH treated summer cows. This indicates a significant seasonal effect, which were attributed to heat stress. It also suggests that inducing extra luteal tissue with GnRH administration was not able to fully overcome the inhibitory effects of heat stress on P₄ secretion. The summer heat stress may have compromised the functioning of the CL of ovulation and the GnRH-induced luteal tissue as well. The mechanism by which heat stress decreases serum P₄ concentrations is unknown but various studies have suggested that decreased gonadotropin secretion (Gilad et al., 1993; Madan and Johnson 1973), which subsequently could compromise the development of the CL (Wolfenson et al., 1993) and decreased blood flow, could limit nutrients and hormonal availability to the CL (Roman-Ponce et al., 1978).

The majority of studies have reported decreased serum P₄ in heat stressed cows (Howell et al., 1994; Imtiaz-Hussein et al., 1992). Howell and colleagues (1994) measured serum P₄ concentrations during the spring and summer in order to compare CL growth between the two seasons in lactating dairy cows. They determined that for the duration of the estrous cycle P₄ concentrations were unaffected by season. However when they analyzed day 6 through day 18, serum P₄ was depressed in the summer. During the summer trial the environmental data indicated that the cows experienced temperatures ranging from 27°C to 40°C, and an average relative humidity of 82.9%, resulting in heat stress. Imtiaz-Hussein and colleagues (1992) reported similar results when they compared nonpregnant Holstein cows, to Jersey cows. Holstein cows had a lower range of P₄ for the duration of the study, and also

had lower luteal phase P₄ concentrations than Jersey cows under heat stress conditions. The study did not address if P₄ concentrations for both breeds were lower in the summer than in the winter. Only the two breeds were compared to one another in the summer, no comparisons within breed were made.

The pregnancy results from the present study did not agree with previous work. There were no differences between the pregnancy rates from the control group and the combined GnRH treated groups during the summer. Willard and colleagues (2003) compared pregnancy rates of all GnRH treated groups (38%) (single injections on day 5, or on day 11) with the control group (19%). The present study had a control pregnancy rate of 66.7%, whereas expected values fall around 20%, and a combined GnRH treated group of 52%. The difference maybe due to the efficiency of service technician or individual cows, or it maybe due to another mechanism that was not identified in this study. None of the cows in the winter study control group became pregnant, whereas the expected value usually reaches around 35%, and the combined GnRH treated groups were 50%. This could again be due to the technician, the cow, or other factors that needs to be identified.

Several studies have been conducted in cattle describing the effects of heat stress on cortisol levels. Evidence shows that there is a difference between cows exposed to acute heat stress as opposed to chronic heat stress (Christison and Johnson, 1972). Christison and Johnson (1972) showed that cows exposed to acute heat stress had a rapid increase in cortisol that leveled off after 2hrs. During chronic heat stress cortisol levels peaked at 12hrs after exposure, and then returned to normal levels after 1 or 2 days and continued to decline to below resting levels. Alvarez and Johnson (1973) found similar results in that cortisol was elevated with acute heat stress, but upon chronic stress levels decreased to normal levels.

Gwazdauskas and Vinson (1979) studied the response of the adrenals to adrenocorticotropin (ACTH) in Holstein heifers during cold temperatures and found that heifers exposed to -2.7°C were no different from heifers exposed to 19°C . This change in environmental temperature from 19°C to -2.7°C was not sufficient to result in a response by the adrenals to ACTH.

In the current study the cows experienced chronic heat stress. There were no differences in serum cortisol levels between treatment groups during the summer. Interestingly in all treatment groups during the summer, around the time of expected estrus there was a slight increase in serum levels. Lyimo and colleagues (2000) showed that cortisol levels increased around the time of estrus, indicating that estrus could be a cause of stress. Upon comparison of average cortisol levels in each treatment group and between seasons it was evident that concentrations were elevated in the hot summer months compared to the cooler months of winter.

Pregnancy rates did not differ between treatment groups in winter. In the meta-analysis of Peters et al. (2000), the conclusion was that most papers reported an improvement in pregnancy rate with GnRH supplementation. Peters and colleagues did not differentiate between studies conducted in winter and summer. The relatively low numbers made it difficult to detect differences in pregnancy rate in the current study. It was therefore difficult to determine if GnRH was beneficial or not to pregnancy rates.

Pregnancy rates during the summer of 2002 did not agree with previous studies. The overall pregnancy rate for the control group ($n=13$) was 62% for the summer, which is exceptionally high, whereas Ron and colleagues (1984) reported that in Israel conception rates decreased from 52% in winter to 24% in the summer. The other treatment groups also

were unexpected, GnRH-D5 (n=6) 16.7%, GnRH-D11 (n=6) 62.5%, and GnRH-D5+11(n=15) 26.7%. Caution has to be taken when interpreting these findings due to the low number of cows that were used in the study. Willard and colleagues (2003) reported that cows receiving GnRH-D5 (n=34) and GnRH-D11 (n=34) had pregnancy rates of 32%, and 38% respectively. The control group had a pregnancy rate similar to that which is often seen during periods of heat stress, 19%. Ullah and colleagues (1996) reported cows treated with GnRH at observed estrus had a pregnancy rate of 28.6% where as the control group was 17.9%, as determined by rectal palpation. They monitored progesterone in subset of cows, for which pregnancy rates were also determined. On d 20, after estrus and AI, the pregnancy rates based on the progesterone levels were 42.8% for the GnRH treated group, and 57.1% for the control group. However, after d 45 pregnancy rates were determined by palpation and were found to be 28.6% for the GnRH treated cows and 14.3% for the control cows, indicating that the control group experienced a 42.8% loss over a 25 day period.

When combining the results from 2002 with those of previous years, pregnancy rates were higher in GnRH treated groups than in controls ($P<0.05$). When results from 2002 were combined with the results from the 2001 experiment the pregnancy rates were 25% (Control), 35% (GnRH-D5) and 34.7% (GnRH-D5+11). No cows received a single injection of GnRH on D11 in 2001. The pregnancy rates were lower in controls than in the two GnRH treated groups when both years were combined. The inconsistent pregnancy rate may be associated with the sample size difference between the two years. The level of heat stress was not different based on the THI values and serum P₄ concentrations were similar. The high pregnancy rates in the 2002 control group are most likely due to chance.

CONCLUSIONS

The administration of supplemental GnRH increased endogenous levels of P₄ during periods of heat stress. However, summer P₄ levels were lower across all treatment groups compared to those of the winter. There were no differences in cortisol concentrations between treatment groups. As expected, levels of cortisol increased around the time of estrus and then gradually decreased with continued exposure to heat stress.

