Effect of climatic conditions on peripheral concentrations of LH, progesterone and oestradiol-17 β in high milk-yielding cows

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Summary. In a subtropical climate, high milk-yielding dairy cows were kept during the summer under ventilated conditions or untreated; during the winter, cows were kept untreated. The afternoon mean rectal temperature for multiparous cows in the three groups was 39.3, 39.8 and 38.9°C, respectively. Each group was significantly different from the other two ($P < 0.05$). Plasma LH concentrations measured every 6 h during the oestrous period in 38 'summer' cows were not significantly different for untreated and ventilated animals. Conception rate was higher ($P < 0.05$) in cows that showed oestrous behaviour before the LH surge reached its peak than in cows in which oestrus coincided with or occurred later than the LH surge. Plasma progesterone levels measured in 62 cows during the oestrous cycle before the first insemination were higher in the winter than in the summer in multiparous, but not in primiparous, cows. Ventilation increased progesterone levels in multiparous and primiparous cows. Plasma oestradiol-17 β levels did not differ between groups until 36 h before the onset of oestrus, when they remained at 4.75 pg/ml in winter and summer-ventilated cows but increased to 6.75 pg/ml in summer untreated cows ($P < 0.01$). Significant negative correlations were found between oestradiol levels observed 12 h before to 12 h after the onset of oestrus and plasma progesterone concentration during both the preceding and the subsequent oestrous cycles.

Introduction

Heat stress has been shown to reduce the fertility of dairy and beef cows (Stott & Williams, 1962; Dunlap & Vincent, 1971; Ingraham, Gillette & Wagner, 1974; Thatcher *et al.*, 1974; Monty & Wolff, 1974; Rosenberg, Herz, Davidson & Folman, 1977). Plasma concentrations of progesterone and LH in heat-stressed cows have been monitored under various experimental and physiological conditions (Madan & Johnson, 1973; Miller & Alliston, 1974; Abilay, Johnson & Madan, 1975; Rosenberg *et al.*, 1977; Wolff-Vaught, Monty & Foote, 1977). However, little information is available on the effects of exposure to elevated ambient temperatures on levels of oestradiol-17 β in peripheral plasma.

The degree of heat stress and its effect on plasma progesterone and LH concentrations is influenced by the length of exposure (Abilay, Johnson & Madan, 1975) and by milk production (Wolff-Vaught, Monty & Foote, 1977). It seemed interesting therefore, to monitor the concentrations of LH, progesterone and oestradiol-17 β in the plasma of high milk-yielding

dairy cows exposed to subtropical summer conditions and to compare them with the values obtained in winter. A further aim of the study was to examine whether forced ventilation, which has been shown to have a beneficial effect on conception during the summer (Folman *et al.*, 1979b) would affect the concentrations of the reproductive hormones.

Materials and Methods

The study involved 119 high milk-yielding primiparous and multiparous dairy cows that calved during May–August ('summer') or during November–December ('winter'). Mean bodyweights recorded 3 days after parturition were 506 ± 8 kg for primiparous and 610 ± 8 kg for multiparous cows. Data relating to the 'summer' cows were collected during July–September, and those for the 'winter' cows during December–March.

During July–September, the mean minimum and maximum ambient temperatures were 21.6 ± 0.2 and $33.6 \pm 0.2^\circ\text{C}$, respectively, and the mean minimum and maximum relative humidity levels were 36 ± 1 and $78 \pm 1\%$. During the December–March period, the mean minimum and maximum ambient temperatures were 9.8 ± 0.4 and $19.3 \pm 0.5^\circ\text{C}$, respectively, and the mean minimum and maximum relative humidity levels were 47 ± 1 and $73 \pm 1\%$.

