ABSTRACT

CAROTHERS, RANDOLPH ERWIN. The Effects of Post-Insemination Progesterone Supplementation on Pregnancy Rates and Serum Progesterone in Dairy Cows Exposed to Mild Heat Stress. (Under the direction of Dr. C.S. Whisnant.)

Progesterone was administered by controlled intravaginal drug release devices (CIDRs) in an attempt to improve pregnancy rates and increase serum progesterone concentrations in heat stressed dairy cows. Two trials were conducted, each trial containing one treatment. Trial one utilized a CIDR from days 5 through 12 after breeding, and trial two utilized a CIDR from days 5 through 19 after breeding. Rectal temperatures and blood samples were obtained from cows every other day throughout the trial. Serum progesterone concentrations were measured with RIA. Pregnancy was checked on approximately day 30 via transrectal ultrasonography. Mean THI values were 73.5 for trial one and 75.1 for trial two. This indicated that cows in both trials were subjected to mild heat stress conditions. Overall serum progesterone concentrations indicated no statistical difference between CIDR treated and control cows in both experiments. Trial one pregnancy rates were 31.8% vs. 33.3% for control and CIDR treated cows respectively, which showed no difference ($p = 0.25$). Trial two pregnancy rates were 33.3% vs. 26.7% for control and CIDR treated cows respectively, which was not statistically different ($p = 0.29$). Providing supplemental progesterone to heat stressed dairy cows after breeding did not improve pregnancy rates or increase serum progesterone concentrations.

THE EFFECTS OF POST-INSEMINATION PROGESTERONE SUPPLEMENTATION ON PREGNANCY RATES AND SERUM PROGESTERONE IN DAIRY COWS EXPOSED TO MILD HEAT STRESS

by
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Chair of Advisory Committee

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BIOGRAPHY

Randolph Erwin Carothers, IV, “Chip,” was born to R.E. and Randy B. Carothers on October 26, 1981. Raised in Patterson Springs, NC, Chip developed an attraction to critters and tractors at an early age.

After graduating Crest High School in 1999, Chip attended Clemson University where he received a Bachelor of Science degree in Animal and Veterinary Sciences and a minor in Agricultural Business Management in 2003. Chip and his wife, Katy, were married in August 2003, just before Chip started his Master’s work at North Carolina State University.

During his time at NC State, Chip taught for the Biological Sciences Department, which proved to be one of the greatest aspects of his graduate career. Chip is a member of the American Society of Animal Science, American Registry of Professional Animal Scientists, North Carolina Cattlemen’s Association, and the National Cattlemen’s Beef Association.

Chip will receive a Master of Science degree from NC State in May 2006, after which he will enter the Class of 2010 at the North Carolina State University College of Veterinary Medicine.

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I would like to thank everyone in my family, especially my wife Katy, to whom this thesis is dedicated.

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Introduction

Reproductive losses attributed to heat stress in dairy cattle are a major problem for dairy producers in North Carolina and throughout the southeastern United States. High producing dairy cows are at an additional disadvantage, with high milk yields exacerbating reproductive deficiencies (Al-Katanani et al., 1999). Attempts to improve reproductive efficiency in heat stressed dairy cows require a combination of approaches, including environmental modifications, reproductive strategies, and intense management.

Luteal insufficiency is known to cause embryonic losses (Villarroel et al., 2004), and chronic heat stress has been shown to reduce corpus luteum size and function (Younas et al., 1993), thereby increasing the odds of embryonic loss in heat stressed cows. Hormonal manipulation of the bovine estrous cycle may provide a means to reduce pregnancy losses associated with heat stress.

Previous research with GnRH administered at estrus (Ullah et al., 1996) or after insemination (Willard et al., 2003; Sweetman 2003) has improved pregnancy rates by inducing accessory corpora lutea, increasing the progesterone concentration, thereby making a more favorable environment for developing embryos. Supplemental progesterone may provide the same benefits as GnRH, only without inducing accessory corpora lutea.

Progesterone is currently administered post-insemination to some dairy cows, allowing for a synchronized return to estrus if the breeding attempt was unsuccessful (El-Zarkouny and Stevenson, 2004). Progesterone can be administered in the form of a controlled internal drug release (CIDR), which has many benefits. A CIDR is easily

inserted and withdrawn, safe to use in pregnant cows, and approved for use in lactating dairy cows in the United States.

The objectives of this study were to determine the effects of administration of CIDR post insemination on pregnancy rates and serum progesterone concentrations in heat stressed dairy cows.

Review of Literature

Previous research has shown that inducing accessory corpora lutea by hormonal manipulation of the bovine estrous cycle can result in improved reproductive efficiency in heat stressed dairy cattle. Methods of increasing serum progesterone levels through endogenous pathways have improved pregnancy rates in heat stressed dairy cattle. Thus, providing exogenous progesterone might also result in increased pregnancy rates. For this study, supplemental progesterone was administered after breeding, assisting the corpus luteum in maintaining a favorable hormonal environment for the conceptus. To understand the background for the current experiment this review of the literature was conducted.

Estrous Cycle

The estrous cycle is defined by Bearden et al. (2004) as "the time between periods of estrus." Estrus, or heat, is the period when a female is sexually receptive to the male. The bovine estrous cycle ranges from 17 to 24 days in length, with 21 days considered average. The estrous cycle is divided into two major phases, with each phase consisting of two periods. The phases are named according to the dominant structure(s) found on the ovary at that time (Senger, 1999). The follicular phase is comprised of proestrus and estrus; and the luteal phase is comprised of metestrus and diestrus.

Follicular phase. The follicular phase is the period of the estrous cycle that spans from regression of the corpus luteum (CL) to ovulation, generally lasting from day -3 to day 0, with day 0 being the day of ovulation or estrus. Proestrus begins after luteolysis

and the subsequent drop in progesterone concentrations. Follicles undergo rapid growth during proestrus, resulting in increasing estrogen concentrations. Effects of estrogen on the reproductive tract and behavioral signs of estrus are seen near the end of proestrus (Bearden et al., 2004).

Estrus is the period of sexual receptivity, easily identified by the female standing to be mounted. Maximum estrogen secretion and behavioral signs are associated with estrus. Ovulation of the dominant follicle occurs near the end of estrus and the beginning of metestrus. Estrus generally lasts for 12 to 18 hours in the bovine and is typically indicated by day 0 of the cycle.

Luteal phase. The luteal phase begins with the end of estrus. Metestrus marks the change of estrogen dominance to progesterone secretion. Ovulation occurs near the beginning of metestrus, resulting in the formation of the CL on the ovary, which is responsible for progesterone secretion. Metestrus lasts about 3 days.

A fully formed CL marks diestrus, with progesterone being secreted at maximum levels. Diestrus generally begins around day 5 and extends through day 16 to 17. Bearden et al. (2004) describes diestrus as preparation of the uterus for pregnancy. Diestrus ends if luteolysis caused by secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) occurs allowing for the estrous cycle to repeat (Senger, 1999). The embryo secretes interferon - τ, allowing the CL to be maintained if the cow is pregnant (Mann et al., 1999).

Estrus Synchronization

Many estrus synchronization protocols have been developed for cattle, all relying on hormonal manipulation of the estrous cycle to allow a greater percentage of cows to

ovulate within a limited time span. There are basically three strategies for estrus synchronization: progestin based protocols, gonadotropin releasing hormone + prostaglandin (GnRH+PG) based protocols, and prostaglandin (PG) based protocols without the addition of GnRH. Additionally, most protocols utilize multiple hormones in an attempt to produce a tighter synchrony of estrus and ovulation. Regardless of the method used to synchronize estrus, the benefits to the producer are similar. Synchronizing estrus gives the option of using timed artificial insemination (TAI), thus reducing or possibly eliminating labor associated with estrus detection. With TAI, all cows are bred at the end of the estrus synchronization protocol, with exact times dependent on the protocol utilized. Estrus synchronization allows for improved herd management, providing groups of cows at similar stages of production rather than having individual cows scattered throughout different stages. Another benefit of estrus synchronization is the production of a uniform calf crop. For dairy producers, this means a group of replacement heifers will be ready to enter production at the same time, simplifying management. For beef calves that will not return to the herd as replacements, uniformity will provide increased revenue, since uniform groups of calves usually bring a premium price when compared to smaller, mixed-age groups of calves.

Progestin based protocols. Progestin based protocols rely on exogenous progesterone to simulate the luteal phase of the estrous cycle. Progesterone can be administered various ways including: intravaginal drug release, such as the EAZI-BREED ™ CIDR ® (CIDR) insert; implants such as Synchro-MateB; or melengestrol acetate, a feed additive. The CIDR is the only method of providing exogenous progesterone that is approved by the US Food and Drug Administration for lactating

dairy cattle (FDA, 2003). For estrus synchronization, progesterone is administered for seven days, ensuring that the CL has at least seven days to develop, and therefore will be responsive to $PGF_{2\alpha}$ (Lucy et al., 2001). Administering progesterone also postpones estrus for cattle that would have otherwise undergone luteolysis during the period prior to the prostaglandin treatment (Roche et al., 1999). Removal of the exogenous progesterone concurrent with prostaglandin administration causes a sharp drop in progesterone concentrations, initiating estrus. The use of progestins in estrus synchronization protocols can initiate estrus and ovulation in some anestrus cows (Anderson et al., 1996; Fike et al., 1997; Imwalle et al., 1998). Anderson and Day (1994), Fike et al. (1999), and Ahmad et al. (1995) reported that the use of progestins can result in persistent follicles which reduce conception rates in cattle. Thus, the use of progestins for more than seven days accompanied with breeding at the subsequent estrus is not recommended (Ahmad et al., 1995).

Progesterone can also be administered to cows after insemination. Most dairy cows will not become pregnant following a single insemination, and providing progesterone after breeding offers a safe way to resynchronize the subsequent estrus. Progesterone can be administered to all cows without knowing their pregnancy status and would not cause abortion, as would be the case if prostaglandin were administered. Stevenson and Mee (1991) investigated whether progesterone-releasing intravaginal devices, inserted on either day 5 or day 13 post-insemination and utilized for an 8 day period would affect pregnancy rates. Pregnancy rates at first service were not different between treatment and control cows, but pregnancy rates of treated cows that returned to estrus were higher than the control cows (60% versus 39%) (Stevenson and Mee, 1991).

Villarroel et al. (2004) examined the effect of post-insemination progesterone supplementation on pregnancy rates in repeat-breeder Holstein cows. This study found that young repeat-breeder Holsteins in late lactation were 3.26 times more likely ($p=0.009$) to become pregnant with progesterone treatment; however, this difference was only seen when the population of cows was stratified by parity and stage of lactation. Chenault et al. (2003) experimented with resynchronization of previously inseminated dairy cows by inserting CIDRs 13 to 15 days post insemination for a 7 day period. Estrus was detected in 34.1% (n=589) of CIDR treated cows compared to 19.3% (n=544) of the control cows ($p=0.001$) during the 3 day period following CIDR removal (Chenault et al., 2003). The estrus response was attributed to CIDR treatment (Chenault et al., 2003).

GnRH + PG based protocols. The use of GnRH in synchronization protocols resulted from protocols used for the treatment of cows with ovarian cysts. Seguin et al. (1976) found that cows with ovarian cysts that were treated with GnRH had increased serum concentrations of luteinizing hormone (LH) and progesterone. The increase in progesterone indicated that the cystic follicle had either luteinized or ovulated, and formed a corpus luteum. GnRH can induce follicular atresia or turnover, and recruit a new wave of follicles (Macmillan and Thatcher, 1991). Pursley et al. (1995) explored the use of GnRH as a means of estrus synchronization to be used concurrently with TAI. Cows were administered GnRH at a random stage of the estrous cycle, followed by $PGF_{2\alpha}$ 7 days later, and a second administration of GnRH 48 hours after $PGF_{2\alpha}$ administration. Pursley et al. (1995) found that 18/20 cows and 13/24 heifers ($p<0.01$) responded to the first GnRH injection by ovulating a dominant follicle. A new follicular wave emerged in 20/20 cows and 18/24 heifers ($p<0.025$) and the same number

responded with luteolysis to a $PGF_{2\alpha}$ injection 7 days after GnRH. All cows and 18/24 heifers ovulated within an 8 hour period in response to the second GnRH injection given 48 hours after $PGF_{2\alpha}$ (Pursley et al., 1995). The OvSynch, CO-Synch, and Select Synch protocols all use GnRH in conjunction with $PGF_{2\alpha}$.

