ABSTRACT

Follicular growth, lifespan of the corpus luteum, and antioxidant status of lactating Holsteins that experienced heat stress were monitored. Eleven multiparous cows, 60 to 110 d in milk, were maintained from 0800 to 1800 h daily in environmental chambers from d 11 to 21 of the estrous cycle. Cows were randomly assigned to a heat stress (mean dry bulb temperature peaked at 38.3°C) or control treatment (mean dry bulb temperatures varied from 20.8 to 25.6°C). Rectal temperature and respiration rates of heat-stressed cows were higher at 1600 h than those of control cows. The length of the estrous cycle and the interval from estrus until luteolysis were not different between treatments. Two of 6 control cows and 1 of 5 heat-stressed cows had extended cycles (>24 d). Heat-stressed cows had more class 1 (2 to 5 mm) follicles from d 11 to 15 of the estrous cycle. Numbers of class 2 (6 to 9 mm) and class 3 (>9 mm) follicles were similar between treatments. Plasma progesterone concentrations were higher for heat-stressed cows until d 19 of the estrous cycle. Treatment did not affect concentrations of α-tocopherol, β-carotene, retinol, retinyl palmitate, or total protein in plasma or concentrations of malondialdehyde in muscle. In conclusion, heat stress did not extend luteal function or the length of the estrous cycle of lactating Holstein cows but did affect follicular growth and progesterone concentrations in plasma. Heat stress did not appear to increase lipid peroxidation or decrease lipid-soluble antioxidant concentrations in blood.

( Key words: free radical, length of estrous cycle, heat stress, lactating Holstein cows )

INTRODUCTION

For dairy cows, heat stress reduces conception rates (4, 36), decreases duration and intensity of estrus (1, 18, 23, 36), and has been reported to alter circulating concentrations of estradiol (37) and follicular dynamics (5, 34, 37). Reports also suggest that heat stress causes the duration of the estrous cycle to be reduced (36) or prolonged (1, 23, 34). Discrepancies in the literature also exist concerning the effects of heat stress on concentrations of progesterone in the blood during the estrous cycle. Some studies (1, 31) have indicated that progesterone concentrations increase under heat stress conditions, others (32, 35) have indicated a decrease in progesterone concentrations, and still others (7, 37) have shown no effect on progesterone concentrations.

Previous researchers (22, 27) have proposed that some of the deleterious effects of elevated temperature are due to increased production of oxygen-derived free radicals. Free radicals compromise cellular function by removing electrons from a variety of molecules. As a result, protein crosslinking, DNA damage, and lipid peroxidation can occur (11, 16). Evidence suggests that the effects of elevated temperature on embryonic development involve changes in the metabolism of free radicals. Exposure of cultured mouse embryos to elevated temperatures causes a reduction in glutathione (3), which is a cytosolic antioxidant (25). Furthermore, effects of elevated temperature on cultured embryos can be reduced by the administration of several antioxidants to the culture medium (2, 3, 13). An increase in the production of free radicals caused by heat stress could also conceivably lead to damage of mammary epithelium and contribute to decreased milk yield (14, 19).

Objectives of the present study were to evaluate whether heat stress altered follicular dynamics, plasma progesterone concentrations, and the length of the estrous cycle in lactating cows and to test whether heat stress increased the production of free radicals. To examine the latter hypothesis, concentrations of several molecules involved in the metabolism of free radicals were monitored, including malondialdehyde, a degradation product of lipid peroxidation (11, 16), and vitamin E and β-carotene, which function to pro-
tect unsaturated fatty acids in membranes (6, 11, 12, 17).

MATERIALS AND METHODS

Assignment of Cows to Treatments

Lactating, multiparous Holstein cows, 60 to 110 DIM, that had not been bred during the present lactation were used in the study. The study was performed during four separate periods from February to April 1996 with a total of 5 heat-stressed cows and 6 control cows. For each period, estrous cycles were synchronized by i.m. injection of 100 μg of GnRH (Cystorelin®, Sanofi Animal Health, Inc., Overland Park, KS) followed by an i.m. injection of 25 mg of PGF2α (Lutalyse®, Upjohn, Kalamazoo, MI) 7 d later. After PGF2α injection, tailheads were chalked (Paintstik®, LA-CO Industries, Inc., Chicago, IL), and cows were observed for estrus. For each period, up to 4 cows that came into estrus were assigned randomly to heat stress or control treatments.