The summer cows, paired according to number of previous lactations and milk yield, were allocated to Group SC (control) or Group SV (ventilated). The winter cows were kept in one group (Group W). The cows were kept in an open shed divided into two parts by a raised concrete surface. Forced dry ventilation was maintained on one side of the shed by fans creating an air velocity of 1.5–3 m/sec. Forced ventilation was operated from 05:30 to 22:00 h, between 1 July and 31 October. The cows were fed 8 times daily and food, including concentrates, was continuously available in the mangers. The mean 122-day milk yields of the multiparous cows were 4183, 4416 and 4538 kg/cow (s.e.m. ± 106 kg) in Groups SC, SV and W, respectively.

Rectal temperatures were measured every 14 days at 05:30 h and 16:00 h. Cows were observed for behavioural signs of oestrus 4 times daily, at 6-h intervals, each observation lasting 30 min. Cows were artificially inseminated, beginning at 60 days *post partum*, when oestrus was detected. Oestrus was defined as the time during which an animal stood when mounted by another cow. All cows were inseminated twice within the oestrous period, <12 and 24 h, respectively, after the first detection of oestrus. Pregnancy was diagnosed by palpation at 45 days after insemination. Details on ambient and body temperatures, feeding, body weight, milk yield, management of reproduction and fertility have been published elsewhere (Folman *et al.*, 1979b).

Plasma progesterone concentrations were determined three times per week in 33 multiparous and 29 primiparous cows, during the oestrous cycle preceding the first insemination. The concentration of LH was determined in 38 cows and that of oestradiol-17 β in 36 cows (from all groups) every 6 and 12 h, respectively, during the oestrous period.

Progesterone and LH assays

Plasma progesterone and LH were measured by radioimmunoassays used routinely in our laboratory. The source and the specificity of the antisera and the method and efficiency of extraction did not differ from those described previously (Rosenberg *et al.*, 1977; Folman *et al.*, 1979a). For the progesterone assay, within- and between-assay coefficients of variation were 8.1 and 18.8%, respectively. All the LH determinations were performed in a single assay which had an intra-assay coefficient of variation of 14.8%. The sensitivities were <0.1 ng progesterone/ml and 2 ng LH/ml.

Oestradiol-17 β assay

Oestradiol was extracted from 4 ml plasma by 4 ml freshly distilled diethyl ether. The mean extraction efficiency, determined by recovery of labelled oestradiol, was 88.5%. Oestradiol was

assayed by a radioimmunoassay kit supplied by Isodan Ltd (Diagnostic Laboratories, Jerusalem, Israel). The antiserum supplied with the kit was produced in rabbits immunized with oestradiol-17 β -6-CMO-BSA. The antiserum was specific, the cross reactivity being 0.2% for oestrone and oestriol, 0.015% for cortisol and 0.0012% for testosterone (oestradiol-17 β = 100%). The [2,4,6,7-³H(n)]oestradiol-17 β (sp. act. 85–100 Ci/mmol) was diluted in buffer so that a 1:120 000 dilution of antiserum bound 48–55% of the labelled hormone. Incubation of the samples with the antiserum and with [³H]oestradiol was performed at room temperature on 3 ml Sephadex columns which were supplied with the kit. The samples were preincubated with the antiserum for 20 min, and then the radioactive oestradiol was added and the reagents were allowed to equilibrate for another 20 min. The antibody-bound oestradiol was then eluted from the columns directly into counting vials, while the free steroid was retained on the columns.

The minimum sensitivity of the assay was 3.0 pg/sample, i.e. 0.75 pg/ml plasma. The percentage recovery of 15, 30 and 60 pg crystalline oestradiol added to 4 ml plasma from an ovariectomized cow was 108 ± 8.3 , 93 ± 4.8 and $78 \pm 3.7\%$, respectively. The intra- and interassay coefficients of variation were 6.8 and 15.5%, respectively, as determined from three reference serum samples which were run in duplicate with each assay.