The OvSynch protocol (Figure 1), developed by Pursley et al. (1995) is primarily used in dairy herds and is the basis of the other GnRH + PG protocols. The OvSynch protocol utilizes an injection of GnRH, followed by administration of prostaglandin one week later. Another GnRH injection is given 48 hours after the prostaglandin, with timed artificial insemination 12 hours after the second injection of GnRH. OvSynch does allow for timed artificial insemination, but higher pregnancy rates can be obtained if estrus detection is performed. Progesterone can be added to GnRH based protocols. Pursley et al. (2001) found that including progesterone in the OvSynch estrus synchronization protocol improved conception rates in non-cycling lactating dairy cows to 55.2% for progesterone treated cows compared to 34.7% for control cows (n=182).

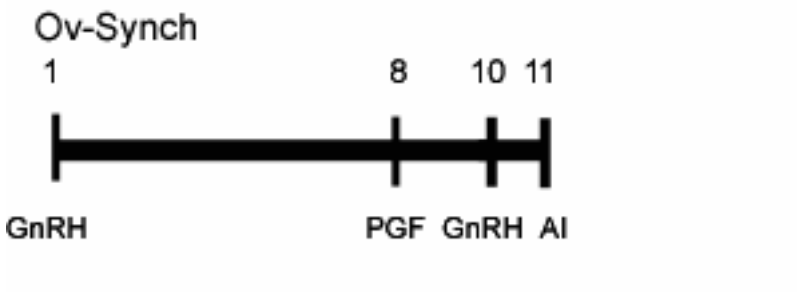

Figure 1. Ov-Synch protocol. Days are identified above the bar; treatments are identified below the bar.

The CO-Synch protocol (Figure 2) is a variation of the OvSynch protocol, designed for beef cattle. The CO-Synch protocol combines the second GnRH injection

with the TAI, reducing the labor involved with synchronization. Similar to OvSynch, CO-Synch starts with an injection of GnRH, followed by prostaglandin one week later. The second GnRH in conjunction with timed artificial insemination is administered 48 – 64 hours after the prostaglandin. Geary et al. (2001) demonstrated that pregnancy rates of cows subjected to the CO-Synch protocol were similar to those subjected to the OvSynch protocol.

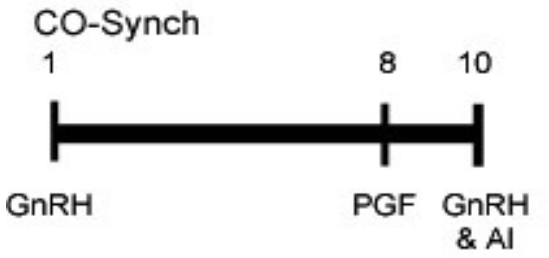

Figure 2. CO-Synch Protocol. Days are identified above the bar; treatments are identified below the bar.

The Select Synch protocol (Figure 3) is essentially the same as OvSynch, but includes estrus detection. The protocol begins with the administration of GnRH. Estrus detection begins 24 to 48 hours before prostaglandin injection. Cows seen in estrus are bred and do not receive the prostaglandin injection. The Select Synch protocol offers the option of a second injection of GnRH and TAI 72 hours after prostaglandin administration, or to detect estrus for the following week and omit the second GnRH injection. These protocols can include a CIDR inserted at the time of the first GnRH injection and removed at the time of prostaglandin administration.

Figure 3. Select-Synch Protocol with addition of CIDR. Days are identified above the bar; treatments are identified below the bar. CIDR is inserted on day 1 and removed on day 6.

Prostaglandin based protocols. The use of prostaglandin to synchronize estrus is the simplest protocol. The most common prostaglandin protocol includes two injections of $PGF_{2\alpha}$ administered 14 days apart. Estrus occurs earlier if the second $PGF_{2\alpha}$ is administered 11 days after the first (Rosenburg et al., 1990). Folman et al. (1990) found that pregnancy rates were greater in cows given two injections 14 days apart compared to those in cows given $PGF_{2\alpha}$ 11 days apart. All cows seen in estrus after the first $PGF_{2\alpha}$ injection are bred and not subjected to the second injection. The single largest drawback to the use of prostaglandins is that these protocols require that cows have a functional CL in order for the prostaglandin to be effective. Anovulatory cows will not respond to prostaglandin injection. If the cow is in the follicular phase of the estrous cycle, prostaglandins will not be effective, as there is no CL to regress. The use of

prostaglandins alone does not provide a synchrony tight enough for TAI (Lucy et al., 1986; Stevenson et al., 1987; Larson and Ball, 1992).

Controlled Internal Drug Release

The EAZI-BREED ™ CIDR ® (CIDR) (Pfizer Animal Health, New York, NY) insert is a T – shaped device that consists of molded silicone over a flexible nylon spine. The CIDR is impregnated with 1.38g of progesterone, which is 10% by weight. The CIDR is placed in the vagina of the cow with a specially designed applicator and is designed to be used in estrus synchronization protocols for a period of seven days. The progesterone is released into the vagina and is absorbed into the blood. Although the CIDR is intended to be used for seven days, several researchers have shown that the CIDR continues to release progesterone for another week: Macmillan et al. (1991) demonstrated that the CIDR releases progesterone for at least 15 days. Colazo et al. (2004) found no difference in fertility between beef cows that were synchronized with a new CIDR compared to those synchronized with a CIDR previously used for 7 days. This indicates the potential for using CIDRs for a two-week period. Numerous studies involving CIDRs report CIDR retention rates above 95% (Chenault et al., 2003; Colazo et al., 2004). Rathbone et al. (2002) demonstrated with ovariectomized Holstein cows that serum progesterone concentrations begin to rise within one hour following CIDR insertion, elevating the concentration to levels normally seen during the luteal phase of the estrous cycle; and progesterone concentrations begin to decrease within one hour after CIDR removal. Martinez (2002) reports plasma progesterone concentrations of 5 to 7 ng/ml within 24 hours after CIDR insertion and 2 to 3 ng/ml after 2 to 3 days until CIDR

removal, with progesterone levels returning to baseline within 12 hours after CIDR removal in ovariectomized cows. The ease of insertion and removal combined with the ability of the CIDR to deliver progesterone for 14 days make the CIDR ideal for long-term progesterone supplementation.

Heat Stress

Heat stress is a major problem of dairy production in tropical and sub-tropical climates. North Carolina and the southeastern United States have weather patterns that can lead to heat stress during the summer and to a lesser extent, the spring and fall. Heat stress alters many parameters, including decreased milk production and reduced reproductive efficiency. As will be discussed later, recent research indicates that hormonal supplementation may be of use in improving reproductive performance in heat stressed cows.

Parameters. Hansen and Arechiga (1999) define heat stress as external forces acting on an animal to raise body temperature from the normal state. In the southern United States and other sub-tropical and tropical areas, these external forces are most notably high temperature and high humidity. Temperature and humidity are used to calculate the temperature humidity index (THI), which serves as an indication of the degree of heat stress experienced by the animal. THI values are calculated with the formula: $^{\circ}F - (0.55 - 0.55\ (RH / 100))\ (^{\circ}F - 58)$ (NOAA, 1976). THI values above 70 are generally classified as heat stress conditions (Armstrong, 1994). Ingraham et al. (1974) reports that the use of average daily THI may not be sufficient since it does not take the number of heating or cooling hours of the day into consideration. The use of

average THI may also not provide an accurate assessment for areas that experience a "wider diurnal flux" (Ingraham et al., 1974). Despite these shortcomings, the use of THI as a measure of heat stress is widely accepted. The effects of heat stress in dairy cattle begin to be seen at 75°F and 75% relative humidity (THI = 72.7). A noticeable decrease in feed intake occurs around 80°F (THI = 77), a dramatic decrease in milk production occurs around 90°F (THI = 85.6), and lethal effects can be observed at 100°F and 80% relative humidity (THI = 95.4). The average summer THI for North Carolina ranges from 70 to 75, which indicates mild heat stress (Armstrong, 1994).

Table 1.Temperature-humidity index. Black background for severe stress, bold font for moderate stress, standard font for mild stress. Adapted from Armstrong, 1994.

		Relative Humidity (%)																				
°F	°C	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
72	22.2																				72	72
73	22.8																		72	72	73	73
74	23.3																72	72	73	73	74	74
75	23.9														72	72	73	73	74	74	75	75
76	24.4			NO STRESS									72	72	73	73	74	74	75	75	76	76
77	25.0											72	72	73	73	74	74	75	75	76	76	77
78	25.6										72	73	73	74	74	75	75	76	76	77	77	77
79	26.1									72	73	73	74	74	75	76	76	77	77	**78**	**78**	**79**
80	26.7							72	72	73	73	74	74	75	76	76	**77**	**78**	**78**	**79**	**79**	**80**
81	27.2					72	72	72	73	73	74	74	75	76	77	77	**78**	**78**	**79**	**80**	**80**	**81**
82	27.8					72	72	73	73	74	75	75	76	77	77	**78**	**79**	**79**	**80**	**81**	**81**	**82**
83	28.3				72	73	73	73	74	74	75	76	77	**78**	**78**	**79**	**80**	**80**	**81**	**82**	**82**	**83**
84	28.9			72	73	73	73	74	75	75	76	77	**78**	**78**	**79**	**80**	**80**	**81**	**82**	**83**	**83**	**84**
85	29.4			72	73	74	74	75	75	76	77	**78**	**79**	**79**	**80**	**81**	**81**	**82**	**83**	**84**	**84**	**85**
86	30.0		72	73	74	74	74	75	76	77	**78**	**78**	**79**	**80**	**81**	**81**	**82**	**83**	**84**	**84**	**85**	**86**
87	30.6		72	73	73	74	75	76	77	77	**78**	**79**	**80**	**81**	**81**	**82**	**83**	**83**	**85**	**85**	**86**	**87**
88	31.1	72	72	73	74	75	75	76	77	**78**	**79**	**80**	**81**	**81**	**82**	**83**	**84**	**85**	**86**	**86**	**87**	**88**
89	31.7	72	73	74	75	76	76	77	**78**	**79**	**80**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**86**	**87**	**88**	89
90	32.2	72	73	74	75	76	77	**78**	**79**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**86**	**87**	**88**	89	90
91	32.8	73	74	75	76	76	77	**78**	**79**	**80**	**81**	**83**	**83**	**84**	**85**	**86**	**86**	**87**	**88**	89	90	91
92	33.3	73	74	75	76	77	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**85**	**86**	**87**	**88**	89	90	91	92
93	33.9	74	75	76	77	**78**	**79**	**80**	**80**	**81**	**83**	**83**	**84**	**85**	**86**	**87**	**88**	89	90	91	92	93
94	34.4	74	75	76	77	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	89	90	91	92	93	94
95	35.0	75	76	77	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	89	90	91	92	93	94	95
96	35.6	75	76	77	**78**	**79**	**80**	**81**	**82**	**83**	**85**	**86**	**87**	**88**	89	90	91	92	93	94	95	96
97	36.1	76	77	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	89	91	92	93	94	95	96	97
98	36.7	76	77	**78**	**79**	**80**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	89	90	91	93	94	95	96	97	98
99	37.2	76	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**87**	**88**	89	90	91	92	93	94	96	97	98	99
100	37.3	77	**78**	**79**	**80**	**82**	**83**	**84**	**85**	**86**	**87**	**88**	90	91	92	93	94	95	97	98	99	
101	38.3	77	**79**	**80**	**81**	**82**	**83**	**86**	**86**	**87**	**88**	89	90	92	93	95	95	96	97	99		
102	38.9	**78**	**79**	**80**	**81**	**83**	**85**	**86**	**86**	**87**	89	90	91	92	93	95	96	97	97			
103	39.6	**78**	**79**	**81**	**82**	**83**	**86**	**86**	**87**	**88**	89	91	92	94	96	96	97					
104	40.0	**79**	**80**	**81**	**82**	**85**	**86**	**86**	**88**	89	90	91	93	95	95	96						
105	40.6	**79**	**80**	**82**	**83**	**86**	**86**	**87**	**88**	89	91	92	93	96	96	97						
106	41.1	**80**	**81**	**82**	**85**	**86**	**86**	**88**	89	90	91	93	94	95	97	98						
107	41.7	**80**	**81**	**83**	**85**	**86**	**87**	**88**	89	91	92	94	95	96	98							
108	42.2	**81**	**82**	**83**	**85**	**86**	**87**	89	90	92	93	94	96	97								
109	42.3	**81**	**82**	**85**	**86**	**87**	**88**	89	91	92	94	95	96	98								
110	43.3	**81**	**83**	**86**	**86**	**87**	**88**	90	91	92	94	96	97									
111	43.9	**82**	**83**	**85**	**86**	**88**	89	91	93	94	95	96	98									
112	44.4	**82**	**83**	**85**	**87**	**88**	90	91	94	94	96	97										
113	45.0	**83**	**86**	**86**	**87**	89	90	92	95	95	96	96										
114	45.4	**83**	**85**	**86**	**88**	89	91	92	94	96	97											
115	46.1	**85**	**85**	**87**	**88**	90	91	94	95	96	98											
116	46.7	**86**	**86**	**87**	89	90	92	94	95	97												
117	47.2	**86**	**86**	**88**	89	91	93	94	96	98												
118	47.3	**86**	**87**	**88**	90	92	93	95	97													
119	48.3	**86**	**87**	89	90	92	94	96	97													
120	48.9	**86**	**88**	89	91	93	94	96	98													
121	49.4	**86**	**88**	90	92	93	95	97														

