SERUM CORTISOL AND ESTRADIOL-17β LEVELS IN BOVINE UNDER HEAT STRESS

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During July, 1989, fourteen lactating, nonpregnant and normally cycling Holsteins were assigned randomly to pens in freestall barn with fan or without fan to observe the effect of forced ventilation on cortisol and estradiol-17β concentrations. After an adjustment period of 21 days, jugular cannulae were inserted and 25 mg prostaglandin (PG)F2α (Lutalyse^R, The Upjohn Co., Kalamazoo, MI) was injected. All animals were in their luteal phase at the time of injection as determined by previous observations for oestrus and palpation. After PGF2α, blood samples were collected at 6 h interval from 0 to 36 h, 4 h intervals from 36 to 88 h and 3 times weekly (MWF) for three weeks thereafter. Blood samples were assayed for cortisol, and estradiol-17β. Daily rectal temperatures and ambient condition data were recorded. Average daily rectal temperatures were lower ($P<0.05$) in the fan (39.10 ° C) than in the control group (39.48 C). Cortisol did not show diurnal pattern and the values did not differ ($P<0.05$) between treatment groups. The values of pre- and post-oestrus estradiol-17β were analysed separately and were also not different in groups.

INTRODUCTION

Heat stress has been reported to affect the reproductive behaviour and efficiency of animals possibly through the alteration of the reproductive hormones (Roman-Ponce *et al.*, 1981). Various scientists have reported that heat-stressed animals undergo different changes like elevated rectal temperature (McGuire *et al.*, 1989), depressed appetite (McDowell, 1972), reduced milk production (Fuquay, 1981) and low reproductive efficiency (Badinga *et al.*, 1985).

The change in body temperature has been listed as an indicator of heat stress, which directly or indirectly may change the hormonal profile of the animals causing the silent and missed periods of oestrus, prolonged or short oestrous cycles (Fuquay *et al.*, 1970) and early embryonic mortality (Ulberg and Burfening, 1967). A previous study (Younas *et al.*, 1992) has also shown an improved estrual response due to longer adjustment period. The objective of this study was to determine the concentrations of cortisol and estradiol-17β under the heat stress conditions if animals are given extended adjustment periods in hot and humid conditions of Mississippi.

MATERIALS AND METHODS

Animals and design of experiment: The experiment was conducted using fourteen

nonpregnant lactating Holstein cows during the month of July, 1989 at the Bearden Dairy Research Centre at Mississippi State University, Mississippi, USA. All the animals were checked for a normal oestrous cycle and were housed in a section of a freestall barn, with or without overhead ceiling fans, for a complete oestrous cycle. Each section contained freestalls with sand bedding, a self-waterer and feed mangers with self-closing head gates. The barn possessed an insulated roof with peak ventilation. Milking time, feeding regime and other managemental aspects were unaltered. Animals were only removed from the barns for twice-daily milkings at 0330 h and 1500 h. A total mix ration of 20% protein was fed in the barns *ad libitum*.

Using the completely random design, the animals were assigned to their respective treatment pens with an overhead fan (fan) or without fan (Control). One animal in the control group had to be removed because of an injury. Twenty-one days of adjustment period was provided before the animals were inserted with intravenous catheters (Angiocath, 14 gauge x 5¼ in, Desert Medical, Inc., Sandy, UT) in their jugular veins. On the same evening at 1800 h the first blood sample was drawn and 25 mg prostaglandin (PG)F2α (Lutalyse^R, The Upjohn Co. Kalamazoo, MI) was injected to each cow. All were judged to be in their luteal phase of their cycles based on previous observations and palpation of ovaries. This luteal phase was confirmed by high progesterone concentration at zero hours. From 0 to 36 h after PGF2α, blood samples were taken at 6 h intervals; 36 to 88 h at 4 h intervals and three times weekly (MWF) at 1400 h thereafter until the end of the oestrous cycle. Animals were observed for oestrus daily for about 30 minutes early morning and late afternoon each day alongwith other observations performed before each bleeding. Additional observations were provided by the staff and milking personnel.