Environmental Modification

Two environmental chambers were used. Each chamber measured 4.5 m × 4.3 m and contained two tie stalls (1.6 m × 1.2 m) with an individual feed bunk and metered water bowl. Mattresses (North Brook Farms, Weedsport, NY) were placed in each stall for cow comfort. Temperature in the rooms was controlled by a microprocessor (Metasys Network Control Unit; Johnson Controls, Milwaukee, WI). Humidity was not controlled but was similar in both rooms. Ventilation of each room was provided by an air-handling unit (model no. 39LD035; Carrier, Syracuse, NY) equipped with an electrical heater for air intake. Air conditioning to both chambers was provided by a 27.7-tonne capacity chiller (30 GT-050 air cooled reciprocating chiller; Carrier). Cows were provided with a continuous supply of fresh air; air was removed from each chamber using an exhaust fan set at 37 m³/min.

Treatments were administered from d 11 through 21 of the estrous cycle. On each day of treatment, cows were placed in the appropriate chamber at 0800 h and removed at 1800 h. Cows were milked at approximately 0600 and 1800 h and were kept in a sand lot adjacent to the chambers from 1800 to 0600 h. Environmental controls for the chambers were engaged each morning at 0600 h to achieve a set point temperature at 0800 h. The set point temperature for the heat stress group was initially 40°C but was modified on a daily basis (±2°C) if target rectal temperatures (40.0 to 40.9°C) were not achieved. The set point temperature for the control chamber varied from 18.3 to 24°C. The chamber used for each treatment was alternated each time a new group of cows was used to avoid confounding the effects of environmental treatment with chamber effects.

Mean dry bulb temperatures for the two chambers are presented in Figure 1. For the heat stress chamber, dry bulb temperature averaged 30.7°C when cows entered the chamber at 0800 h and rose to a mean temperature of 38.3°C by 1500 h. Actual temperatures were <40°C (initial set point temperature) because the set point temperature was reduced on some days to maintain rectal temperatures between 40.0 and 40.9°C and because the heating system was insufficient to raise dry bulb temperatures to the set point temperature on cold days. For the control chamber, dry bulb temperature averaged 20.8°C when cows entered the chamber at 0800 h and rose to a mean temperature of 25.6°C by 1500 h. Thus, dry bulb temperature in the afternoon often rose above the set point temperature because of the inability of the chamber to cool the air adequately.

Water and Feed Intake

Daily water intakes for the period during which cows were in chambers were measured using meters attached to the water bowls. Cattle were fed a TMR of corn silage, whole cottonseed, alfalfa hay, and soybean meal. The TMR contained 18.4% protein and 0.35 Mcal/kg of NE L. While cows were in chambers, feed intake was measured as follows. Feed was...
weighed out each morning before the cows entered the chambers, and orts were collected and weighed after cows exited the chambers in the evening. Cows were provided with sufficient feed to allow ad libitum consumption. Cows were fed in groups at night in the adjacent sand lot.

Rectal Temperatures and Respiration Rates

Rectal temperatures were measured twice daily, at 0800 and 1600 h, using a glass mercury thermometer (Nasco, Ft. Atkinson, WI). Respiration rates were measured daily, at 0800 and 1600 h, by counting the number of flank movements in 1 min.

Ultrasound Examination

Ovarian structures were monitored daily from d 11 through 21 at 1630 h by intrarectal ultrasonography using a real time linear ultrasound scanner (Aloka Echo-Camera SSD 500; Wallingford, CT) equipped with a 7.5-MHz intrarectal probe. At each ultrasound examination, diagrams of the relative position of each follicle and the corpus luteum were drawn. Follicles ≥2 mm were detectable. Data collected by ultrasound included the total number of follicles in each class size [class 1 (2 to 5 mm), class 2 (6 to 9 mm), and class 3 (≥9 mm)].

Detection of Estrus and Blood Sampling

While cows were in the environmental chambers, paint stick and Kamar heat detectors (Kamar®, Inc., Steamboat Springs, CO) were applied as aids to detect estrus. Twice daily visual observation, before cows entered the chambers in the morning and after they were removed from the chamber in the evening, began once luteolysis occurred as determined by ultrasonic examination of the corpus luteum. Cows were removed from the chambers on d 21 of the estrous cycle and returned to their appropriate milking groups. Observations for the detection of estrus occurred twice daily thereafter.

For all cows, jugular blood samples (30 to 40 ml) were collected daily at 1615 h from d 11 to 21 to determine concentrations of progesterone, β-carotene, retinol, and retinyl palmitate were determined by HPLC (9, 28). Total protein was determined by the Bradford procedure (8) using the Bio-Rad protein assay (Bio-Rad, Hercules, CA).