Effects on reproduction. Reproduction is one of the first production parameters to suffer when an animal is subjected to heat stress. Ingraham et al. (1974) concluded that average daily THI values greater than 70 before breeding are associated with a linear decline in conception rates. Ingraham et al. (1976) again demonstrated a relationship between impaired reproductive efficiency in dairy cows and elevated THI values immediately prior to breeding. Reduced duration of estrus, decreased intensity of estrus, and decreased fertility are all attributed to heat stress. Her et al. (1988) demonstrated that of cows cooled with forced ventilation and sprinkling beginning 1 day prior to a synchronized estrus through 8 days post-insemination, 70% exhibited estrus behavior compared to 45% of non-cooled cows ($p<0.05$). Fewer cooled cows were classified as anestrus (12%) when compared to the non-cooled control cows (33%) ($p<0.05$) (Her et al., 1988). Conception rates between cooled and control cows did not differ (Her et al., 1988). This data concurs with Younas et al. (1993) who reported that 71.4% of cows cooled by fans showed estrus, compared to 33% of control cows that showed estrus. Younas et al. (1993) did have small sample sizes (treatment n = 7, control n = 6), so this must be considered when reviewing these data. Howell et al. (1994) reported no difference in the length of the luteal phase between cows measured in spring compared to those measured in summer. De Rensis et al. (2003) reported that the majority of studies demonstrated a decrease in motor activity and other behavioral signs of estrus in heat stressed cows. A decrease in the percentage of cows exhibiting estrus behavior leads to a decrease in estrus detection, giving the appearance of acyclicity. Imtiaz-Hussain et al. (1992) reported that only 36.8% of cows in summer were visually detected in estrus, but all cows remained cyclic based on progesterone profiles. Cows not detected in estrus are

not bred, contributing to a greater number of days open, impairing reproductive efficiency. De Rensis et al. (2003) states that there is an increase in the proportion of inseminations that do not result in pregnancy during periods of heat stress. Jordan (2003) reports that Ulberg and Burfening (1967) noted a decreased conception rate (61% to 45%) when rectal temperatures 12 hours after breeding were increased by 1^{o}C. Exposing cows to an environmental temperature of 32.2°C for 72 hours after breeding resulted in rectal temperatures of 40°C and a conception rate of 0% (Ulberg and Burfening, 1967). In contrast, cows exposed to an environmental temperature of 21.1°C, had rectal temperatures of 38.5°C and conception rates of 48% (Ulberg and Burfening, 1967).

Decreased fertility is compounded by increased mature equivalent milk yields (Al-Katanani et al., 1999). Al-Katanani et al. (1999) analyzed 90 day non-return rates and grouped cows based on the level of their mature equivalent milk yield and found that of cows bred in July, a 44.9% non-return rate was reported for cows producing <4536 kg of milk, while cows producing 4536 – 9072 kg had a 13.5% non-return rate, and cows producing >9072 kg had a non-return rate of 5.3%.

Effects on follicles. It has been proposed that some of the infertility associated with heat stress may be a result of damage to the developing oocyte (Al-Katanani et al., 2002b). Badinga et al. (1993) experimented with effects of heat stress on follicular development in dairy cows. Cows were subjected to shade or no shade and synchronized to ovulate (Badinga et al., 1993). Follicular development was monitored daily by ultrasonography for 7 days following estrus, and cows were ovariectomized on day 8 (Badinga et al., 1993). Shaded cows showed an increased dominant follicle size (16.4 ± 0.7 mm) and more fluid in the follicle (1.6 ± 0.1 ml) than the unshaded group (14.5 ± 0.6

mm, 1.1 ± 0.1 ml) (Badinga et al., 1993). Badinga et al. (1993) also found that subordinate follicles in the unshaded group were larger and contained more fluid than subordinate follicles in the shaded group. Despite these differences, Badinga et al. (1993) reported that acute heat stress from day 1 through day 7 of the estrous cycle had no effects on the patterns of growth of dominant and subordinate follicles in the first wave. Badinga et al. (1994) found that first wave dominant follicles in dairy cows in Florida were larger in April than in June, August, or November. Badinga et al. (1994) noted that the larger dominant follicles seen in April were associated with earlier regression of the largest subordinate follicle and a more notable decrease in the number of medium size follicles by day 9 of the estrous cycle. The ovulatory follicles were largest in November, but preovulatory follicles grew at a faster rate in June than April, August, or November (Badinga et al., 1994). Badinga et al. (1994) states that it is unclear whether these changes are correlated with reduced reproductive performance seen in summer months. Wolfenson et al. (1995) observed follicular growth throughout the entire estrous cycle and demonstrated that heat stress does alter follicular dynamics and that follicular dominance is depressed. Wolfenson et al. (1995) reports that alterations of follicular development can have interactions with other components of the reproductive system, possibly causing the lowered fertility associated with heat stress. Heat stress was shown to alter the patterns of growth in both first and second wave dominant follicles; although the average size was not affected, the size of the first wave dominant follicle decreased earlier in heat stressed cows (Wolfenson et al., 1995). Heat stressed cows experienced an increase in the size of the second wave dominant follicle sooner than control cows, which implied that the dominant follicle emerged earlier (Wolfenson et al., 1995). Heat stress

changed the number of follicles in different size categories, with heat stressed cows having fewer small follicles than control cows during the second wave ($p < 0.10$) (Wolfenson et al., 1995). A change in the size, number, and timing of follicles altered the hormonal environment of the cow, which may be one of the underlying causes of reduced fertility. Wilson et al. (1998) determined the effects of controlled heat stress on follicular growth and development of the preovulatory follicle in lactating Holsteins. Wilson et al. (1998) carried out the experiment in climatic control chambers, keeping humidity and light schedules identical for heat stress and thermoneutral cows. Dominant follicles were smaller and subordinate follicles were larger in heat stressed cows (Wilson et al., 1998). Wilson et al. (1998) concluded that heat stress inhibited follicular growth and dominance. Chronic heat stress appears to have different effects on follicles than acute heat stress, and this may explain some of the discrepancies in the previous literature. Wilson et al. (1998) stated that the effects of heat stress on reproduction can carry over to fall because developing follicles that are damaged by heat stress will ovulate 40 to 50 days later, and may result in a subfertile oocyte. Al-Katanani et al. (2002b) devised two experiments to test if oocyte quality is affected by heat stress and to evaluate seasonal variations in oocytes. To test the effect of season, Holstein ovaries were collected from a slaughterhouse in various months (April to September classified as warm season, October to March classified as cool season) and oocytes were harvested. Oocytes were fertilized in vitro, with cleavage rate and blastocyst development recorded (Al-Katanani et al., 2002b). The cleavage rate was increased for the warm season oocytes compared to cool season oocytes ($p < 0.001$), but the rate of blastocyst development was lower for warm season oocytes compared to cool season oocytes ($p < 0.001$) (Al-Katanani et al., 2002b).

The second experiment consisted of three groups of non-lactating Holstein cows housed in three different environments: heat stressed (provided shade in summer), cooled (summer), and winter, for 42 days prior to slaughter (Al-Katanani et al., 2002b). It should be noted that cows were exposed to heat stress before the experiment began. Cows underwent their respective treatments for 42 days, since that is the time necessary for an early antral follicle to develop into a preovulatory follicle (Al-Katanani et al., 2002b). Cows were slaughtered on day 18 to 19 of the estrous cycle after estrus synchronization and oocytes were recovered for use in in-vitro fertilization (IVF) (Al-Katanani et al., 2002b). The number of oocytes was similar for all three groups and there was no difference between groups in cleavage rate (Al-Katanani et al., 2002b); however, a highly significant difference was observed in blastocyst development rate, with summer cows (heat stressed and cooled) having fewer oocytes that developed into blastocysts; There was no difference in proportion of blastocyst development between the heat stressed and cooled groups (Al-Katanani et al., 2002b). Al-Katanani et al. (2002b) also found a significant difference in oocyte quality between seasons, indicating that oocyte competence is compromised in heat stressed cows. Al-Katanani et al. (2002b) concluded that cooling cows for 42 days before oocyte collection was not effective in overcoming the adverse effects of heat stress. The oocytes were likely damaged in a stage of development before cooling was initiated, or effects on oocyte quality were the result of other factors unrelated to heat stress.

Effects on embryos. Bovine embryos are particularly susceptible to heat stress during the first week of development, both in vivo and in vitro. Rivera and Hansen (2001) exposed bovine oocytes and embryos in vitro to temperatures consistent with the

body temperature of heat stressed cows to determine effects of increased temperature in development. Oocytes and embryos were cultured at 38.5°C, and then challenged with heat shock temperatures (40°C or 41°C; 39.5°C or 40.5°C) during fertilization, at the one-cell stage, and the two-cell stage. Embryos were also cultured in incubators set to mimic the body temperature of a heat stressed cow, with exposure to 38.5°C for 5 hours, 39.5°C for 5 hours, 40.5°C for 5 hours, and 39.5°C for 9 hours (Rivera and Hansen, 2001). Embryos that were exposed to heat shock of 40°C or 41°C during fertilization were cultured at 38.5°C for the remainder of the 8 day experiment (Rivera and Hansen, 2001). One-cell and two-cell embryos were exposed to heat shock for periods of 3, 6, 9, or 12 hours, after which they were cultured at 38.5°C (Rivera and Hansen, 2001). Oocytes that were fertilized at 41°C had lower cleavage rates ($p < 0.01$) and had a lower proportion of blastocyst development ($p < 0.01$) than oocytes fertilized at 38.5°C (Rivera and Hansen, 2001). One-cell embryos exposed to 41°C for 12 hours had reduced cleavage rates ($p < 0.01$) when compared to all other times and temperatures. Blastocyst development rates for one- and two-cell embryos were impaired ($p < 0.01$) following exposure to 41°C for 9 and 12 hours (Rivera and Hansen, 2001). Embryo development was not impaired for the pattern of temperatures similar to those experienced by heat stressed cows from 52 to 84 hours after insemination, but exposure to this pattern for 192 hours significantly reduced the development of blastocysts (Rivera and Hansen, 2001). This study confirmed the deleterious effects of heat on developing embryos and presented evidence that chronic heat stress and acute heat stress resulted in different reproductive outcomes. Ealy et al. (1993) superovulated lactating Holstein cows and exposed them to one of 4 treatments (heat stress on day 1, 3, 5, or 7 of pregnancy) or control. Cows