A Hygrothermograph (Model H 311, Weather Measure Corporation, Sacramento, CA) was used to collect the ambient temperature and relative humidity data in the research barns. Animals comfort indices were determined by calculating Temperature-Humidity Index (THI) using the formula [THI = Temp. - 0.55 (1 - RH) (T - 14)] as described by Lutgens and Tarbuck (1982). Temperature-Humidity Index has usually been used by the U.S. Weather Bureau and other researchers to give a numerical indication of human as well as animal comfort based on the conditions of temperature and humidity. Rectal temperatures were taken before each bleeding by a high speed, electronic, rectal thermometer (M211, GLA Agricultural Electronics, San Louis Obispo, CA).

Blood collection and analysis: A 10 ml blood sample was drawn at each bleeding after discarding the first 2 to 3 ml containing the anticoagulant. Sodium citrate (3.5%) as an anticoagulant was used between the bleedings to fill the catheters. Some samples towards the end of the experiment were collected from the tail (coccygeal artery or vein) when indwelling cannulae stopped working. Blood collection tubes (Vacutainer^R 6480, Becton Dickinson, Rutherford, NJ) were used for the collection of samples and were immediately placed in an insulated box containing crushed ice. Samples were taken to the laboratory, refrigerated for few hours and were centrifuged (Model CRU-5000, Damon/IEC Division, Needham Heights, MA) for 20 minutes at 1200 x g at 4 ° C. The serum was then pipetted off, placed in labelled vials and frozen at -20 ° C until the time of assay.

Cortisol assay: For cortisol determination a commercial kit "Animal Cortisol Radioimmunoassay (RIA) Procedure" (Cambridge

Medical Technology Corporation, 575 Middlesex Turnpike, Billerica, MA) was used. The kit primarily developed for *in vitro* diagnostic testing of cortisol, has been validated for serum and plasma levels of cortisol. Separation of the bound and free radiolabelled antigen was accomplished by aspirating the liquid in the tubes. The quantity of unknown unlabelled antigen was then determined by comparing the radioactivity in the tubes containing known standards in the assay system. Anti-cortisol coated yellow polypropylene ready to use tubes (12 x 75 mm) and ready made standards were provided with the kit. Controls coated with preservatives for the assay were provided with the kit. Their original concentrations were diluted and gave the values within the expected ranges.

Radioactive cortisol tracer ^{125}I-labelled in buffer, containing bovine serum albumin (BSA) and a displacing agent (4.5 μCi ^{125}I, 110 ml/vial) was used. Before the start of the actual assay, the standards were tested and parallelism was observed. The samples were assayed in duplicate. Anti-cortisol serum tubes were labelled, 25 μl of cortisol standard and unknowns were added to the bottom of the respective tubes. Every tube then received 1000 μl ^{125}I, which were vortexed and incubated for overnight at room temperature (25 ° C). This overnight incubation was modified and validated in our laboratory. With the exception of total count (TC) tubes, the liquid was aspirated and radioactivity was counted for one minute in Gamma Counter (Model 4/200, Micromedic Systems, Inc., Horsham, PA). Counts per minute (CPM) were merged in the RIA programme in the laboratory and the values for the samples were calculated from log-logit value procedure. The average sensitivity of the cortisol assays was 0.93 ng/ml. The intraassay coefficient of variation was 5.45%, while the intraassay coefficient of variation was 5.88%.

Estradiol assay: The radioimmunoassay procedure described by Cox *et al.* (1986) was used to determine the concentration of estradiol-17β in serum samples. A single extraction was done in duplicates from serum samples (500 μl) using 4 ml ethyl acetate and evaporated extracts were measured. Values were corrected for extraction losses and the average percentage recovery for estrogen was 88% after addition of 3,000 CPM labelled hormone, E2-6 [0-carboxymethyl]-oximino 2-[^{125}I] iodohistamine with a specific activity of 2,000 Ci/mmol (Amersham Corp., Arlington Heights, IL). Approximately 12,000 CPM of ^{125}I-Estradiol-17β in 100 μl of buffer was added to each tube. Estradiol standards, 17β-estradiol E8875, (Sigma chemical Co., St. Louis, MO) were used in each standard curve. The antiserum, E2 Rabbit Antiserum E2-6 # 3, lot 0403-061873, (Eli Lilly Co., Indianapolis, IN) was used at a 1:1,000,000 dilution in 100 μl.