When all treatment groups that received GnRH supplementation were combined there appeared to be a beneficial effect on pregnancy rates for lactating dairy cows in the summer. A comparison of individual groups indicated GnRH-D11 and GnRH-D5+11 treated cows had greater pregnancy rates than the control cows, but there was no difference between the GnRH-D5 treated cows or the controls. This may indicate that administering GnRH later, around D11 post insemination, is more beneficial to improving pregnancy rates, rather than an earlier injection on D5. However, the winter study indicated that supplemental GnRH had no significant effect on pregnancy rates but low numbers may have contributed to these findings. This result may indicate that GnRH treatment is not beneficial to pregnancy rates in lactating dairy cows when overall reproductive performance is normal or above normal. Rather it may be a benefit in situations when reproductive performance is substandard. Overall it appears that when control group pregnancy rates are low, there is some benefit to supplementing cows with GnRH. However the exact day of administration for optimal pregnancy has yet to be determined.

This study is part of an ongoing research project looking for possible mechanisms to improve fertility during the summer in dairy cattle. Follow up experiments need to be

performed to observe the effects of heat stress on serum estradiol and prostaglandin metabolite. The study needs to be repeated in subsequent summers to increase sample size and help identify potential mechanisms that decrease fertility in lactating dairy cows, and develop protocols to improve pregnancy rates.

APPENDIX

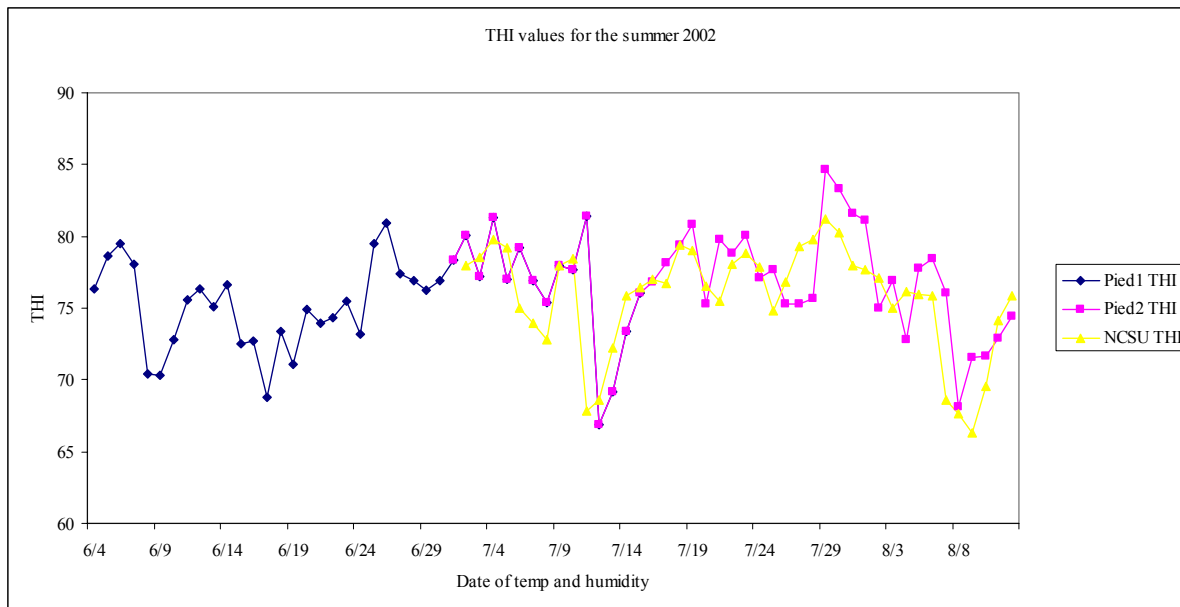


Figure 1 Mean THI values during the summer trials at the DEU, and the North Carolina State Piedmont Research center

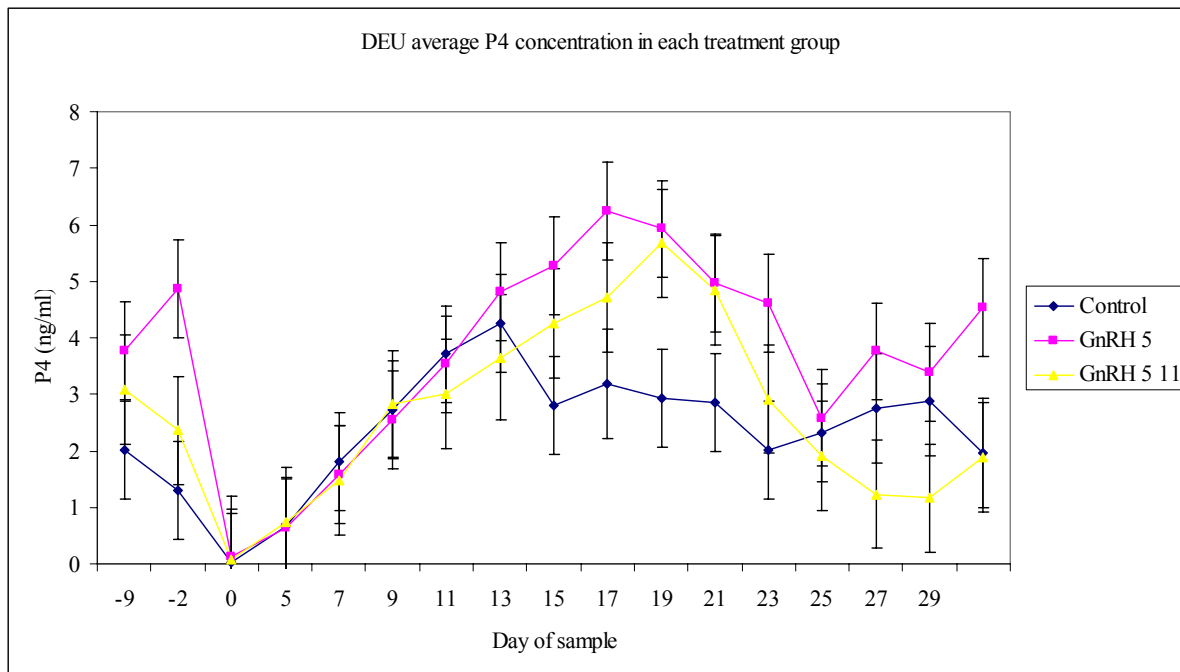


FIGURE 2.1 Mean P₄ concentrations from NCSU-DEU in each treatment group during the summer