selected to undergo heat stress were placed in an unshaded lot for 7 hours; cows were housed in free stall barns to maintain thermoneutral temperatures the remainder of the time. Embryos were non-surgically retrieved on day 8 and evaluated for viability (Ealy et al., 1993). Maternal heat stress on day 1 resulted in fewer viable embryos ($p = 0.07$ complete data set, $p = 0.05$ reduced data set) (Ealy et al., 1993). Percentages of embryos recovered were: 70.9% control, 54.9% day 1, 60.3% day 3, 62.6% day 5, and 82% day 7 (Ealy et al., 1993). These data demonstrated an interaction of maternal heat stress and embryonic survival as well as the embryo's increased resistance to the effects of heat stress as it matures. Ealy et al. (1993) hypothesized that embryos may gain increased thermotolerance by inclusion of heat shock proteins during periods of heat stress, which early embryos are not capable of producing. Ealy et al. (1993) theorized that embryos should be able to produce heat shock proteins after the genome is activated, which occurs between the 8 to 16 cell stage, corresponding to day 3 of pregnancy. Biggers et al. (1987) examined the effects of heat stress on embryos in beef cattle, measuring progesterone concentration, CL weights, conceptus weight, and pregnancy rates. Cows in that study were placed in chambers to maintain constant temperature and relative humidity (RH) for two heat stress groups (37°/33°C, 27%RH; 37°C/33°C, 38%RH) and a control (22°C, 35%RH). Cows were harvested on day 17, allowing for recovery of the uterus and contents. Pregnancy rates and progesterone concentrations were not significantly different between groups, however, conceptus weights ($p < .01$) and CL weights ($p < .10$) were decreased in the heat stress groups compared to controls (Biggers et al., 1987). Biggers et al. (1987) concluded that embryonic development was altered based on the

decreased weights of conceptuses from the heat stressed cows. This study supported the concept of adverse effects of heat stress on embryo development in beef cattle.

Effects on hormones. The hormonal environment within the heat stressed cow has been shown to be variable. Conflicting data exist about the effects of heat stress on hormone concentrations of heat stressed cows. Abilay et al. (1975) reported that CL function, measured by progesterone concentration, was elevated in heat stressed cows, while Wise et al. (1988) reported that CL function remained static, and Younas et al. (1993) reported that CL function was impaired. Howell et al. (1994) observed no difference in the growth, maximum size, or regression rate of the CL when comparing lactating Holsteins measured in spring or summer. They also found no difference in progesterone concentrations when averaged across the entire estrous cycle, but did see a significant difference in progesterone concentrations of cows in summer being lower than progesterone concentrations of cows in spring when days 0 – 5 and >18 were omitted. Howell et al. (1994) states that reduced secretion of progesterone is characteristic of chronic exposure to heat stress. Howell et al. (1994) observed lower luteal progesterone secretion during the summer but noted that neither the length of the luteal phase nor the size of the CL was altered. Imtiaz-Hussain et al. (1992) observed a decrease in progesterone concentrations in Holsteins exposed to chronic heat stress, and hypothesized that reports of progesterone concentrations increasing during heat stress was most likely a response to acute heat stress. Trout et al. (1996) reported an increase in serum progesterone in cows subjected to heat stress, but this study was conducted during 4 separate periods from February to April inside environmental chambers and only followed the cows for one estrous cycle. The cows would not have been acclimatized to

the heat stress conditions when entering the study in February, so the heat stress can be classified as acute. This agrees with Imtiaz-Hussain and colleagues (1992) who proposed that serum progesterone would increase in response to acute heat stress and decrease for cows exposed to chronic heat stress. Wilson et al. (1998) tested the effect of heat stress on hormones in environmental chambers, but similar to the work presented by Trout et al. (1996) it most likely tested the effects of acute heat stress since the experiment was conducted in October and April. Wilson et al. (1998) observed an increase in serum progesterone in heat stressed cows after day 16, with luteolysis (as measured by progesterone < 1ng/ml) delayed by 8.7 days ($p < 0.05$). Heat stressed cows did have a lower serum concentration of estradiol than control cows ($p < 0.001$) (Wilson et al., 1998). Wilson et al. (1998) theorized the differences in estradiol were a result of reduced follicle size which was associated with decreased steroidogenesis within the theca and granulosa cells in heat stressed cattle. Roman-Ponce et al. (1981) did not find significant changes in levels of progesterone and estradiol between heat stressed and shaded cows, but noted that the small changes may be responsible for changes in uterine blood flow which could directly contribute to lowered fertility.

Methods to Alleviate Effects of Heat Stress

Environmental. The simplest methods of reducing the effects of heat stress include environmental modifications. Most dairies utilize a combination of shade, fans, and mist sprayers to reduce the ambient temperature the cows are exposed to. Bond and Kelly (1955) reported that heat load on an animal could be reduced as much as 50% with shading. Roman-Ponce et al. (1977) and Collier et al. (1981) reported higher rectal

temperatures and lower milk production in cows with no shade when compared to shaded cows. The use of fans and misters in concert is a very effective means of cooling cows. West (2003) reported that Seath and Miller (1948) recognized the benefits of air movement and wetting the animal to assist in cooling. Seath and Miller (1948) induced heat stress in cows and then compared rectal temperatures of cows subjected to different treatments, consisting of: fans, wetting the cow, fans and wetting, or no treatment. Cows subjected to fans and wetting demonstrated the greatest decrease in rectal temperatures, fans alone or wetting alone showed similar intermediate decreases in rectal temperatures, and the control cows showed the smallest decrease in rectal temperatures (West, 2003). Some studies have examined the effects of air conditioning on cows (Thatcher, 1974; Hahn et al., 1969). Although milk yield increased (Thatcher, 1974), this method of environmental control was not economical (Hahn et al. 1969). Most studies have evaluated the effectiveness of cooling strategies by measuring the resulting changes in milk production. Evaporative cooling has been shown to be effective in cooling cows in arid environments (Takamitsu et al., 1987; Ryan et al., 1992), but the effectiveness decreased as humidity increased. Taylor et al. (1986) experimented with evaporative cooling in Florida and reported decreased air temperature, rectal temperature, and respiration rates in heat stressed dairy cows. Although temperatures were decreased, milk yields did not improve, offering dairy producers little economic incentive to invest in evaporative cooling. Tarazon et al. (2004) experimented with bathing cows to improve production and reproduction during summer months in Mexico. Cows were bathed with 25L of 26^{o}C to 29^{o}C water once a day for 60 days. Cows that underwent the bath treatment had fewer days open and fewer services per conception; however, these

numerical differences were not statistically significant (Tarazon et al., 2004). Milk yields were not different between bathed and control cows (Tarazon et al., 2004).

Pharmacological. Pharmacological methods have been used in an attempt to decrease hyperthermia and embryonic losses induced by heat stress. Sakurada and Hales (1998) utilized indomethacin, a prostaglandin synthesis inhibitor, in an attempt to minimize hyperthermia in sheep exposed to heat stress conditions. Sakurada and Hales (1998) found that indomethacin only benefited sedentary animals by lowering rectal temperatures to levels comparable to those of the physically fit animals. Soto et al. (2003) reported that despite the limited success of indomethacin in reducing the incidence of hyperthermia in sheep, the influence of prostaglandin synthesis on heat stress induced hyperthermia remains unknown. Soto et al. (2003) experimented with flunixin meglumine, another prostaglandin synthesis inhibitor, in an attempt to reduce the increases in body temperature and respiration rates of dairy cows subjected to heat stress. Lactating Holstein cows were moved to a closed barn with no cooling mechanisms on the day of the trial at 0500 (Soto et al., 2003). Flunixin meglumine was administered at 0800 and 1300 and rectal temperatures were recorded every two hours (Soto et al., 2003). Treatment with flunixin meglumine had no effect on rectal temperatures or respiration rates (Soto et al., 2003). Cows in this trial had rectal temperatures of 39.4°C to 41.3°C, typical of summer rectal temperatures (Soto et al., 2003). Soto et al. (2003) proposed that more severe heat stress may have resulted in a larger effect of treatment. Guilbault et al. (1987) inhibited prostaglandin secretion by administering flunixin meglumine. Flunixin meglumine inhibited synthesis of $PGF_{2\alpha}$, as assessed by serum concentrations of PGF metabolites which decreased after flunixin meglumine injection (Guilbault et al., 1987).

$PGF_{2\alpha}$ reached the lowest concentration 4 hours after flunixin meglumine injection and increased until the next injection of flunixin meglumine 12 hours later (Guilbault et al., 1987). Manipulation of the uterus is known to cause a release of $PGF_{2\alpha}$, which is detrimental to successful embryo transfer programs (Wann and Randel, 1990). Schrick et al. (2001) utilized flunixin meglumine prior to embryo transfer in beef cattle to minimize the release of $PGF_{2\alpha}$ and demonstrated an increase in pregnancy rates in treated cows (63.8%) compared to control cows (51.1%) ($p = 0.0005$). Purcell et al. (2005) used flunixin meglumine in conjunction with CIDRs in an attempt to increase pregnancies from embryo transfer in beef cattle. Flunixin meglumine was administered 2 to 12 minutes prior to embryo transfer and CIDRs were inserted immediately after embryo transfer (Purcell et al., 2005). CIDRs remained in the vagina for 13 days (Purcell et al., 2005). Mean pregnancy rate for flunixin meglumine treated cows was 72.3% compared to 63% for control cows ($p < 0.01$) (Purcell et al., 2005). In summary, flunixin meglumine has been shown to effectively inhibit prostaglandin synthesis in cows.

Timed artificial insemination and embryo transfer. Reproductive management tools are available to reduce the effects of heat stress on cows. Imtiaz-Hussain et al. (1992) reported that only 36.8% of cows in a summer study were visually detected in estrus, but all cows remained cyclic based on progesterone profiles. The development and refinement of estrus synchronization has enabled producers to time inseminate cows that would otherwise not be inseminated because they did not exhibit estrus behavior. De la Sota et al. (1998) evaluated the use of TAI on lactating dairy cows exposed to heat stress and found that the insemination rate for the control group was only $18.1 \pm 2.5\%$ compared to 100% for TAI cows ($p < 0.01$). Days open decreased for cows subjected to

TAI (77.6 ± 3.8 versus 90.0 ± 4.2, $p < 0.05$), days to first insemination decreased for TAI cows (58.7 ± 2.1 versus 91.0 ± 1.9, $p < 0.05$), and the overall pregnancy rate increased for the TAI cows (18.2% versus 10.6%, $p < 0.05$) (de la Sota et al., 1998). De la Sota et al. (1998) concluded that while the embryo is not protected from heat stress in these protocols, TAI eliminates the limitation of estrus detection in heat stressed cows and results in a net economic gain compared to traditional breeding plans. Cartmill et al. (2001) compared pregnancy rates between heat stressed dairy cows that were subjected to TAI and those who were bred on detection of estrus. TAI cows had a 100% AI submission rate compared to 58.7% for the estrus detection group (Cartmill et al., 2001). Conception rates did not differ between the two groups at 27 to 30 days after breeding, but the TAI group had a 33% pregnancy rate compared to 17.9% for the control at the same time (Cartmill et al., 2001). The TAI cows had a higher pregnancy rate than the control due to the fact that a larger number were inseminated. Of the cows diagnosed as pregnant on day 27 to 30 of gestation, a greater percentage of the embryos were aborted in the TAI group (Cartmill et al., 2001). Cartmill et al. (2001) concluded that TAI does improve pregnancy rates at 27 to 30 days because success did not depend on estrus detection. However, they observed no difference in pregnancy rates between TAI and controls by days 40 to 50 after breeding.