Analysis of Malondialdehyde

Concentration was determined in the tissue biopsy collected on d 21 by measuring thiobarbituric reactive products (21). Values for malondialdehyde were based on the use of 1,1,3,3-tetramethoxypropane (Sigma Chemical Co., St. Louis, MO) as the external standard (29). Samples were prepared by suspending approximately 1 g of tissue (exact wet weight determined) in 9 ml of 1.15% (wt/vol) KCl. Samples were then homogenized for 30 to 45 s using an electric homogenization probe (Omni, Waterbury, CT). If not completely homogenized, samples were re-homogenized for an additional 30 to 45 s.

Statistical Analysis

Data were analyzed by least squares analysis of variance using PROC GLM of SAS (33). The mathematical model included effects of treatment, cow (period × treatment), day, period, and their interactions. In the initial analyses, period and its interactions were found to be nonsignificant, and these terms were subsequently removed from analyses.

RESULTS

General Responses to Heat Stress

At 0800 h, rectal temperatures were similar for cows maintained under heat stress and control condi-
tions (Table 1). At 1600 h, heat-stressed cows had higher \((P = 0.0001)\) rectal temperatures than did control cows. Respiration rates were higher for heat-stressed cows at 0800 \((P = 0.0022)\) and 1600 h \((P = 0.0001)\). Heat stress decreased \((P = 0.0119)\) feed intake and water consumption \((P = 0.0338)\) during the 10-h period each day that cows were in the chambers. No effect of heat stress on plasma protein concentration (an indirect measure of hemodilution) was detected. Milk yield was not different between heat-stressed and control cows, but the change in milk yield (i.e., daily milk yield minus milk yield during the 3 d before the trial started) tended \((P = 0.1091)\) to be more negative for heat-stressed cows.

**Length of the Estrous Cycle and Follicular Development**

No differences \((P > 0.10)\) were observed in the length of the estrous cycle or the interval from estrus to luteolysis between treatments. Two of 6 control cows had estrous cycles that were \(>24\) d in length, and another 2 cows had estrous cycles that were \(24\) d in length. One heat-stressed cow had an extended cycle of \(26\) d. In all cases, these longer cycles were due to prolonged luteal lifespan as determined by circulating concentrations of progesterone (results not shown).

When data from all days were analyzed together, day of the estrous cycle \((P = 0.0193)\) affected the number of class 1 follicles and tended to affect the numbers of class 2 \((P = 0.0826)\) and 3 \((P = 0.0977)\) follicles (Figure 2). Heat stress had no effect \((P > 0.10)\) on the number of class 1, 2, or 3 follicles, but...
there was a tendency \( (P = 0.1090) \) for an interaction of treatment and day for the number of class 1 follicles. Subsequent analysis revealed that, for the period from d 11 to 15 only, heat-stressed cows had more \( (P = 0.0583) \) class 1 follicles. From d 16 to 21, there was an interaction of treatment and day \( (P = 0.0476) \) because heat-stressed cows had more class 1 follicles on d 20 and 21 but not on other days.

**Progesterone Concentrations**

There was an interaction of treatment and day \( (P = 0.0599) \) that affected plasma concentrations of progesterone (Figure 3). Concentrations of progesterone were higher for cows under heat stress from d 11 to 14 and from d 16 to 17 and were lower for heat-stressed cows from d 19 to 21. In a second series of analyses, there was an effect of treatment \( (P = 0.0206) \) when concentrations of progesterone were examined from d 11 to 15 only, and there was an interaction of treatment and time \( (P = 0.0156) \) for concentrations of progesterone from d 16 to 21.

**Antioxidant Status**

Heat stress did not affect plasma concentrations of \( \alpha \)-tocopherol, \( \beta \)-carotene, retinol, or retinyl palmitate (Table 2). Similarly, heat stress did not affect concentrations of malondialdehyde in muscle on d 21 of the estrous cycle \( (249 \pm 59.4 \text{ nmol/g of wet weight for control cows vs. } 266 \pm 72.8 \text{ nmol/g of wet weight for heat-stressed cows}; P = 0.8624) \).

**DISCUSSION**

No evidence existed suggesting that the length of the estrous cycle was altered by heat stress conditions. Lack of an effect of heat stress on the length of the estrous cycle was not due to an insufficient heat stress treatment. Although results could possibly be different if the heat stress conditions were greater, the heat stress treatment was sufficient to reach target rectal temperatures of 40.0 to 40.9°C, to increase respiration rate, and to decrease feed intake. Moreover, the decrease in milk yield after cows entered the chamber tended to be greater for cows under heat stress than for control cows. The rectal temperatures experienced were similar to those experienced by cows under heat stress in conditions similar to commercial conditions \((14, 15, 18, 19)\). Control cows did experience a slight increase in rectal temperature in the afternoon compared with the morning, which could represent some heat stress in this group as well. The rise in temperature in the afternoon for control cows may also represent diurnal variation unrelated to heat stress. In any case, a clear difference in thermal environment and animal physiological responses to the environment were established.