A solution of dextran-coated charcoal (0.04% dextran, 0.4% activated charcoal in phosphate-buffered saline) was used for separation of bound and free hormone after a 24 h incubation at 4 ° C. The tubes were centrifuged at 2500 x g for 20 minutes. The supernatant was counted in Gamma Counter. The estradiol concentrations in the samples were calculated using a log-logit transformation of the standard curve. The intraassay coefficient of variation was 19.81%, while the intraassay coefficient of variation was observed as 13.21% in this assay. Average sensitivity of the assays was 0.25 pg/ml.

Statistical analysis: The general linear model (GLM) for the analysis of variance (SAS, 1990) was used. Cortisol values were analysed using GLM procedures as a split-plot design in time within treatments. The effects of fan group were tested with error

term in animals within fan group. Estradiol-17β values were divided into pre-oestrus and post-oestrus values (15 d after oestrus) until the next observed oestrus. Timing of the pre-ovulatory surge of LH was used to identify the pre-oestrus and post-oestrus values, in the animals which did not exhibit oestrus. One-way analysis of variance was used to analyse the estradiol data. Split-plot analysis over time was used to analyse the rectal temperature and THI data. The least significant difference or Student-Newman-Keuls (SNK) tests (SAS, 1990) were used for mean comparison where significant differences were found.

RESULTS AND DISCUSSION

Ambient temperature and humidity: During the course of study, the ambient temperature and relative humidity in the research barns were recorded. Ambient temperature ranged from 22.20 to 36.11 °C, relative humidity from 45 to 100% and THI from 21.95 to 32.80 during the experiment. Mean values for ambient temperature, relative humidity and THI are shown in Figure 1.

The mean diurnal ambient temperature ranged from 25.84 ± 0.27 °C (0400 h) to 31.24 ± 0.41 °C (1600 h) and relative humidity from 70.24 ± 2.32% to 94.56 ± 0.71%

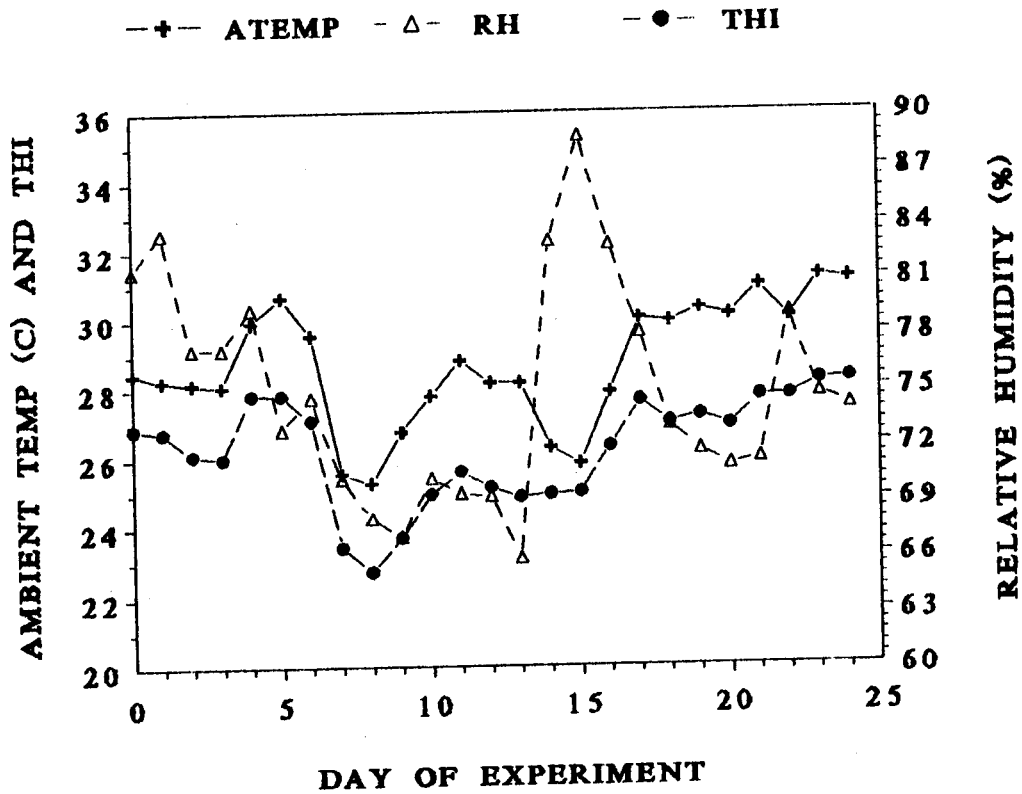

Fig. 1. Average daily ambient temperature (ATEMP), relative humidity (RH) and temperature-humidity index (THI) of the barns throughout the experiment.