Embryo transfer (ET) has been used to negate effects of heat stress on reproduction. Gordon et al. (1987) and Monty and Racowsky (1987) found that early embryonic mortality is a major factor responsible for pregnancy losses in heat stressed animals, and that ET offered a potential to bypass this period of early embryonic loss. Putney et al. (1989) demonstrated that pregnancy rates at day 21 of gestation in heat

stressed cows were 47.6% for ET compared to 18% for AI ($p < 0.001$). Pregnancy rates decreased somewhat by 40 days, with 29.2% of ET cows pregnant compared to 13.5% of AI cows (Putney et al., 1989). Despite these decreases, pregnancy rates for ET cows remained higher than the average pregnancy rates of 10 to 15% for cows bred by AI during periods of heat stress. Putney et al. (1989) concluded that ET is a potential means of bypassing early embryonic losses associated with heat stress, but also noted that embryos continue to be sensitive to heat stress beyond the post-transfer period. Drost et al. (1999) compared frozen embryos from superovulated donor cows to frozen in vitro-produced embryos, with TAI as a control. Donors were superovulated and embryos were collected in periods of cool weather, eliminating the effects of heat stress on early embryo development. Conception rates on day 22 of gestation were not different between recipients that received in vivo-produced embryos (60.4 ± 7.1%), in vitro-produced embryos (54.2 ± 7.1%), and TAI (60.7 ± 5.4%) ($p > 0.05$); however, conception rates on day 42 of gestation differed significantly between recipients that received in vivo-produced embryos (35.4%), in vitro-produced embryos (18.8%), and TAI (21.4%) (Drost et al., 1999). Ambrose et al. (1999) tested the efficacy of timed ET (TET) using fresh and frozen in vitro-produced embryos to improve pregnancy rates in heat stressed cows. The use of in vitro-produced embryos offers advantages to obtaining embryos from superovulated donors: in vitro-produced embryos can be produced at a substantially reduced cost since oocytes can be obtained from slaughterhouse ovaries, meaning that management and synchronization of donor cows is not required. The pregnancy rates were 6.7 ± 3.2% for TAI, 17.5 ± 3.0% for TET-Fresh, and 6.1 ± 3.8% for TET-Frozen (Ambrose et al., 1999). The difference between TET-Fresh and TET-Frozen was highly

significant (Ambrose et al., 1999). Similar to findings presented by Drost et al. (1999), Ambrose et al. (1999) demonstrated that embryo transfer using frozen in vitro-produced embryos yielded results similar to TAI. Al-Katanani et al. (2002a) found that TET of fresh IVF embryos can improve pregnancy rates for cows exposed to heat stress, but TET with frozen IVF embryos yielded pregnancy rates similar to TAI. TAI resulted in a 6.2 ± 3.6% pregnancy rate, TET-Fresh resulted in a 19.0 ± 5.0% pregnancy rate, and TET-Frozen resulted in a 6.5 ± 4.1% pregnancy rate (Al-Katanani et al., 2002). There was no difference between TAI and TET-Frozen, but TET-Fresh was significantly different from both of these (Al-Katanani et al., 2002a).

Hormonal Supplementation. Hormones have been administered to heat stressed dairy cows in an attempt to improve reproductive performance. Human chorionic gonadotropin (hCG), GnRH, and progesterone have been utilized to provide supplemental progesterone. HCG and GnRH work in a similar manner, causing the next dominant follicle to ovulate and thereby produce an accessory CL to provide endogenous progesterone. Ullah et al. (1996) studied the effects of GnRH administered to heat stressed cows at estrus. Lactating Holstein cows in Mississippi were assigned to GnRH or control in the late summer to test the effects on reproduction (Ullah et al., 1996). It was hypothesized that GnRH treatment would overcome the suppressed preovulatory LH surge, reduced secretion of progesterone, and lowered fertility associated with heat stress (Ullah et al., 1996). Ullah et al. (1996) found that serum progesterone concentrations were higher in GnRH treated cows compared to controls on day 10 of gestation (5.0 ± 0.9 versus 3.4 ± 0.7 ng/ml), day 15 of gestation (5.7 + 1.1 versus 3.7 ± 0.9 ng/ml), day 25 of gestation (4.6 ± 1.2 versus 2.9 ± 0.9 ng/ml), and day 30 of gestation (6.1 ± 0.9 versus 2.2

± 0.6 ng/ml) ($p < 0.05$). Ullah et al. (1996) attributed these differences in treated cows before day 20 directly to the GnRH treatment. Conception rates to the first breeding were 28.6% for GnRH treated and 14.3% for control ($p < 0.05$) (Ullah et al., 1996). All cows were subjected to identical environmental conditions, so these differences cannot be attributed only to environmental modifications. Willard et al. (2003) continued the work with GnRH to determine the optimal time for administration on heat stressed dairy cows. GnRH was administered on either day 5 or day 11 following insemination (Willard et al., 2003). Day 5 was chosen so that the first wave dominant follicle would ovulate, forming an accessory CL to provide supplemental progesterone early in the cycle; day 11 was also chosen to induce an accessory CL, but was also timed to coincide with maternal recognition of pregnancy which follows 4 to 5 days later (Willard et al., 2003). The pregnancy rates for GnRH treated cows in this study were significantly higher than those of the control (GnRH-d5 32%, GnRH-d11 38%, and control 19%) ($p < 0.08$) (Willard et al., 2003). Serum progesterone and CL number were increased for GnRH treated cows (Willard et al., 2003). Dickerson et al. (2004) reported that GnRH increased the number of CL and serum progesterone rates, but did not improve pregnancy rates in heat stressed cows. Sweetman (2003) demonstrated an increase in pregnancy rates and serum progesterone of heat stressed dairy cows given GnRH. Lopez-Gatius et al. (2004) administered supplemental progesterone to high yielding dairy cows in an attempt to reduce pregnancy losses. The embryo is dependent on progesterone from the CL for roughly 200 days and accessory CL improves pregnancy rates (Lopez-Gatius et al., 2004). Supplementing progesterone should have similar effects as accessory CL. Lopez-Gatius et al. (2004) inserted progesterone-releasing intra-vaginal devices (PRIDs; 1.55g

progesterone) into cows after pregnancy diagnosis between 36 and 42 days after breeding and utilized the PRIDs for 21 days. Pregnancy loss was reduced to 5.3% in PRID treated cows compared to 12% of control cows (Lopez-Gatius et al., 2004). Lopez-Gatius et al. (2004) theorized that the pregnancy rates would have been higher if fewer cows had been bred during the warm season, because cows that became pregnant during the warm season were 1.6 times more likely to lose the pregnancy.

Based on the available literature, it was concluded that using a CIDR to increase serum progesterone concentrations during the luteal phase may be beneficial to reproductive performance in heat stressed dairy cows. The following experiment was designed to test this hypothesis.

Materials and Methods

The North Carolina State University Institutional Animal Care and Use Committee approved all procedures described herein.

Animals

Trial 1. Lactating Holstein (n = 40) and Jersey (n = 6) dairy cows (n = 46) at two locations (North Carolina State University Dairy Educational Unit (DEU) n = 17; North Carolina Department of Agriculture and Consumer Services Piedmont Research Station (PRS) n = 29) were randomly assigned to the treatment (n = 24) or control (n = 22) groups. Cows averaged 140.9 ± 9.9 days in milk (DIM) at breeding with 59% being repeat breeders. Lactation numbers ranged from 1 to 5. Estrus was synchronized by following the OvSynch protocol, with GnRH (Fertagyl ®, 100μg i.m.; Intervet Inc., Millsboro, DE) administered on d -10, $PGF_{2\alpha}$ (Lutalyse ®, 5ml i.m.; Pfizer Animal Health, New York, NY) administered on d -3, a second dose of GnRH (100μg i.m.) administered on d -1, and timed AI on d 0. Cows seen in estrus were bred on estrus; the remainder underwent TAI on day 0. Cows were bred to one of two bulls. Cows assigned to the treatment received a CIDR insert 5 days after insemination and it remained in place for 7 days. Cows were milked twice daily, fed a total mixed ration, and were provided water ad libitum.

Table 2. Distribution of cows for trial 1.

Table 2. Cow Distribution: Trial 1

	Control	CIDR
Mean DIM	147.4	135.0
Lactation Number (minimum-maximum)	1 - 5	1 - 5
Lactation Number	2.2	2.0
Times Bred (minimum-maximum)	0 - 3	0 - 3
Times Bred	1.09	0.87

Trial 2. Lactating Holsteins (n = 30) at the Piedmont Research Station Dairy were randomly assigned to either treatment (n = 15) or control (n = 15) groups. Cows averaged 93.8 ± 9.1 DIM at breeding with 16.7% being repeat breeders. Lactations ranged from 1 to 4. Using the OvSynch protocol, estrus was synchronized and cows not seen in estrus were subjected to TAI. Cows exhibiting estrus were bred 12 hours after estrus was detected. Cows were bred to one of two bulls. Cows assigned to the treatment group received a CIDR insert 5 days after insemination that remained in place for 14 days. Cows were milked twice daily. Cows were fed a total mixed ration and were provided water ad libitum.

Table 3. Distribution of cows for trial 2.

Table 3. Cow Distribution: Trial 2

	Control	CIDR
Mean DIM	94.5	89.3
Lactation Number (minimum-maximum)	1 - 4	1 - 3
Lactation Number	1.9	1.8
Times Bred (minimum-maximum)	0 - 4	0 - 4
Times Bred	0.23	0.23
Mean Milk Production (lbs. / day)	76.49	75.54

Sample Collection

Sample collection procedures were similar for both trials. Blood samples were taken by coccygeal venipuncture and collected into 10mL Vacutainer ® (Becton, Dickinson and Company, Franklin Lakes, NJ) blood collection tubes that contained no additive. Blood samples were collected on days: 3, 5, 7, 10, 12, 14, 17, 19, 21, 24, 26, and 28 of gestation for trial 1. Blood samples were collected on days: -9, -2, 0, 6, 8, 11, 13, 15, 18, and 20 of gestation for trial 2, with estrus denoted as day 0. Samples were refrigerated and centrifuged approximately 24 hours later. Serum was decanted after

centrifugation and stored at -20 °C until radioimmunoassays were performed. Rectal temperatures were taken in conjunction with blood samples at the PRS Dairy.

The daily minimum and maximum temperature and relative humidity were measured and recorded each day at the PRS Dairy. Temperature and humidity data for DEU were obtained from the National Weather Service in Raleigh, NC. These data were used to calculate the temperature – humidity index, which is provided by the formula: THI = °F – (0.55-0.55 (RH/100)) (°F – 58). Both average daily temperature and average daily relative humidity were used to calculate THI.

Cows not seen in estrus were checked for pregnancy via transrectal ultrasonography at approximately 30 days after breeding. Cows exhibiting estrus behavior after breeding were assumed to be open.

Radioimmunoassay

Serum progesterone concentrations were measured with Coat-a-Count progesterone RIA kits (Diagnostic Products Corporation, Los Angeles, CA), as validated for bovine serum by Whisnant and Burns (2002). The Cobra II Auto-Gamma Counter (Packard Instrument Company, Boston, MA) was used for assays. Assays for trial 1 were conducted over 4 days. The intra-assay coefficient of variation was 5.75%. The inter-assay coefficient of variation was 8.67%. Assays for trial 2 were conducted over 2 days. The intra-assay coefficient of variation was 5.4%. The inter-assay coefficient of variation was 8.27%.

Statistical Analysis

For statistical analysis of progesterone concentrations, PROC GLM of SAS 8.2 (SAS Institute Inc., Cary, NC) was used. Pregnancy data were analyzed using the chi-square option in PROC FREQ of SAS 8.2. PROC UNIVARIATE of SAS 8.2 was used to calculate mean values for DIM, lactation, and number of times bred. The statistical model included day, treatment, and day x treatment interaction.

Results

Environmental Data

Trial 1. The environmental data showed that cows at PRS were subjected to mild heat stress, with an average THI of 73.5. Cows at DEU were subjected to mild heat stress, with an average THI of 75.7. The THI values at PRS ranged from 64.5 to 78.5, with 73.9% of days measured classified as heat stress conditions. THI values at PRS ranged from 68.5 to 79.8, with 93.2% of days measured classified as heat stress conditions. The mean temperature for the trial at PRS was 24.7°C, with values ranging from 12.8°C to 33.9°C. The mean temperature for the trial at DEU was 25.8°C, with values ranging from 16.1°C to 35.5°C. The mean relative humidity at PRS was 70.0%, with values ranging from 31% to 90%. It should be noted that 90% was the maximum detectable reading at PRS. The mean relative humidity at DEU was 74.9%, with values ranging from 28% to 100%. The mean rectal temperature for the trial was 38.6°C, with individual readings ranging from 36.4°C to 40.4°C.