Statistical analysis

Data on hormone concentrations and rectal temperatures were processed by analysis of variance followed by Duncan's multiple range test. The standard error of the mean (s.e.m.) for each variable was calculated from the mean square error value. Linear correlation coefficients were calculated between concentrations of different hormones. The χ^2 test was used to evaluate differences in conception rate.

Results

Rectal temperature and conception rate

At 16:00 h the mean rectal temperature of multiparous cows in Groups SC, SV and W was 39.8, 39.3 and 38.9°C $\pm$ 0.05, respectively (means significantly different from each other, $P \leq 0.05$). In primiparous cows the mean rectal temperatures were 39.6, 39.4 and 38.9°C $\pm$ 0.06, respectively (means significantly different from each other, $P \leq 0.05$). At 05:30 h, rectal temperatures differed only slightly between the groups. Conception rates of the multiparous cows in Groups SC, SV and W were 22, 52 and 80%, respectively (groups significantly different from each other, $P \leq 0.05$). In primiparous cows the conception rates of the three groups were 50, 35 and 72%, respectively (N.S.) (Folman *et al.*, 1979b).

Table 1. LH concentrations (mean $\pm$ s.e.m., ng/ml) in plasma of 'summer' cows during the oestrous period at which the first insemination was performed

Group	No. of cows	Time relating to first observation of standing oestrus		
		−6 h	0 h	+6 h
Multiparous cows				
Control (SC)	9	3.1 ± 1.5	11.8 ± 4.7	15.3 ± 4.3
Ventilated (SV)	13	3.5 ± 0.7	5.8 ± 1.1	8.1 ± 2.2
Primiparous cows				
Control (SC)	7	4.4 ± 0.6	8.6 ± 3.2	15.7 ± 4.9
Ventilated (SV)	9	6.8 ± 1.4	7.5 ± 1.4	12.6 ± 5.8

Plasma LH concentration

The highest LH concentrations, measured during the oestrous period, occurred between 6 h before and 6 h after the onset of oestrus and are presented in Table 1. The peak concentration appeared 6 h before onset of oestrus in 4 of the cows (11%), at the onset of oestrus in 18 cows (47%) and 6 h following the onset of oestrus in the other 16 cows (42%). When the relationship between conception and the time interval from the beginning of oestrus to the LH peak was considered, the number of fertile inseminations was higher in cows in which standing oestrous behaviour had started 6 h before the LH surge reached its peak (11/16) than in those in which onset of oestrus coincided with or occurred after the LH peak (7/22: $\chi^2 = 4.64$, $P \leq 0.05$). Of the cows that did not conceive, 5 showed oestrus before the peak and 15 at or after the peak.

Plasma progesterone concentration

The plasma progesterone concentrations during the oestrous cycle preceding the first insemination are presented in Text-fig. 1. It can be seen that throughout the cycle the level of progesterone in multiparous cows is higher during the winter than during the summer, with that of cows in Group SV being intermediate between those of Groups SC and W. In primiparous cows Group W cows had a higher progesterone level than did Group SC cows during Days 2–3 of the cycle, a similar level during Days 4–9 of the cycle and a lower level during the rest of the luteal phase of the cycle. As in multiparous cows, ventilation tended to increase the progesterone concentrations in summer cows.

Text-fig. 1. Mean plasma progesterone concentrations in multiparous and primiparous cows during the oestrous cycle before the first insemination. The standard errors are indicated above the curves. *Significantly different from value in Group SC, $P < 0.05$. **Significantly different from values for Groups SC and SV, $P < 0.025$.

Plasma oestradiol-17 β

The average concentration of oestradiol at any time before, at or after oestrus did not differ significantly between primiparous and multiparous cows nor between Groups SV and W; the

data from these cows were therefore pooled. The results in Text-fig. 2 indicate that plasma oestradiol-17 β concentrations increased slowly in all groups from 60 to 36 h before the onset of oestrus. During the 36 h preceding oestrus, oestradiol concentrations in cows in Groups SV and W remained at the level of 4.75 pg/ml. In Group SC cows, however, oestradiol-17 β levels continued to increase, reaching a peak of 6.75 pg/ml at the onset of oestrus ($P \leq 0.01$).