at 1600 h and 2400 h, respectively. Temperature-humidity indices ranged from 25.47 ± 0.27 (0400 h) to 28.38 ± 0.35 (1600 h) in the experimental barns; ambient temperature rose from 0800 h until 1600 h with relative humidity dropping during that period. Relative humidity increased at night when ambient temperatures were dropping. Temperature-humidity indices increased slowly from 0400 h to 1600 h and then dropped slowly for the remainder period at night.

Many researchers have suggested a reduction in fertility during the periods of high ambient temperatures (Monty and Wolff, 1974) but most deleterious effects occur shortly prior to and immediately after breeding (Dutt, 1964). Ambient temperatures affect the reproductive efficiency of the cattle by altering the oestrous cycle (Fuquay, 1981). When ambient temperature increases above 25 ° C, the average length of the oestrous cycle may increase to as much as 25 days, with a range of 19 to 45 days (Gangwar *et al.,* 1965). Tucker (1982) also reported in a review that ambient temperatures above 27 ° C lengthen the oestrous cycle, decrease duration and intensity of oestrus, decrease fertility and increase embryonic mortality in dairy cattle.

Ambient temperatures above the thermoneutral zone of European cows (28 ° C) drastically reduce the conception rates (Fuquay, 1981). In Arizona, Wise *et al.* (1988b) reported a high correlation between breeding efficiency and climatic variables. Similar results with high correlation to breeding failure at maximum temperature 2 d prior to breeding or on the day of breeding (Gwasdauskas *et al.,* 1975) have been shown in dairy cows. Keeping this critical time in view, short term cooling of dairy cows for 1 to 6 d after breeding increased the breeding efficiency in heat-stressed cows by 35% as compared to 58% in controls (Stott and Wiersma, 1976). Several researchers (Ulberg and Burfening, 1967) reported that the deleterious effects of high ambient temperature do not occur if body temperatures of the cows do not increase. Therefore, keeping body temperatures of the cows near normal will help to eliminate decreased fertility rates.

Higher relative humidity accentuates the effect of high ambient temperatures by impairing the heat loss system of the animals (Fuquay, 1981). Some researchers have reported that animals suffer more heat-stress during the afternoon because of high THI (Vincent, 1972). The resulting index is based on the fact that values in excess of 25 indicate that most animals will feel uncomfortable, while a THI between 15 and 20 is considered within the comfort zone (Maunder, 1970). The temerature-humidity index in our study had a diurnal range of 21.95 to 32.80 with all points above the comfort zone, as was indicated by elevated rectal temperatures. The increase in relative humidity is an important factor in lowering the breeding efficiency in August in certain areas compared to months with even higher temperatures.

Rectal temperature: Diurnal rectal temperatures during the first 88 h after PGF2α ranged from 38.85 to 39.70 ° C in control group and 38.57 to 39.42 ° C in fan group. Diurnal rectal temperatures tended to be lower in early morning but higher at 1400 h and 1800 h. The diurnal rectal temperatures were lower in fan group and also cooled down faster at night in fan as compared to control cows. rectal temperatures recorded at all 1400 h observations are shown in Figure 2 which shows that rectal temperatures in control cows were significantly higher ($P<0.05$) than fan cows for the entire experiment. Mean average rectal temperature recorded across the experimental period ranged from 37.83 to 41.22 ° C with a mean

of 39.08 ± 0.03 °C for the fan group and 38.44 to 41.05 °C with a mean of 39.45 ± 0.03 °C in the control group.

(Berman *et al.*, 1985) or a combination of sprinklers and fans (Flamenbaum *et al.*, 1986 and Her *et al.*, 1988).