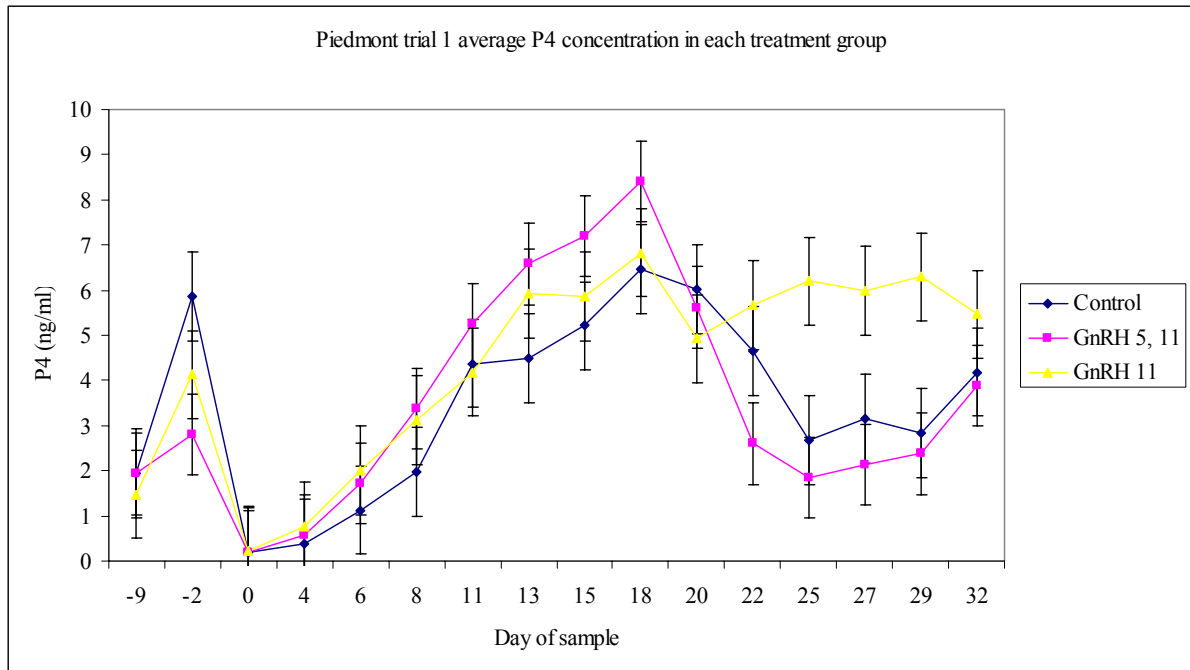


FIGURE 2.2 Mean P₄ concentrations from NCDA-Piedmont trial 1 in each treatment group during the summer

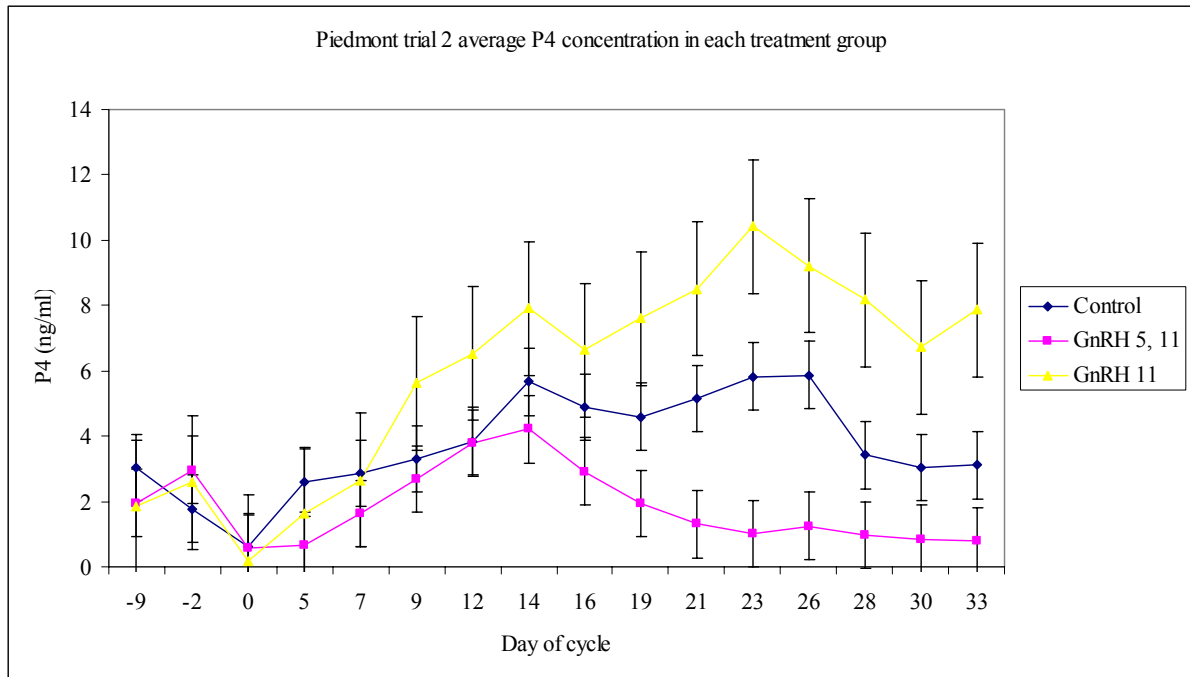


FIGURE 2.3 Mean P₄ concentrations from NCDA-Piedmont trial 2 in each treatment group during the summer