Heat stress increased the number of small (2 to 5 mm) follicles during d 11 to 15 of the estrous cycle. This effect could represent compromised function of the dominant follicle \((5)\) or differences in length of the estrous cycle (and number of follicular waves) between treatments. More control cows had estrous cycles >23 d, which also explains why cows exposed to heat stress had more class 1 follicles at d 20 and 21. The observations that heat-stressed cows had more class 1 follicles during d 11 to 15 is in contrast to findings that heat stress reduced \((34)\) or tended to

![Figure 3. Least squares means (±SEM) for plasma progesterone concentrations from d 11 to 21 of the estrous cycle in cows exposed to control (●) or heat stress (○) conditions.](image-url)
reduce (37) the number of class 1 and class 2 follicles measured from d 11 of the estrous cycle. Differences in experimental design could explain some discrepancies between the current results and earlier studies. In the study of Wolfenson et al. (37), heat stress started at d 1, and cows were treated either with a progesterone implant and prostaglandin F₂α on d 12 or prostaglandin F₂α on d 14. The design for the current experiment was very similar to that of Wilson et al. (34), except that heat stress was more severe in the present study.

Heat stress generally increased progesterone concentrations in plasma between d 11 and 17 of the estrous cycle. After d 18, concentrations of progesterone were higher for control cows because luteolysis tended to be delayed in this group. The increased concentrations of progesterone in heat-stressed cows in the present study were not likely to have been caused by hemoconcentration because the concentration of plasma protein was similar for both groups. The effects of heat stress on progesterone concentrations in blood have been highly variable (1, 7, 31, 32, 35, 37). Progesterone concentrations in blood are dependent on luteal output of progesterone, possible adrenal release of progesterone, metabolism in the liver and other organs, and the degree of hemodilution or hemoconcentration. Heat stress can lead to a redistribution of blood away from viscera (24) and can cause either dilution, concentration, or no effect on blood plasma (14, 19, 24, 30). Much of the diversity in the observed effects of heat stress on circulating progesterone concentrations is likely a reflection of the magnitude of hyperthermia as well as the variable effect of heat stress on assorted aspects of physiological function that determine progesterone concentrations.

Heat stress often causes increased water intake in lactating cows (15, 24, 30). In the present study, however, heat stress decreased water intake during the time that cows were in the chamber. One possibility is that the decline in feed consumption or milk yield caused by heat stress led to decreased water intake. Yousef and Johnson (38) have illustrated a situation in which heat stress caused an increase in water consumption by nonlactating cows and a decrease in water intake by lactating cows. However, it is more likely that water intake was increased by heat stress but was not observed because water intake was measured only while cows were in the chambers. In one study (30), heat stress tended to cause a shift in drinking from day to night. In the present experiment, cows frequently drank soon after leaving the chambers.

One major objective was to determine whether heat stress increased the metabolism of free radicals. This question is important because of the importance of free radicals and antioxidants in various aspects of cellular function. Results indicate that the heat stress imposed was not sufficient to increase the production of free radicals in membranes. Malondialdehyde, which is a product of lipid peroxidation (11, 16), did not increase with heat stress. Also, no effects of heat stress on plasma concentrations of α-tocopherol, β-carotene, or products of β-carotene metabolism (retinol and retinyl palmitate) were observed. These antioxidants limit actions of free radicals in cellular membranes (6, 11, 12, 17). Although comparable with values from another study (26), plasma concentrations of α-tocopherol were higher than more typical values for lactating cows (10), and depletion of fatsoluble antioxidants by heat stress possibly could have occurred if these antioxidants were more limiting.

In conclusion, the application of a heat stress treatment that was sufficient to alter rectal temperatures, respiration rates, and feed intakes had no effect on the length of the estrous cycle but increased the number of small follicles during midcycle and increased plasma concentrations of progesterone. Heat stress also had no effect on the production of malondialdehyde or circulating concentrations of α-tocopherol, β-carotene, retinol, or retinyl palmitate.

ACKNOWLEDGMENTS

The authors thank Mary Russell, Ed Fredriksson, and Charlene Roomes for their assistance with the cows during the experiment; Nancy Wilkinson and Al Boning for performing the HPLC assays; Rocio Rivera for performing plasma protein analyses; Jesse John-son for performing the progesterone radioimmuno-assay; and W. W. Thatcher for allowing us access to the ultrasound equipment and the radioimmunoassay laboratory and for valuable advice regarding data analysis. The authors thank North Brook Farms (Weedsport, NY) for donating the comfort mats that were installed in the environmental chambers.

REFERENCES