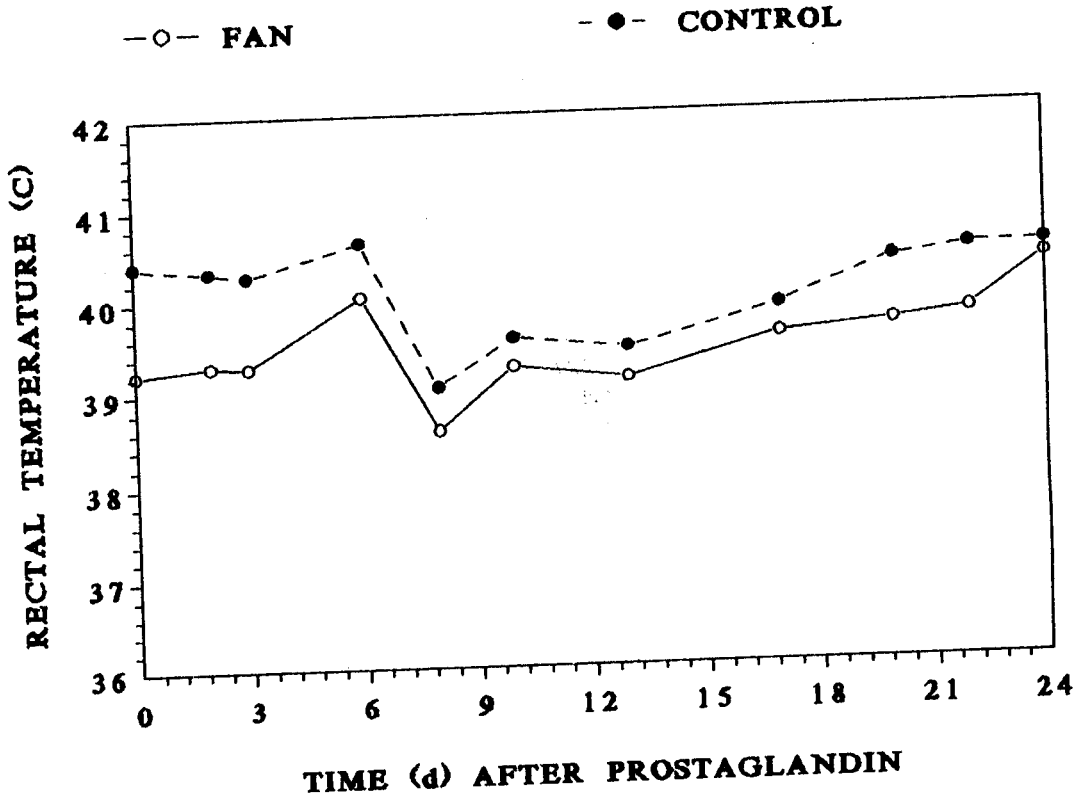

Fig. 2. Average rectal temperature (RTEMP) of fan and control group animals taken at 1400 h daily.

The forced ventilation provided by the ceiling fans decreased the rectal temperature (0.37 °C) in the fan group as compared to controls. The lower rectal temperatures for the fan group in this study are similar to those reported earlier for lactating dairy cows under fans (Gonzalez *et al.*, 1981 and Younas, 1988) and were more pronounced than those observed with simple cross ventilation (Fuquay *et al.*, 1979). Similar reductions in rectal temperatures have been reported at other stations from the use of fans

Higher rectal temperatures have been reported for dairy cows near midnight than during midday (Roman-Ponce *et al.*, 1977). Minimum rather than maximum ambient temperatures are more important as hormonal indicators of physiological stress (Fuquay *et al.*, 1979). Fuquay (1981) reported in his review that night temperature, though coolest in the diurnal temperature cycle, may affect productivity also when it fails to drop into the thermoneutral range. This would seem to be a particular problem

when high humidity reduces heat loss by radiation and evaporation.

nulae were removed and the animals faced a new style of bleeding.