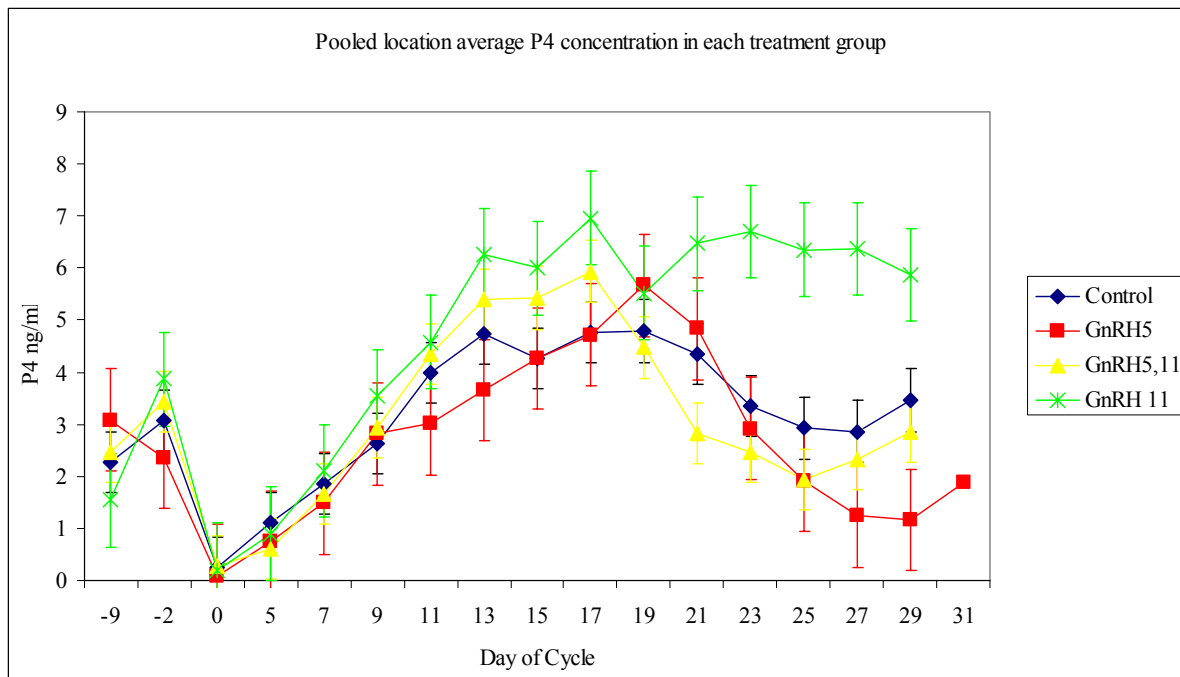


FIGURE 2.4 Mean P₄ concentrations from all locations in each treatment group during the summer

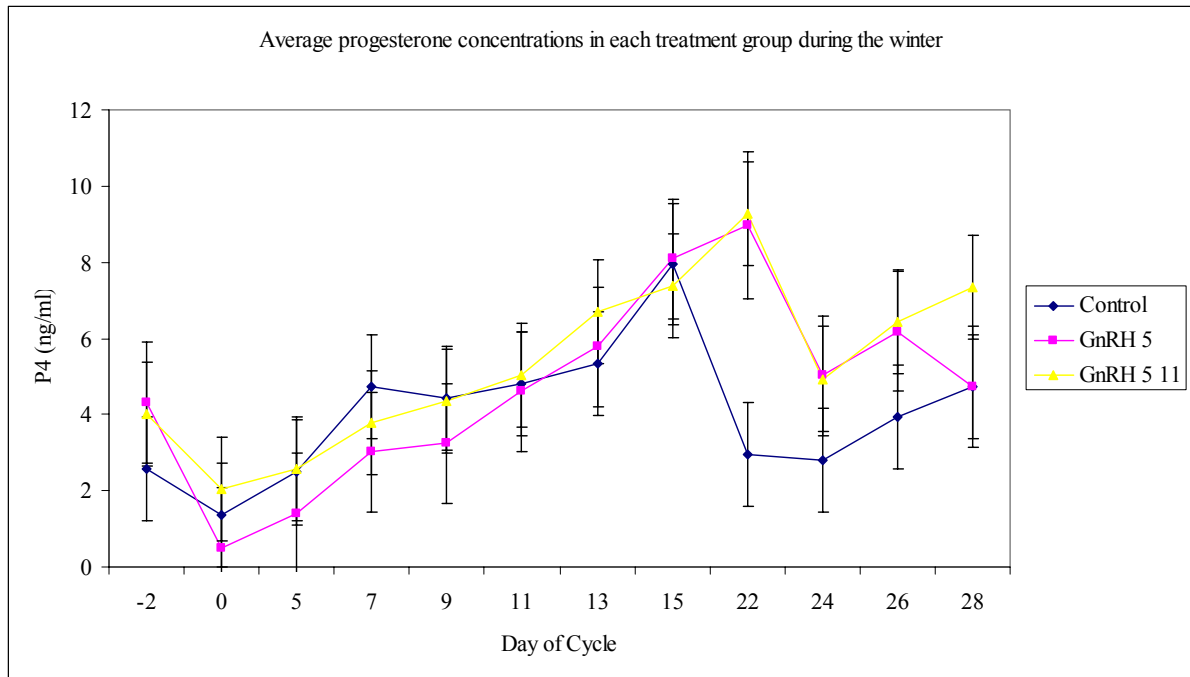


FIGURE 3 Mean P₄ concentrations from NCSU-DEU in each treatment group during the winter

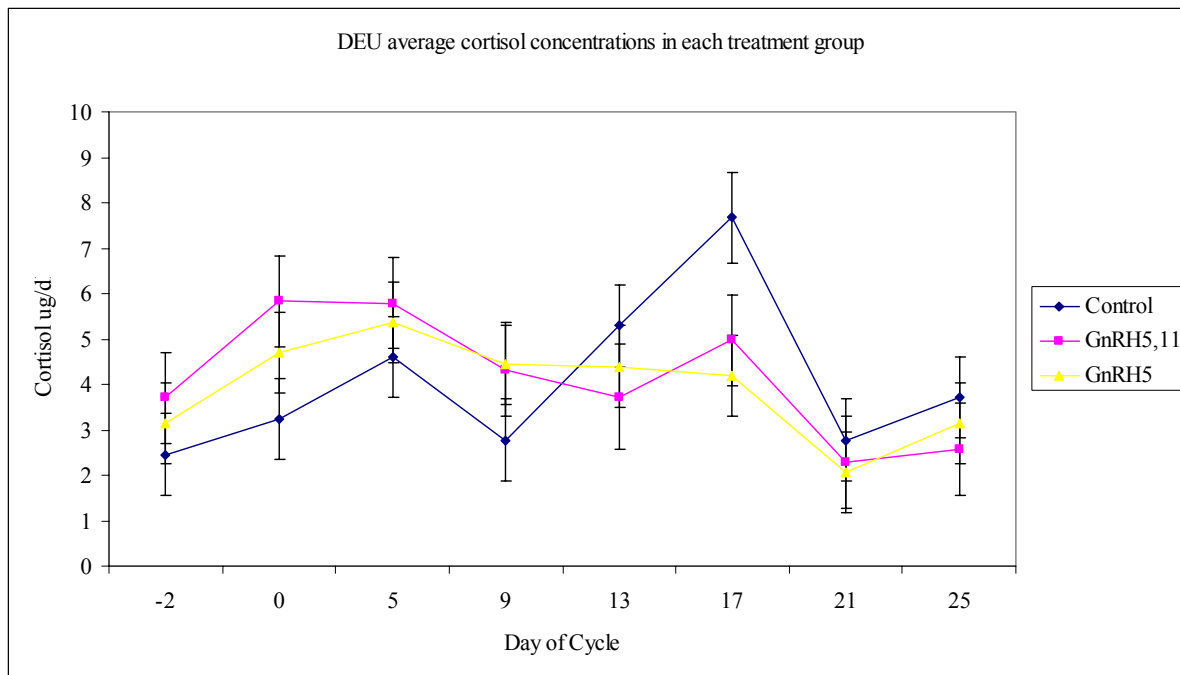


FIGURE 4.1 Mean cortisol concentrations from NCSU-DEU in each treatment group during the summer