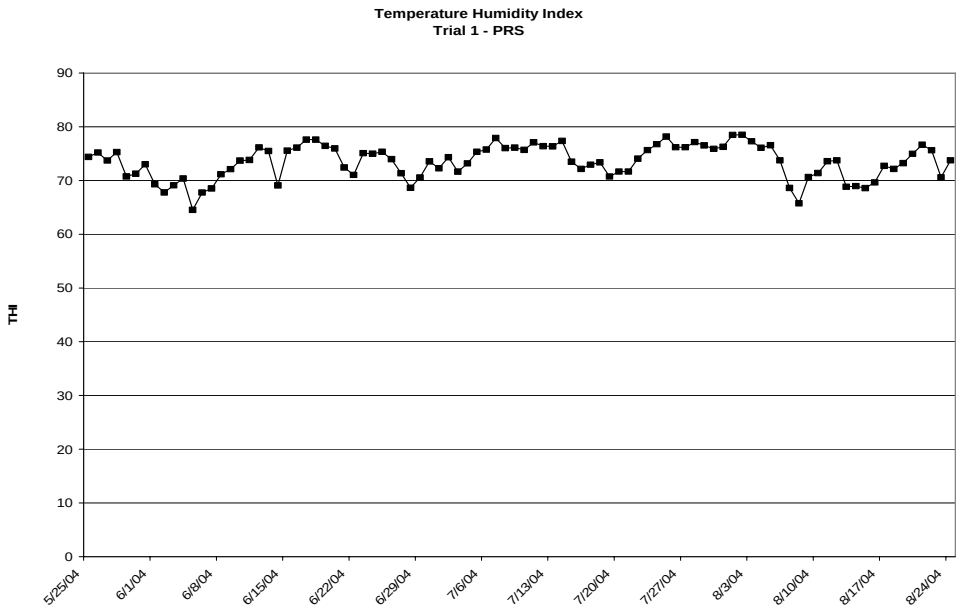

Figure 4. Temperature humidity index values at PRS for trial 1.

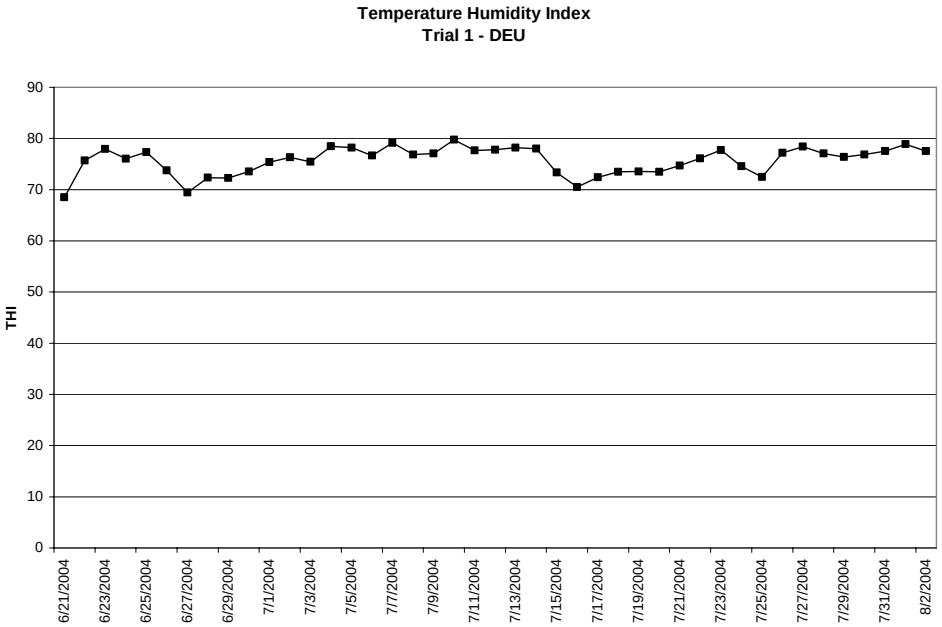

Figure 5. Temperature humidity index values at DEU for trial 1.

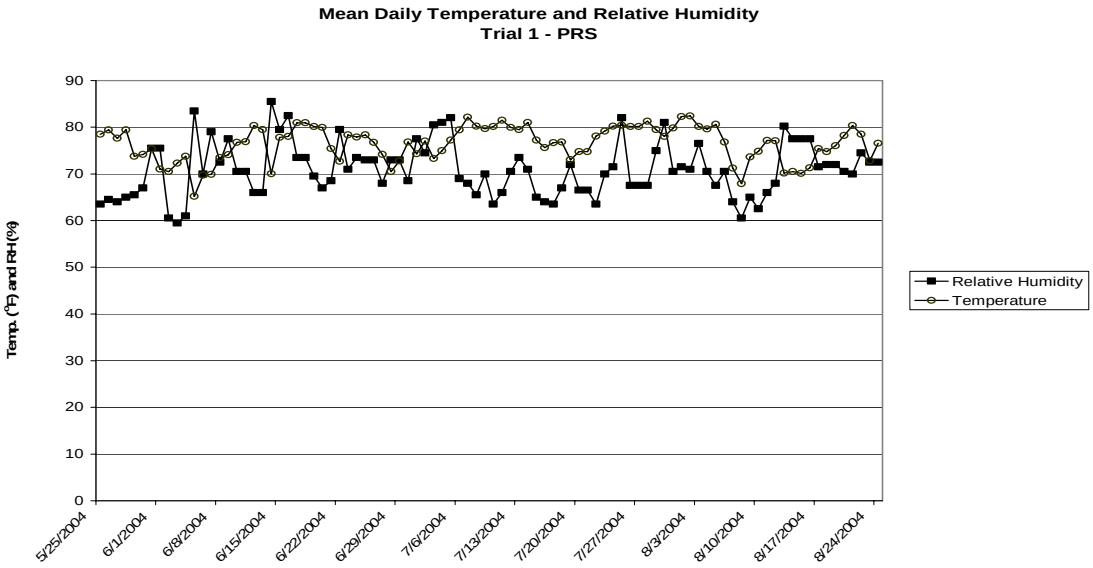

Figure 6. Mean daily temperature and relative humidity at PRS for Trial 1.

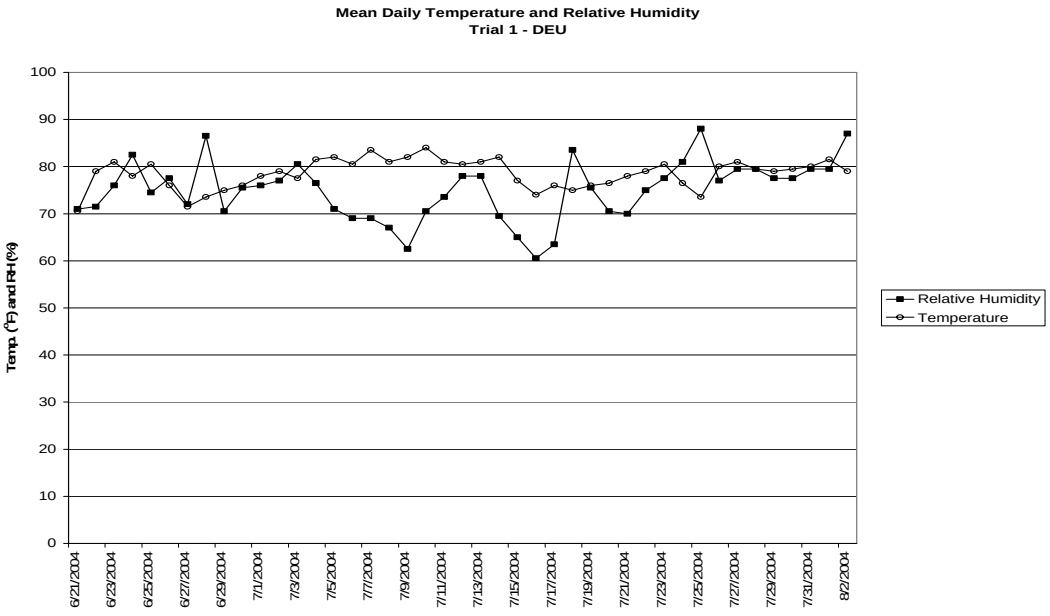

Figure 7. Mean daily temperature and relative humidity at DEU for trial 1.

Trial 2. The environmental data for trial 2 show that cows were subjected to mild heat stress, with a mean THI of 75.1. The THI values ranged from 66.61 to 80.5, with 90.1% of days measured classified as heat stress conditions. The mean temperature for the trial was 25.1°C, with values ranging from 8.3°C to 34.4°C. The mean relative

humidity for the trial was 67.4%, with values ranging from 26% to 90%. Again, the maximum detectable reading from the barometer used was 90%. The mean rectal temperature for the trial was 38.8°C, with a range of 37.2°C to 40.7°C.

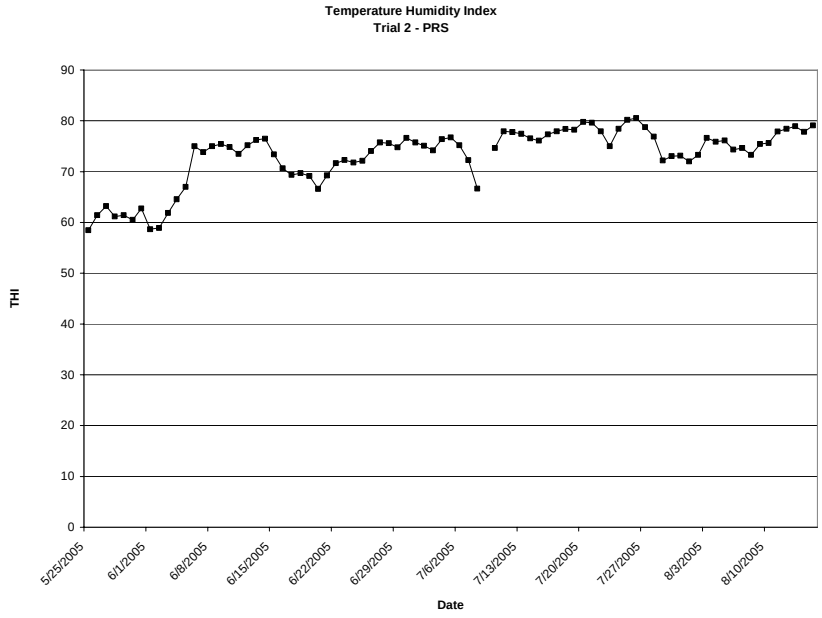

Figure 8. Temperature humidity index values for trial 2.

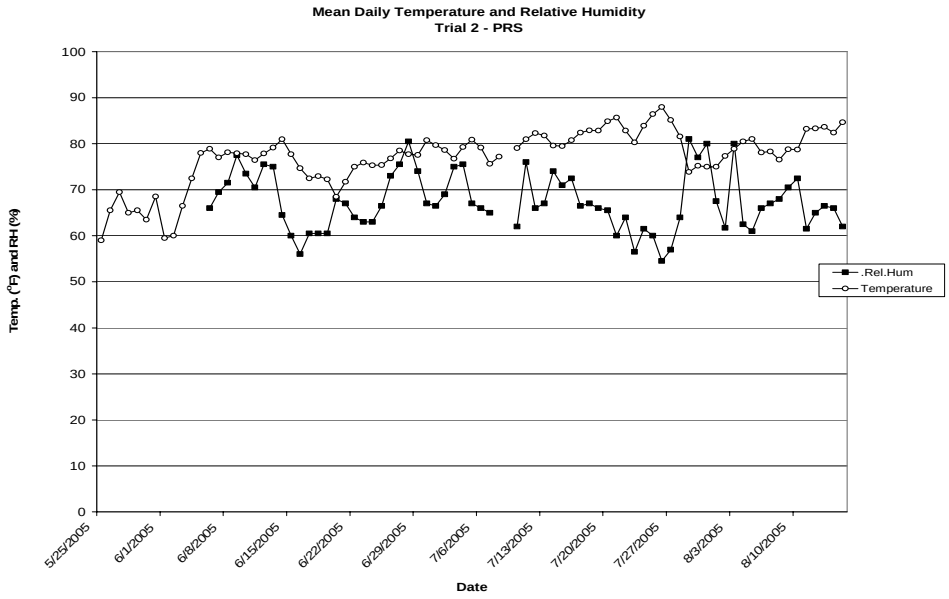

Figure 9. Mean daily temperature and relative humidity for trial 2.