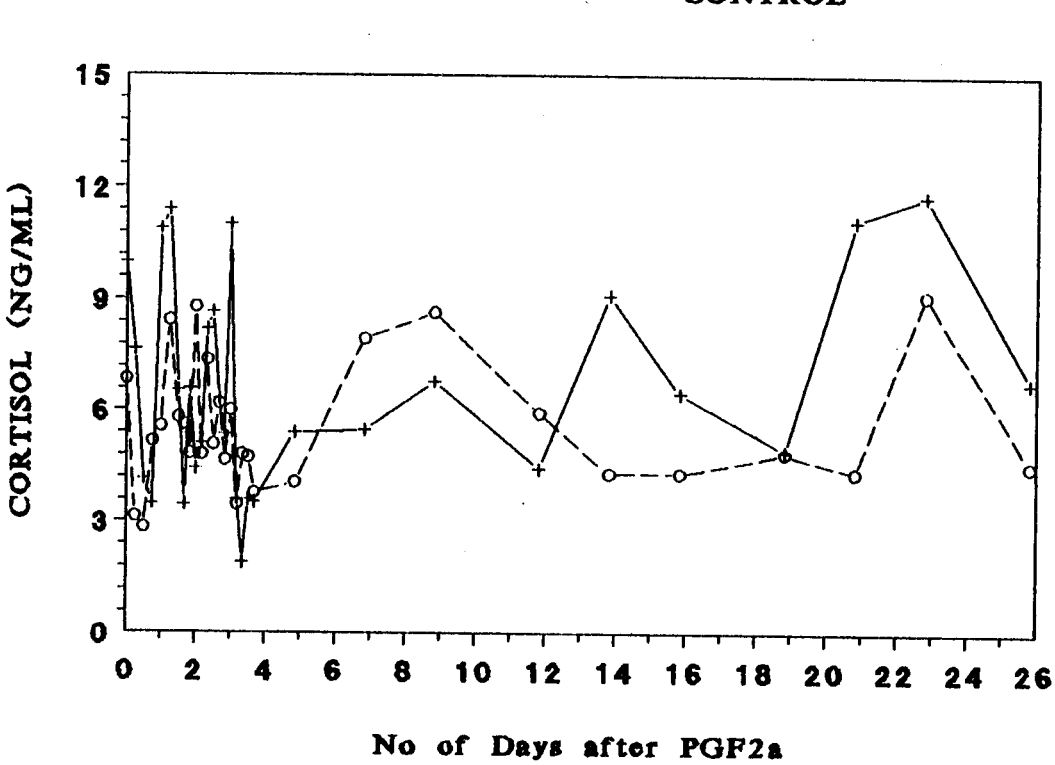

Fig. 3. Average serum concentrations of cortisol in fan and control groups.

Cortisol: Average serum cortisol values are presented in Figure 3. Serum cortisol concentrations for the fan and control groups were 6.60 ± 0.58 ng/ml and 5.53 ± 0.59 ng/ml, respectively. No significant differences were observed in serum cortisol concentrations in fan and control groups ($P<0.05$). Cortisol values showed no diurnal pattern and fluctuated considerably throughout the study. A small increase was observed in some animals towards the end of the bleeding schedule, however, this was not seen in the mean values. The increase near the end could be due to the tail bleeding through the coccygeal artery after can-

Under varying physiological conditions it is difficult to accurately assess adrenal function due to variability in basal glucocorticoid levels (Gwazdauskas and Vinson, 1979). However, Abilay and Johnson (1973) in a study of Guernsey heifers, whose oestrous cycles were heat synchronised under two controlled conditions (18.2 °C, 55% RH and 33.5 °C, 55% RH), reported decreased values of mean plasma cortisol. Decreased cortisol levels have also been reported by Abilay *et al.* (1975) and Roman-Ponce *et al.* (1981). These decreases have been implicated as causative hormonal agents in the negative influence that heat stress has on

reproductive efficiency. Similarly, Barb *et al.* (1991) who studied the cortisol changes in sows maintained in environmental chambers, found suppressed serum cortisol values in heat-stressed sows (30 ° C) as compared to those maintained in the control chamber (22 ° C). Such suppression of cortisol may be due to the lack of responsiveness of the hypothalamic-pituitary-adrenal axis by exposure to evaluated ambient temperature (Seren *et al.*, 1988).