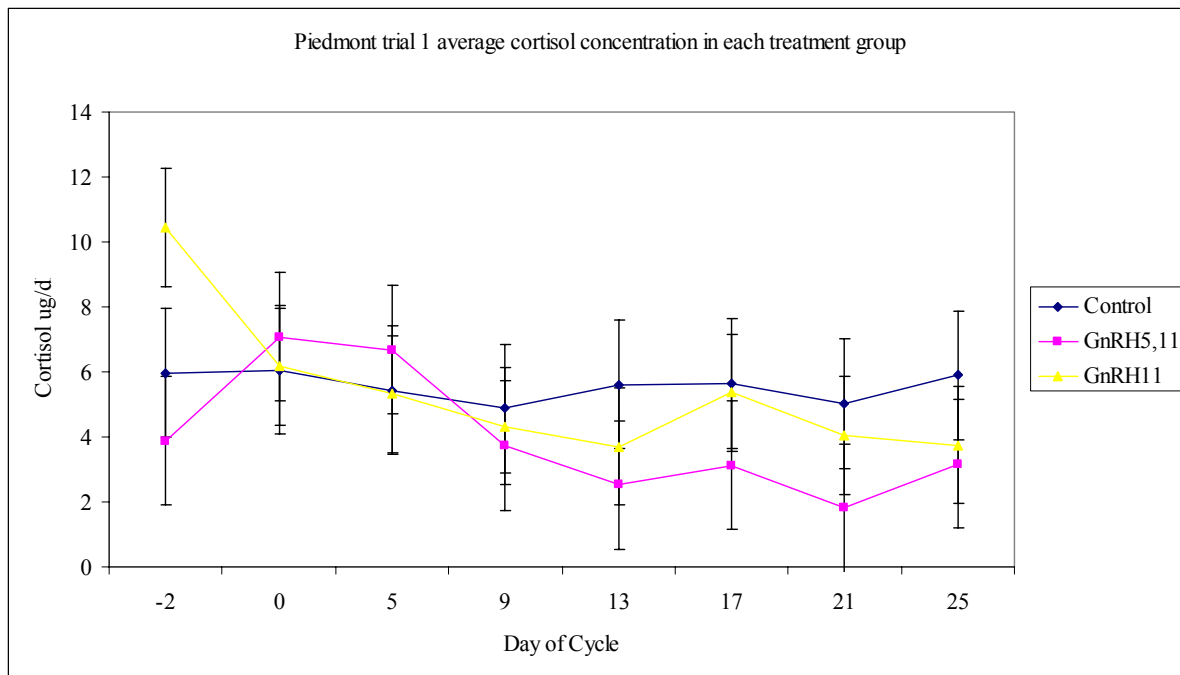


FIGURE 4.2 Mean cortisol concentrations from NCSU-Piedmont trial 1 in each treatment group during the summer

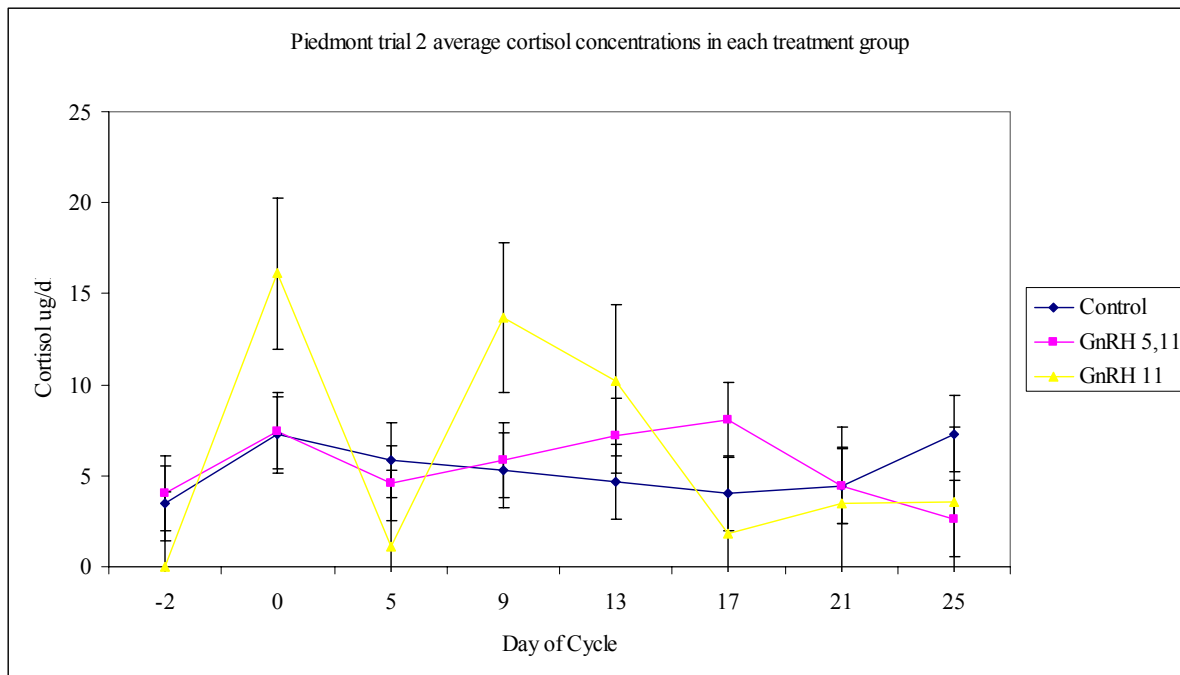


FIGURE 4.3 Mean cortisol concentrations from NCSU-Piedmont trial 2 in each treatment group during the summer