Progesterone Concentrations

Trial 1. There was no significant difference in serum progesterone concentrations between CIDR treated and control cows (Figure 10). A post hoc analysis comparing serum progesterone concentrations between pregnant and open cows also yielded no significant difference (Figure 11). Mean serum progesterone concentrations indicated luteal function in both groups before CIDR insertion for the CIDR treated group. As expected, progesterone concentrations were highest on days 7 through 17, which correspond with diestrus. Progesterone concentrations for CIDR treated cows remained static immediately after CIDR removal, indicating the presence of a CL.

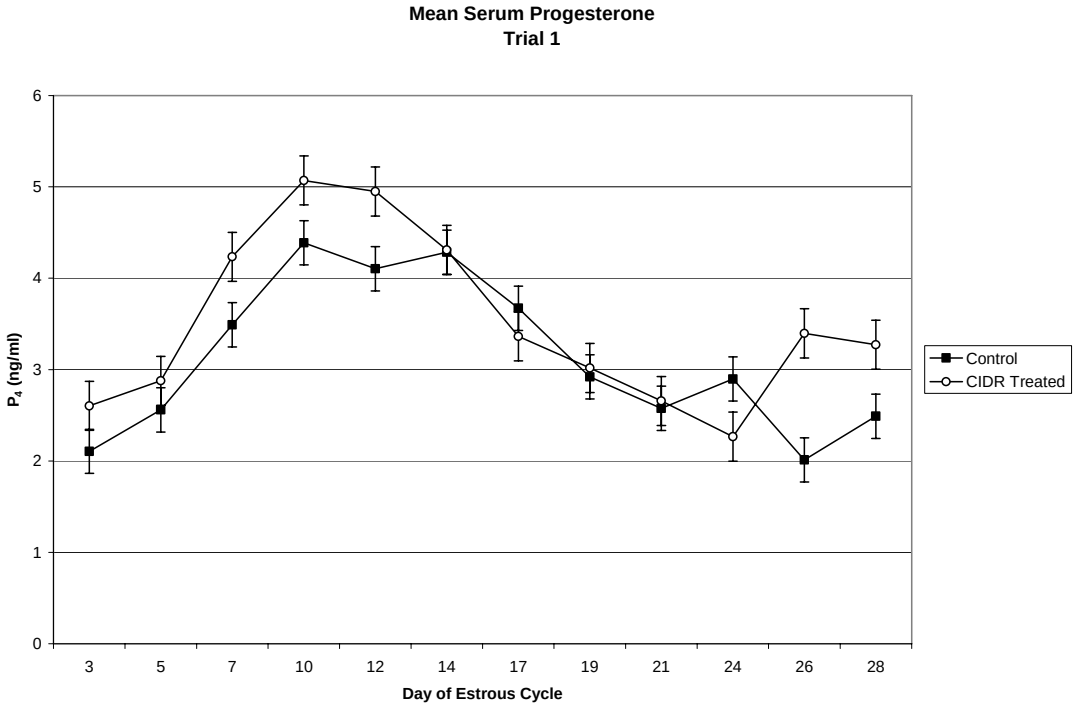

Figure 10. Mean serum progesterone concentrations (ng/ml) by treatment for cows in trial 1.

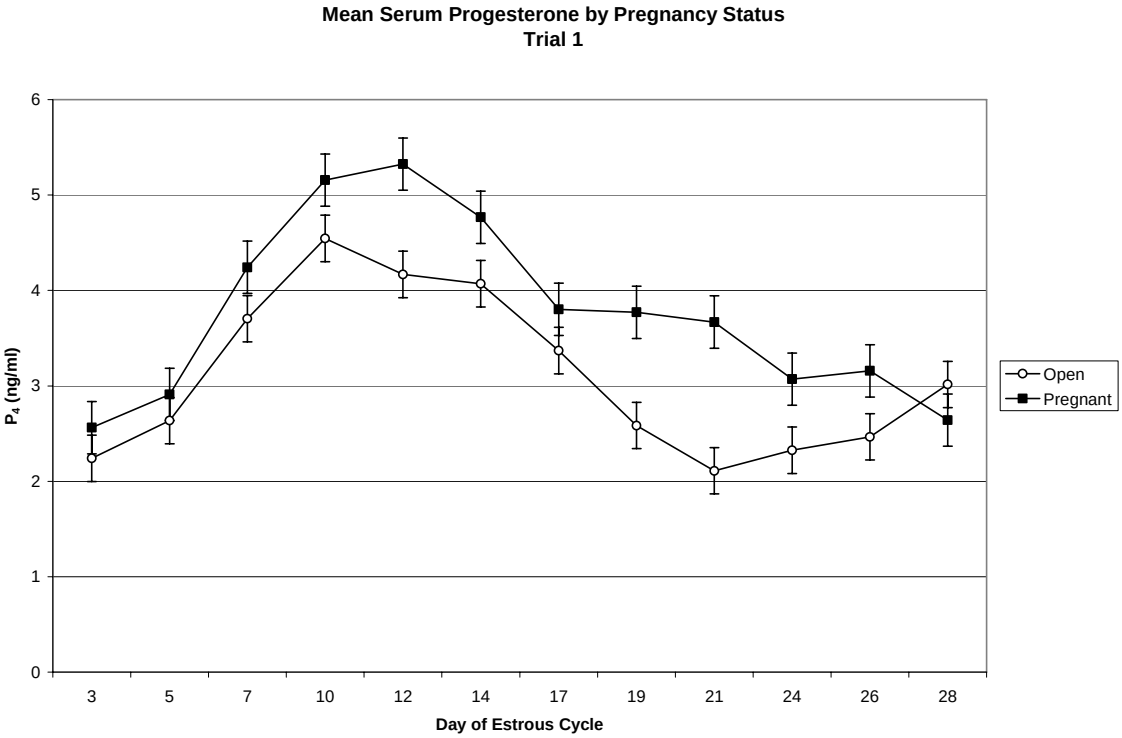

Figure 11. Mean serum progesterone concentrations (ng/ml) by pregnancy status for cows in trial 1.

Trial 2. Overall there was no significant difference in mean serum progesterone concentrations when CIDR treated and control cows were compared. The control group had a mean serum progesterone concentration of 3.4167 ng/ml compared to 2.0420 ng/ml for the CIDR treated group ($p = 0.0255$) on day -2, which was 2 days before synchronized estrus (Figure 12). A post-hoc comparison of mean serum progesterone concentrations between pregnant and open cows showed no difference (Figure 13). Mean serum progesterone concentrations indicated luteal function and response to $PGF_{2\alpha}$ on day 0. Serum progesterone concentrations did not increase as expected in the CIDR treated group immediately after CIDR insertion, in fact, they remained below 2 ng/ml, considered to be the threshold of luteal function. Progesterone concentrations increased

on day 8 to levels normally seen during diestrus, peaked on day 11, and remained elevated through day 18.

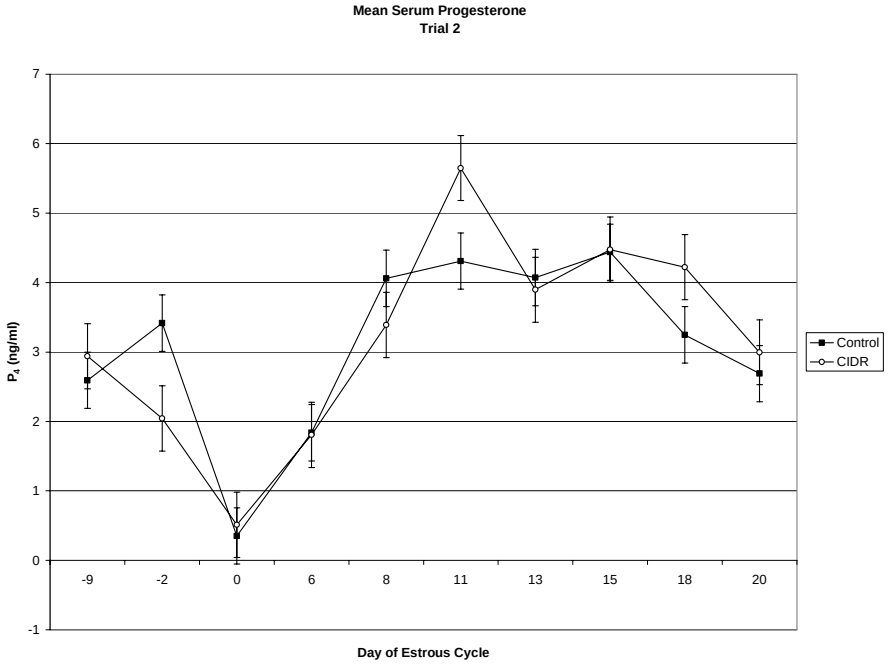

Figure 12. Mean serum progesterone concentrations (ng/ml) by treatment for Holstein cows in trial 2.

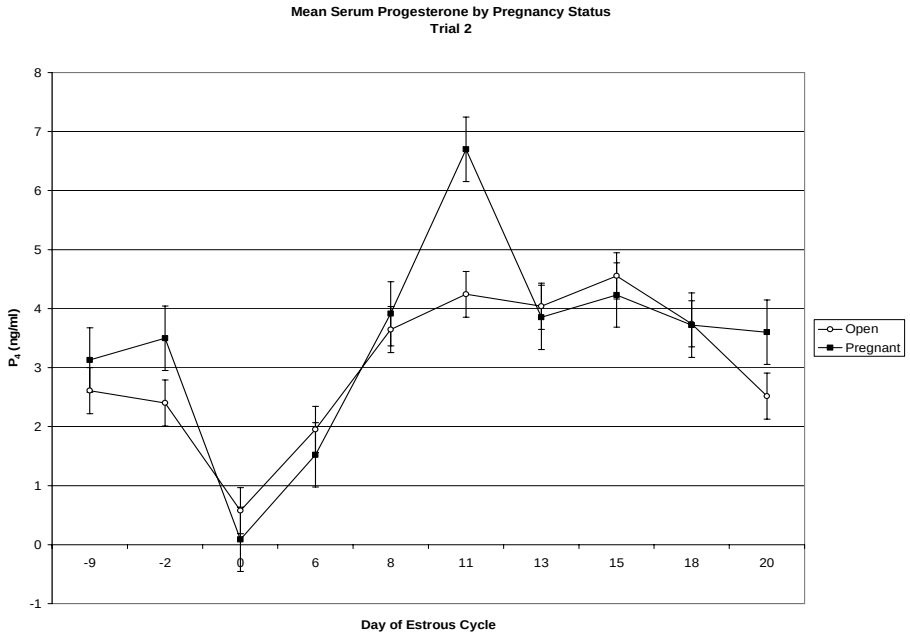

Figure 13. Mean serum progesterone concentration (ng/ml) by pregnancy status for Holstein cows in trial 2.

Pregnancy Rates

Trial 1. There was no difference in pregnancy rates between control and CIDR treated cows. The percentage of pregnant cows in the control group was 31.82% (7/22), compared to 33.33% (8/24) for CIDR treated cows (p = 0.2451) (Figure 14).