Text-fig. 2. Plasma oestradiol-17 β concentrations (mean $\pm$ s.e.m.) during 60 h before and 24 h following the onset of oestrus in cows. * $P \leq 0.05$; ** $P \leq 0.005$, compared with the other group.

In the 12 h following the onset of oestrus, oestradiol-17 β levels decreased to approximately 2.75 pg/ml in Groups SV and W and 3.5 pg/ml in Group SC, with a further decrease during the next 12 h.

A significant negative correlation was observed, in multiparous cows, between the oestradiol-17 β levels around the onset of oestrus and the progesterone concentrations during the luteal phase of the preceding cycle (Table 2). No such correlation was found in primiparous cows.

Table 2. Correlation coefficients (r) between the plasma concentration of oestradiol-17 β during oestrus and the concentration of progesterone during the preceding oestrous cycle in 21 multiparous cows

Time at which oestradiol was evaluated	Days of cycle		
	0-7	8-14	15-21
12 h before oestrus	-0.50*	-0.42	-0.25
At the onset of oestrus	-0.19	-0.48*	-0.29
12 h after onset of oestrus	-0.52*	-0.55**	-0.25

* $P \leq 0.05$; ** $P \leq 0.01$.

In cows of the winter group, but not those of the summer group, a highly significant negative correlation was found between oestradiol-17 β concentrations 12 h after the onset of oestrus and progesterone concentrations on Days 10-13 of the following cycle (Table 3). All these cows had been inseminated during the oestrous period in which oestradiol-17 β was measured, but only 5 of the 8 cows conceived. For the 5 pregnant cows, the correlation coefficient between

oestradiol-17 β concentrations 12 h after onset of oestrus and progesterone concentrations on Days 12–13 following insemination was $r = -0.83$ ($P \leq 0.05$).

Table 3. Correlation coefficients (r) between the plasma concentration of oestradiol-17 β during oestrus and the concentration of progesterone after oestrus in 8 cows of the winter group

Time at which oestradiol was evaluated	Days after oestrus		
	8–9	10–11	12–13
12 h before oestrus	–0.58	–0.60	–0.89**
At the onset of oestrus	–0.65*	–0.46	–0.53
12 h after onset of oestrus	–0.74*	–0.95**	–0.90**

* $P \leq 0.05$; ** $P \leq 0.01$.

Discussion

The time at which the LH peak occurred in the present study, in relation to the onset of oestrus, is similar to that reported in other studies (Henricks, Dickey & Niswender, 1970; Christenson, Echternkamp & Laster, 1975; Schams, Schallenger, Hoffmann & Karg, 1977). In cows that conceived at the first insemination, the LH peak appeared later, relative to the onset of oestrus, than in cows that did not conceive. However, no correlation was found between the interval from insemination to the LH peak and the outcome of insemination.

As in our previous study (Rosenberg *et al.*, 1977), the results of the present study indicate that progesterone concentrations of multiparous cows are higher during the winter than during the summer. Furthermore, during the summer, forced ventilation that significantly decreased body temperature tended to increase plasma progesterone concentrations in multiparous and primiparous cows (Text-fig. 1). In primiparous cows progesterone concentrations during the second half of the oestrous cycle were higher in summer than in winter. The effect of summer conditions on progesterone concentrations in primiparous cows resembles the results of Abilay *et al.* (1975), who reported increased plasma progesterone levels in heifers exposed to heat stress in climatic chambers.