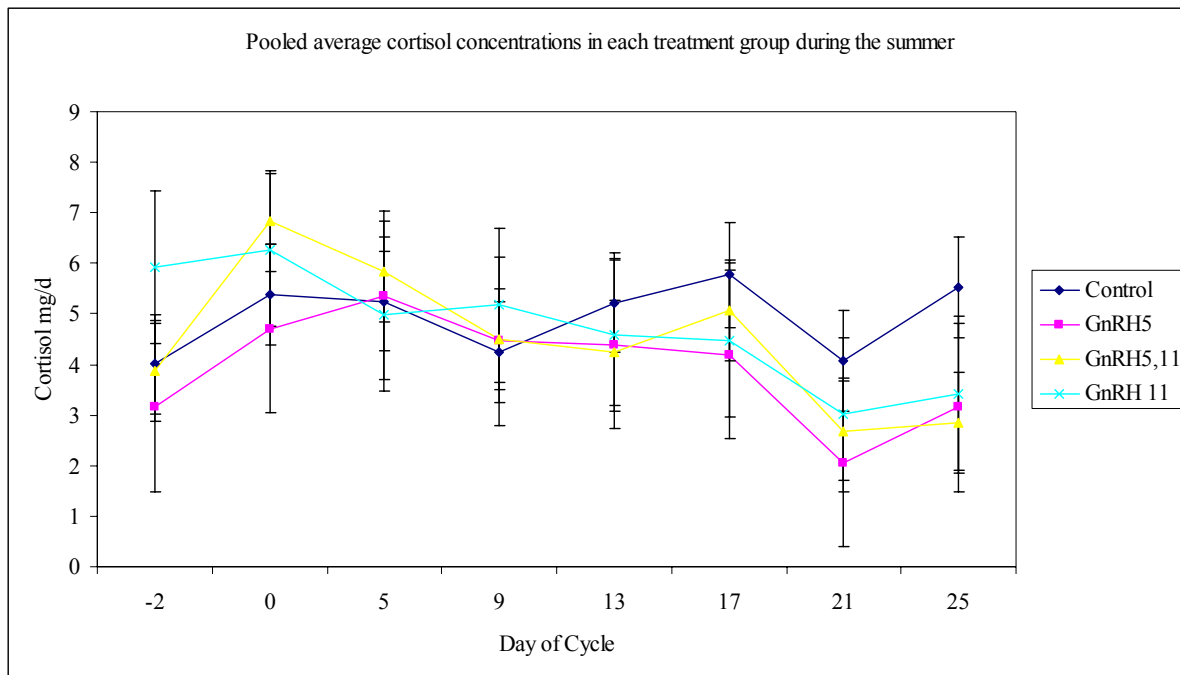


FIGURE 4.4 Pooled average cortisol concentrations between treatment groups

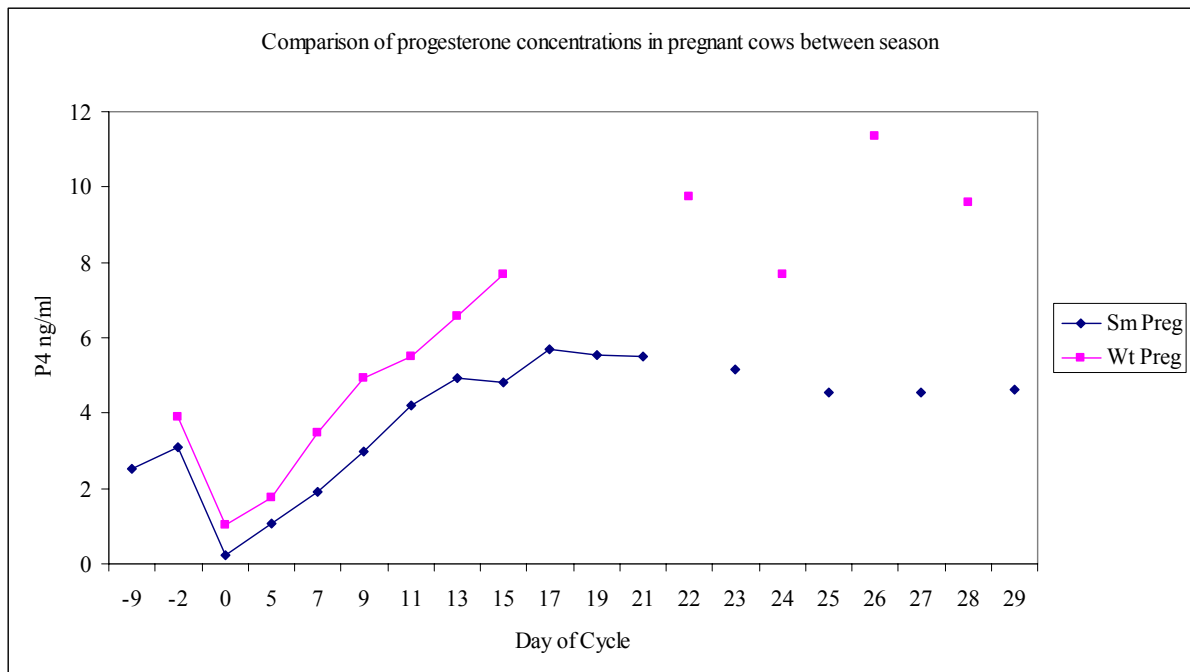


FIGURE 5.1 Comparison of average P₄ concentrations in pregnant cows between season