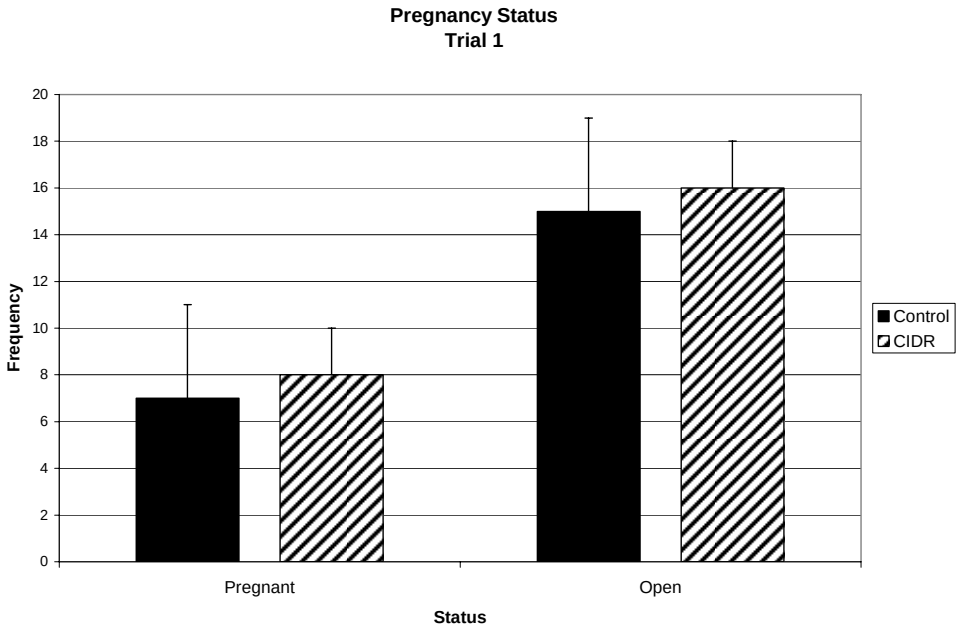

Figure 14. Frequency of pregnant cows in trial 1.

Trial 2. The control group presented with 33.33% (5/15) pregnant, compared with 26.67% (4/15) pregnant in the CIDR treated group. There was no statistical difference in pregnancy rates between the two groups (p = 0.2865) (Figure 15).

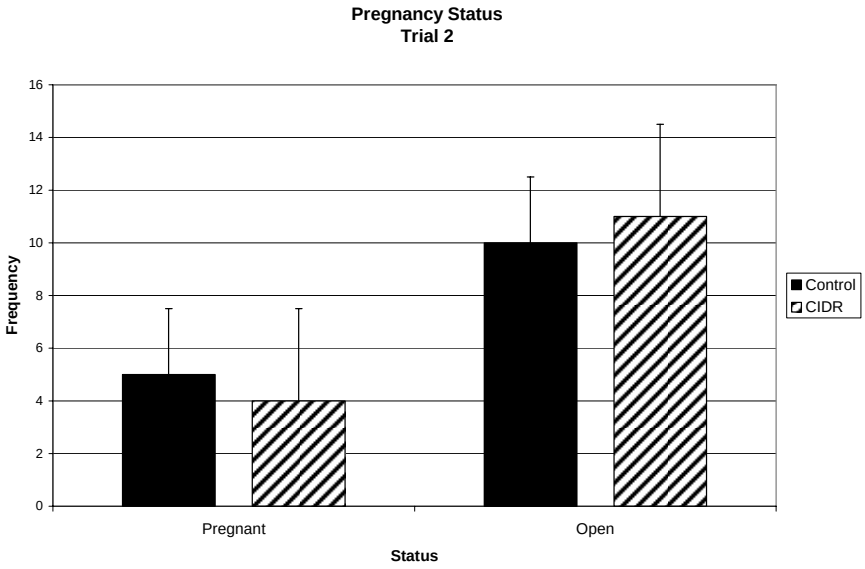

Figure 15. Frequency of pregnant cows in trial 2.

Table 4. Mean serum progesterone concentrations (ng/ml) of CIDR treated and control cows from trial 1.

	CIDR Treated	Control
Day 3	2.60[a]	2.10[a]
Day 5 (CIDR In)	2.87[a]	2.55[a]
Day 7	4.23[a]	3.49[a]
Day 10	5.07[a]	4.38[a]
Day 12 (CIDR Out)	4.94[a]	4.10[a]
Day 14	4.31[a]	4.28[a]
Day 17	3.67[a]	3.36[a]
Day 19	3.01[a]	2.92[a]
Day 21	2.65[a]	2.57[a]
Day 24	2.89[a]	2.26[a]
Day 26	3.39[a]	2.01[a]
Day 28	3.27[a]	2.49[a]

Different superscripts denote significant difference ($p < 0.05$)

Table 5. Mean serum progesterone concentrations (ng/ml) of pregnant and open cows from trial 1.

	Pregnant	Open
Day 3	2.56[a]	2.24[a]
Day 5 (CIDR In)	2.91[a]	2.63[a]
Day 7	4.24[a]	3.70[a]
Day 10	5.15[a]	4.54[a]
Day 12 (CIDR Out)	5.32[a]	4.16[a]
Day 14	4.76[a]	4.07[a]
Day 17	3.80[a]	3.37[a]
Day 19	3.77[a]	2.58[a]
Day 21	3.66[a]	2.11[a]
Day 24	3.07[a]	2.32[a]
Day 26	3.15[a]	2.46[a]
Day 28	3.01[a]	2.64[a]

Different superscripts denote significant difference ($p < 0.05$)

Table 6. Mean serum progesterone concentrations (ng/ml) of CIDR treated and control cows from trial 2.

	CIDR Treated	Control
Day -9	2.93[a]	2.59[a]
Day -2	2.04[b]	3.41[a]
Day 0	0.51[a]	0.35[a]
Day 6(CIDR In)	1.80[a]	1.83[a]
Day 8	3.39[a]	4.06[a]
Day 11	5.64[a]	4.31[a]
Day 13	3.89[a]	4.07[a]
Day 15	4.47[a]	4.43[a]
Day 18	4.22[a]	3.24[a]
Day 20 (CIDR Out 19)	2.99[a]	2.68[a]

Different superscripts denote significant difference ($p < 0.05$)

Table 7. Mean serum progesterone concentrations (ng/ml) of pregnant and open cows from trial 2.

	Pregnant	Open
Day -9	3.12[a]	2.61[a]
Day -2	3.49[a]	2.40[a]
Day 0	0.09[a]	0.57[a]
Day 6(CIDR In d5)	1.52[a]	1.95[a]
Day 8	3.91[a]	3.64[a]
Day 11	6.69[a]	4.24[a]
Day 13	3.85[a]	4.04[a]
Day 15	4.23[a]	4.55[a]
Day 18	3.72[a]	3.74[a]
Day 20 (CIDR Out d19)	3.60[a]	2.51[a]

Different superscripts denote significant difference ($p < 0.05$)

DISCUSSION

The specific objective of this study was to test the effects of supplemental progesterone administered post-insemination on pregnancy rates and serum progesterone concentration in heat stressed dairy cows. Based on the success Willard et al. (2003) and Sweetman (2003) had utilizing GnRH to improve reproduction in heat stressed dairy cows, it was reasoned that exogenous progesterone should produce similar results. Reproductive performance of dairy cows is severely affected by heat stress and these problems are compounded by high milk production. Pregnancy rates of heat stressed dairy cows can be as low as 10%, which is a drastic difference from cool weather.

Environmental data collected during this study indicated that cows in this study were subjected to mild heat stress (Armstrong, 1994). For trial one, the average THI throughout the study was 73.5 and 73.9% of the days were classified as heat stress conditions. THI values ranges from 64.5 to 78.5, which varies from no stress to moderate heat stress. Cows in trial two were subjected to heat stress conditions 90.1% of the days measured. The average THI throughout trial two was 75.1, with a range of *66.6* to 80.5, again, covering no stress to moderate heat stress. Rectal temperatures ranged from 36.4°C to 40.7°C, indicating that the cows had elevated temperatures at some points. While not extreme temperatures, these were within the range that can exert a negative influence on reproduction (Rivera and Hansen, 2001).

Two trials were conducted, one leaving CIDRs in place for 7 days beginning 5 days after insemination; the second trial also had CIDRs inserted on day 5 after breeding, but the CIDR remained in for 14 days. If the CIDR was inserted at breeding, the negative feedback loop would detect the elevated amounts of progesterone and initiate luteolysis

sooner than expected. Inserting the CIDRs 5 days after insemination gives the CL approximately 5 days to develop, meaning that the CL should be fully formed and secreting progesterone for a normal lifespan. The second trial was designed along the same premise as the first trial but to increase the length of progesterone supplementation so that there was progesterone at luteal levels during the time of maternal recognition of pregnancy, which occurs about day 14 to 16 of gestation.

Overall serum progesterone concentrations for trial 1 showed no significant difference between control cows and those treated with a CIDR for 7 days beginning 5 days after estrus. The CIDR treated group had numerically higher serum progesterone concentrations at each sampling, but these differences were not statistically significant. Observed progesterone profiles followed the expected pattern, with the highest progesterone concentrations on days 7 through 17, corresponding with diestrus when progesterone secretion peaks. Samples taken before CIDR insertion indicated normal luteal function. Mean progesterone concentrations remained static after CIDR removal, indicating the presence of a functional CL.

The overall progesterone concentrations for trial 2 showed no difference between CIDR treated cows and control cows. When analyzing individual days, however, the control group had an elevated mean progesterone concentration on day -2 (3.4 compared to 2.0 ng/ml, $p = 0.0255$). This is somewhat of an anomaly since $PGF_{2\alpha}$ was administered on the previous day. Mean serum progesterone concentrations of 0.51 ng/ml and 0.35 ng/ml on day 0 indicated luteal response to $PGF_{2\alpha}$. Serum progesterone did not increase immediately when CIDRs were inserted. Rathbone et al. (2002) and Martinez (2002) reported that progesterone concentrations increased almost immediately

after CIDR insertion in ovariectomized cows, peaking at 5 – 7 ng/ml within 24 hours of insertion. El-Zarkouny and Stevenson (2004) also reported no difference in serum progesterone concentrations in a study similar to this one. Progesterone concentrations increased on day 8 to levels normally seen during diestrus, peaked on day 11, and remained elevated through day 18. A post hoc analysis was performed for both trials to determine the influence of pregnancy status on progesterone concentrations. For both trials there was no difference in progesterone concentrations between pregnant and open cows.

One goal of this study was to improve pregnancy rates in heat stressed dairy cows by providing supplemental progesterone after breeding. For both trials, CIDR treatment did not improve pregnancy rates. Dickerson et al. (2004) reported that administration of GnRH after breeding failed to improve pregnancy rates in heat stressed dairy cows, indicating that progesterone supplemented through endogenous or exogenous methods alone may not improve pregnancy rates under heat stress conditions. Although the CIDR treatment did not improve pregnancy rates, it is important to note that CIDR treatment did not decrease pregnancy rates. These results provide further evidence that CIDRs can be used safely to resynchronize cows of unknown pregnancy status. Anecdotal evidence from trial one showed excellent resynchrony of the cows that did not conceive to the first service.

CONCLUSION

Providing supplemental progesterone after breeding to heat stressed dairy cows did not improve pregnancy rates or increase serum progesterone concentrations. Progesterone supplementation for 7 days or 14 days did not result in increased pregnancy rates, and neither treatment was successful in raising mean serum progesterone concentrations.

Although this study did not improve pregnancy rates or increase serum progesterone, it should be noted that pregnancy rates and serum progesterone concentrations for CIDR treated cows were not negatively influenced by CIDR treatment. This provides further supporting evidence for the safety of using progesterone administration to resynchronize cows of unknown pregnancy status. Altering the timing of progesterone supplementation may have yielded different results.

Impaired reproductive performance in heat stressed dairy cows is a complex problem that warrants further research. A combination of methods previously tried may yield different results when applied in concert. Supplementing progesterone through endogenous methods such as GnRH administration appears to provide a greater benefit than exogenous progesterone, thus further trials comparing the effectiveness of the two should be conducted.

The use of prostaglandin synthesis inhibitors may also be beneficial to reproduction in heat stressed dairy cows, but has not been used in dairy production due to milk withdrawal times. In addition, an economic analysis is needed to determine the

most cost-effective methods for improving reproductive performance of dairy cows during heat stress.

In conclusion, it is recommended that the use of post-insemination progesterone supplementation for the sole purpose of improving pregnancy rates in heat stressed cows be further researched since two trials are not enough to make definite conclusions.

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