The effects of different climatic conditions on plasma progesterone concentrations seem conflicting and are difficult to explain. The physiological state of the cow as well as the severity of the heat stress seem to affect progesterone levels. Wolff-Vaught *et al.* (1977) reported that, in milking cows kept under arid conditions, progesterone levels were elevated in summer compared with winter, while in non-milking cows progesterone concentrations were lower in summer. The cows in the study of Wolff-Vaught *et al.* (1977) were exposed during the summer to temperatures ranging from 30 to 47°C, while the heifers in the experiment of Abilay *et al.* (1975) were kept in climatic chambers at 35.5°C for two cycles. The mean ambient temperature range during the summer in the present study was 22–34°C and the cows were not exposed to continuous heat. It is possible that the response to constantly hot or acutely elevated temperatures is different from that to moderately or intermittently high temperatures. In the study of Abilay *et al.* (1975) progesterone levels were not elevated during the second oestrous cycle of exposure to heat, which may reflect an acclimatization effect. It is also possible that, under conditions of severe stress, some progesterone is secreted from the adrenal gland (Thatcher, 1974).

The oestradiol-17 β concentrations measured in this study were similar to the levels reported by Glencross, Munro, Senior & Pope (1973) and by Chenault, Thatcher, Kalra, Abrams & Wilcox (1975). Our results indicate that heat stress significantly increased plasma oestradiol-17 β

levels, whereas ventilation tended to keep the level low and similar to that of the winter cows. Different results were presented by Gwazdauskas, Abrams, Thatcher, Bazer & Caton (1974), who found that heifers kept in climatic chambers at a constant temperature of 32°C had lower ($P \leq 0.10$) oestradiol-17 β levels than those kept at 21.3°C.

The reason for the effect of heat stress on plasma oestradiol-17 β levels is at present unknown. Oestradiol-17 β reduces the uterine temperature in heifers (Gwazdauskas *et al.*, 1974) and raises the uterine blood flow in ewes (Rosenfeld, Killam, Battaglia, Makowski & Maschia, 1973). It has also been shown that, after an intramuscular injection of oestrogens to ewes, intraluminal uterine temperatures were decreased (Abrams, Caton, Clapp & Barron, 1970), and that conception rate could be related to uterine temperature at insemination (Thatcher, 1974). An increase in oestradiol-17 β concentrations in response to heat stress could provide a mechanism by which excess heat would be dissipated from the reproductive organs into the periphery. It may also be postulated that a suppression of basic LH secretion by heat stress, as reported by Madan & Johnson (1973), may enhance the rate of development of the preantral follicle and increase oestrogen production by the preovulatory follicle which develops from it. Results of experiments in mice and hamsters indicate that a decrease in LH concentration may enhance follicular development and induce superovulation (Terranova & Greenwald, 1981). A decrease in basic LH levels would also result in reduced luteotrophic support of the corpus luteum (Snook, Brunner, Saatman & Hansel, 1969). This could be expected to inhibit progesterone production and reduce peripheral progesterone concentration, as observed in the multiparous cows of the summer control group.

A statistically significant negative correlation was found in multiparous cows of the present study, between plasma progesterone levels at the beginning and the middle of an oestrous cycle and the plasma concentration of oestradiol-17 β on the day of oestrus at the end of that cycle (Table 2). A significant negative correlation between progestagen and oestradiol levels was also observed during the last 8 days of the oestrous cycle by Chenault *et al.* (1975). It may be assumed that during the first part of the cycle progesterone is inhibiting the production and/or release of gonadotrophins, thus affecting the development of follicles and their oestradiol production (Hobson & Hansel, 1972; Beck, Smith, Seguin & Convey, 1976; Convey, Beck, Neitzel, Bostwick & Hafs, 1977; Hauger, Karsch & Foster, 1977). Lower progesterone levels may be related to higher gonadotrophin levels, greater follicular development, and greater oestradiol production at the end of the cycle. The high negative correlation coefficients between oestradiol-17 β and progesterone in the subsequent cycle (Table 3) may suggest that oestradiol is involved in, or related to, the maturation of the preovulatory follicle and the development of the ensuing corpus luteum.

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