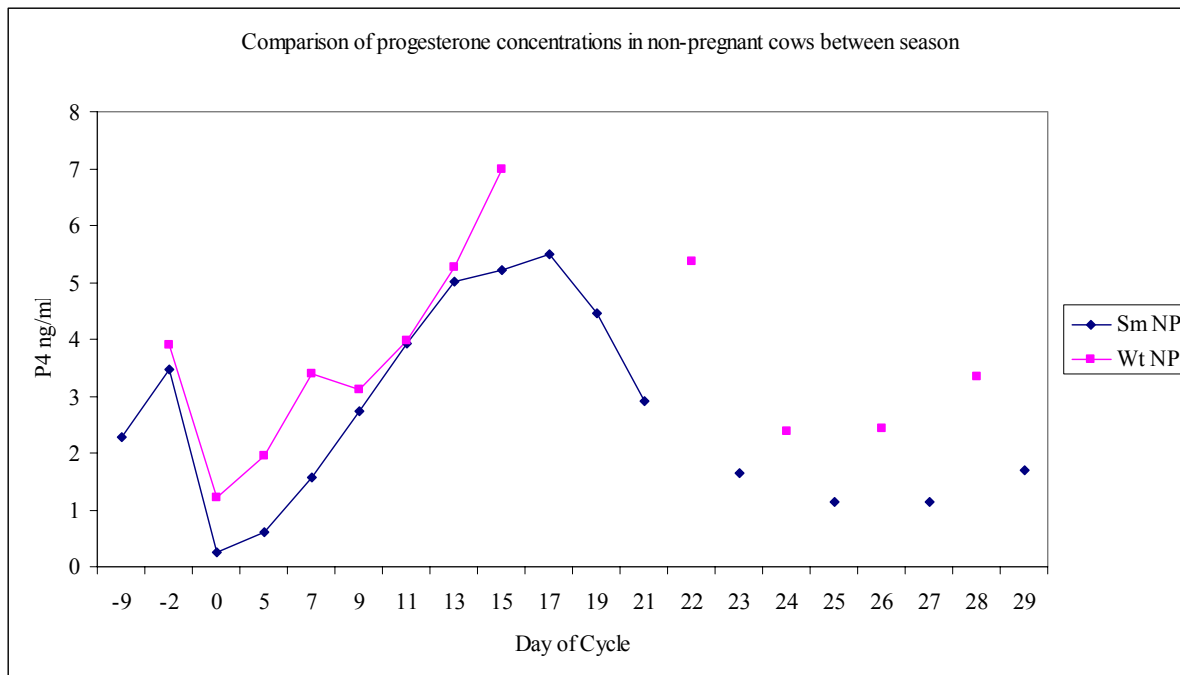


FIGURE 5.2. Comparison of average P₄ concentrations in non-pregnant cows between seasons

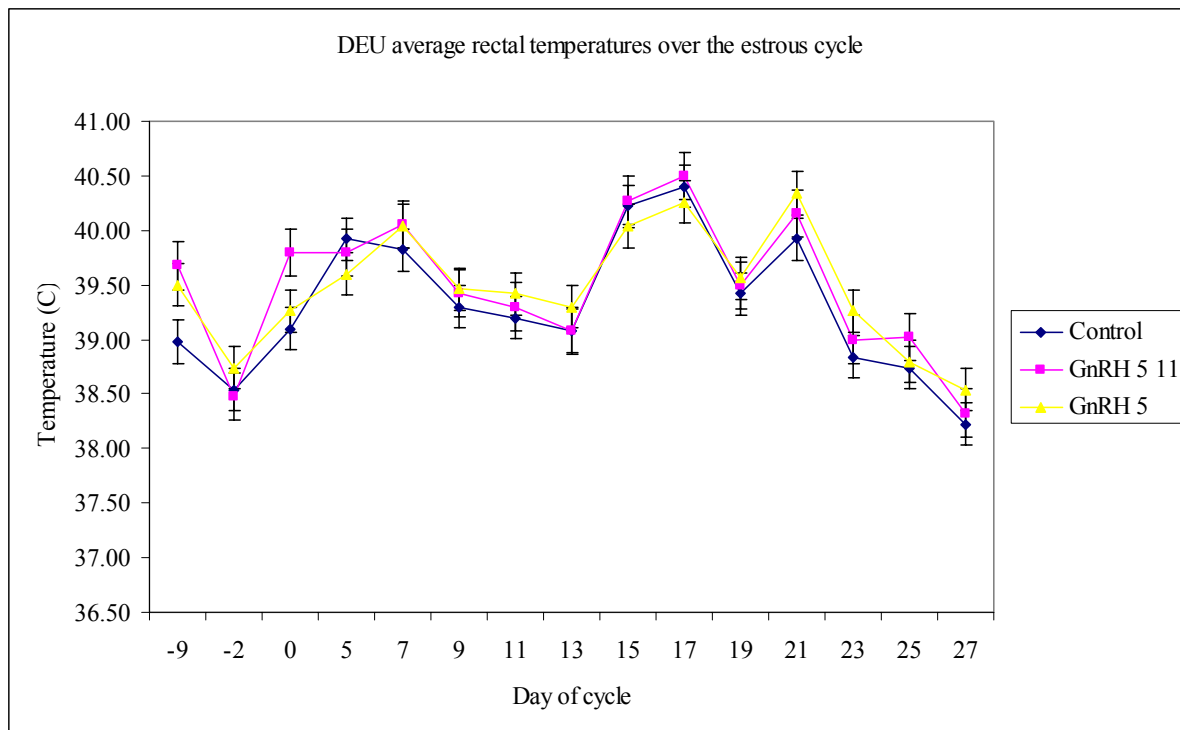


FIGURE 6.1 Comparison of average rectal temperatures between treatment groups at NCSU-DEU during the summer

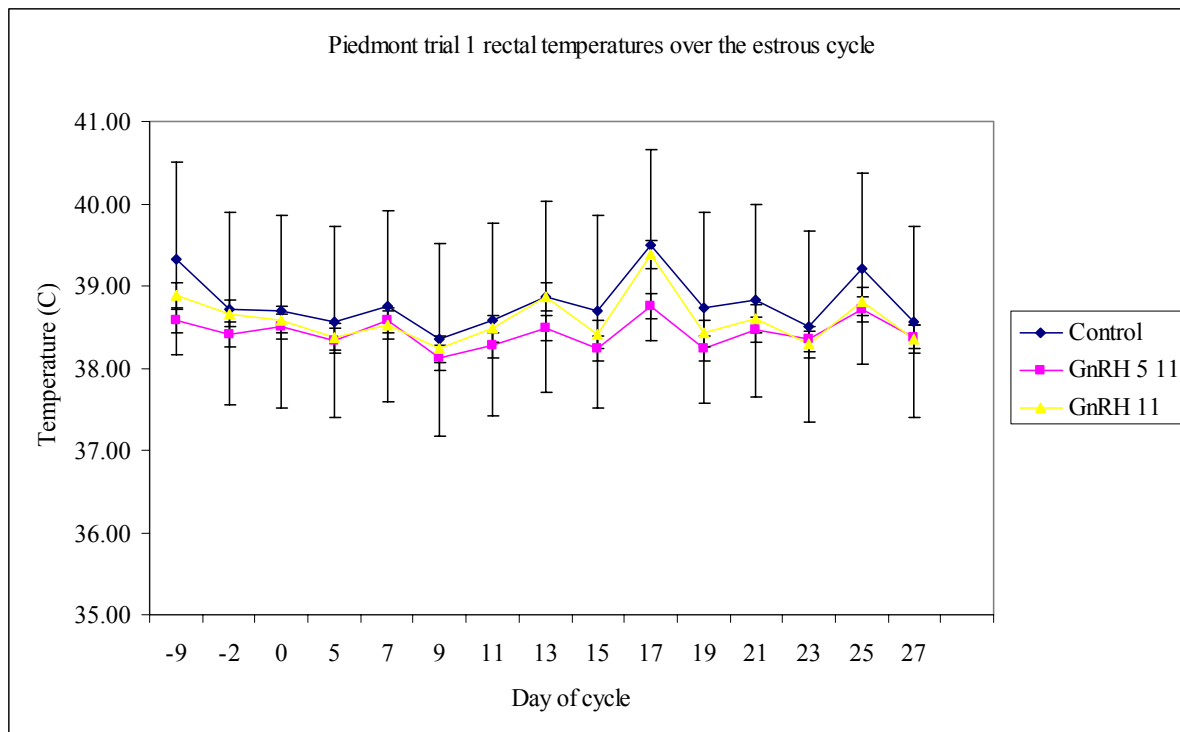


FIGURE 6.2 Comparison of average rectal temperatures between treatment groups at NCSU-Piedmont trial 1 during the summer