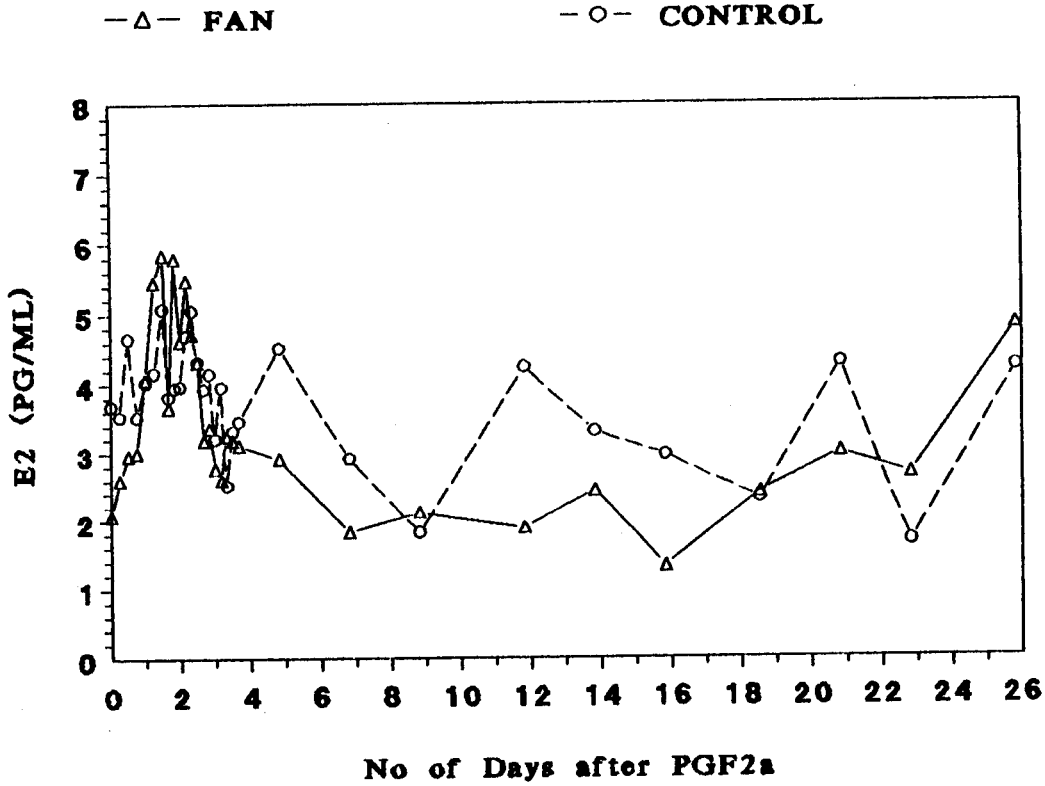

Fig. 4. Average serum concentrations of cortisol in fan and control groups.

Wise *et al.* (1988a & 1988b) got contrasting results in two experiments. In the first study, they found higher serum cortisol concentrations in heat-stressed cows as compared to cows maintained under cooling. Yet in another study, they reported no difference in serum cortisol values between cooled and control cows. Stott and Robinson (1970) reported a short-lived surge in cortisol and progesterone followed by a return to basal values. These studies suggest that heat stress does not appear to evoke the classic stress-induced cortisol response and therefore, may not be directly involved in the etiology of the impaired reproductive efficiency that is seen during the heat stress.

Estradiol-17β: Mean values for pre-oestrus and post-oestrus estradiol-17β were 3.86 ± 0.42 pg/ml and 2.36 ± 0.63 pg/ml for the fan group and for the control group were 4.52 ± 1.09 pg/ml and 2.88 ± 0.64 pg/ml. Estradiol-17β concentrations were compared

with the pre-oestrus and 15 d post-oestrus values in each treatment. The average concentrations for each group are presented in Figure 4. Pre-oestrus values in fan group ranged from 0.28 to 10.33 pg/ml and from 0.62 to 13.34 pg/ml in the control group. Post-oestrus values ranged from 0.25 to 15.26 pg/ml in the fan and 0.32 to 13.02 pg/ml in the control groups. Both pre-oestrus and post-oestrus values tended ($P<0.10$) to be higher in the control than in the fan group.

While concentrations between cows were quite variable, neither a measured pre-ovulatory surge of LH nor detected oestrus appeared related to this variation. Except for one cow in the fan group, peak concentration during the 88 h period after PGF2α was as high or higher than values usually reported during oestrus (Wise *et al.*, 1988a). In another similar study, they reported that cooling cows in summer did not affect estradiol-17β concentration during the oestrous cycle (Wise *et al.*, 1988b), which supported our observations. Based on these studies, heat stress-related changes in the oestrous cycle do not appear to be related to follicle growth as monitored by estradiol values. Reduced estrogen secretion has been observed in heat-stressed pregnant dairy cows (Collier *et al.*, 1982). However, estradiol values have been observed to be higher in heat-stressed cows ($P<0.05$) during the 36 h preceding the onset of oestrus (Folman *et al.*, 1983).

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