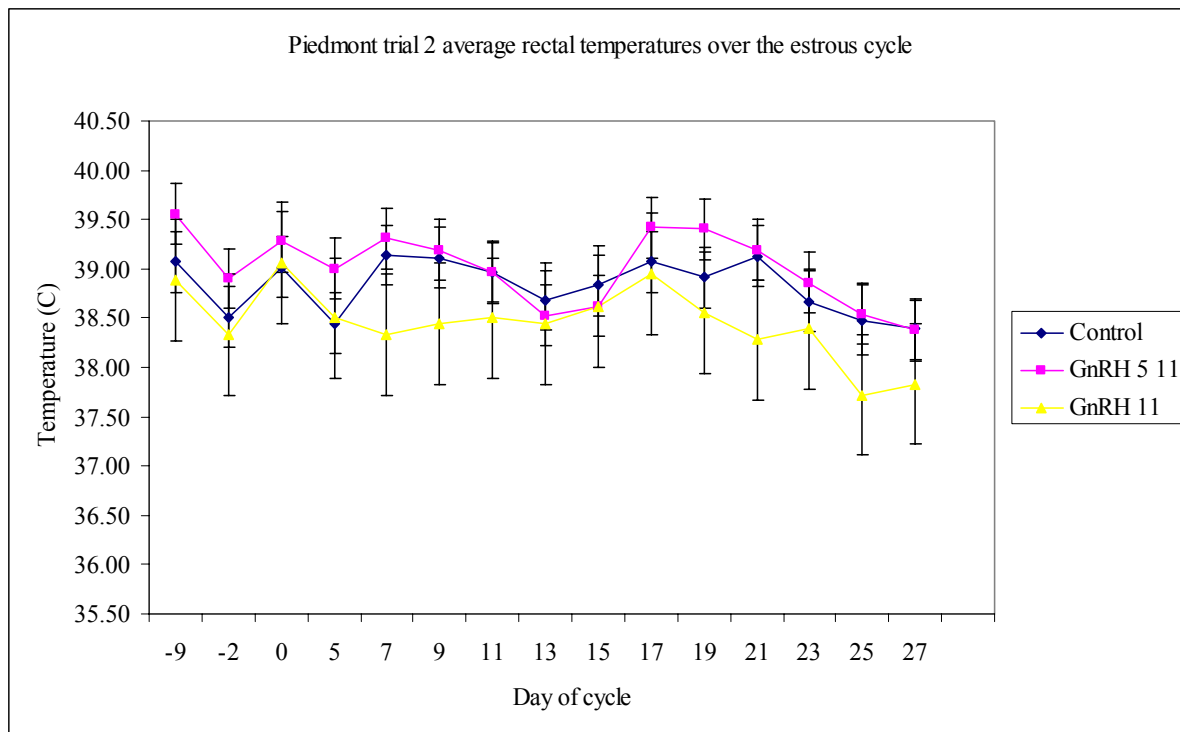


FIGURE 6.3 Comparison of average rectal temperatures between treatment groups at NCSU-Piedmont trial 2 during the summer

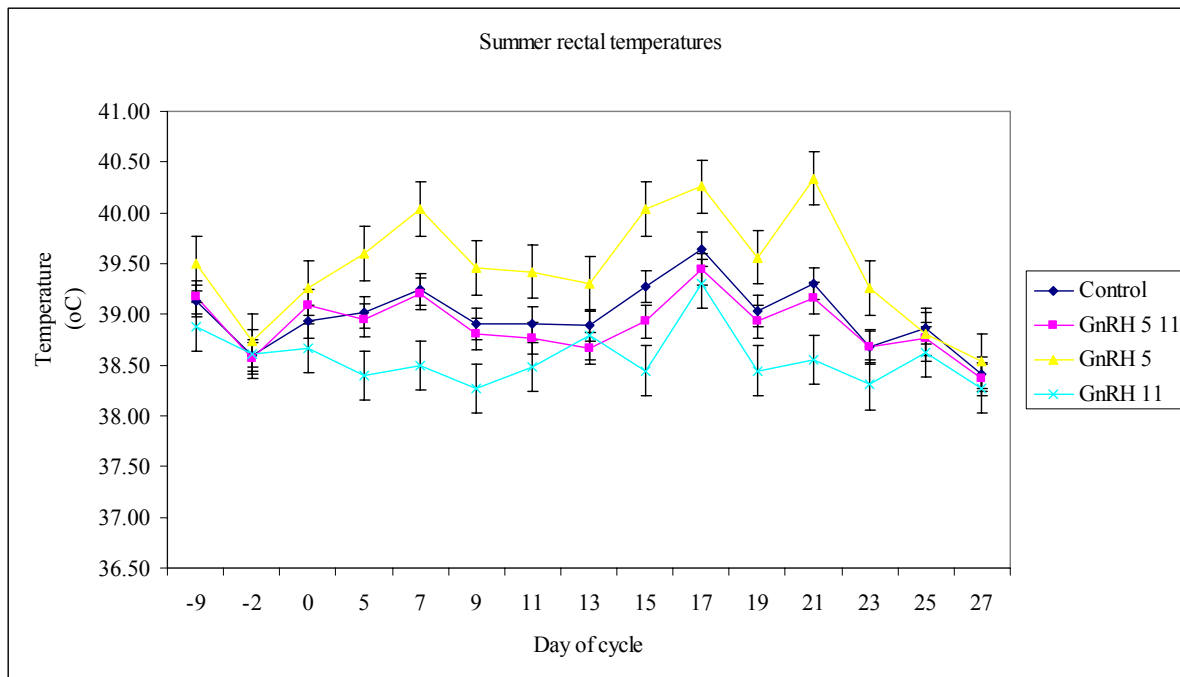


FIGURE 6.4 Pooled Rectal temperatures between treatments during the summer

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