Effect of heat stress on tonic and GnRH-induced gonadotrophin secretion in relation to concentration of oestradiol in plasma of cyclic cows

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Effects of acute and seasonal heat stress on tonic and GnRH-induced LH and FSH secretion were examined during the early follicular phase of the oestrous cycle of cows ($n = 40$). Prostaglandin $F_{2\alpha}$ was injected on day 11 ± 1 of the oestrous cycle and on the next day blood samples were collected at intervals of 15–20 min for 14 h, and i.m. injection of GnRH was given after 7 h. Treatments compared were control versus acute heat stress during blood sampling in winter, and cooled versus chronic heat stress in summer. Before GnRH injection, chronic heat stress in summer did not affect basal concentrations of plasma LH, but did lower LH pulse amplitude. However, in cows with low plasma oestradiol (1.9 ± 0.2 pg ml $^{-1}$), the mean and basal concentrations and amplitude of tonic LH pulses were reduced by heat stress (3.1, 2.1 and 4.8 versus 1.9, 1.4 and 2.5 ng ml $^{-1}$, respectively). In cows with high plasma oestradiol (6.3 ± 0.5 pg ml $^{-1}$), these parameters were not affected. In chronically heat stressed cows in summer, GnRH-induced increases in plasma LH and FSH concentrations were the same as in the cooled controls. However, in cows with low plasma oestradiol, mean concentrations of FSH in plasma (31.8 versus 25.5 ng ml $^{-1}$), the peak of the GnRH-induced FSH and LH surge (FSH 47.4 versus 35.6 ng ml $^{-1}$, LH 50.7 versus 37.3 ng ml $^{-1}$) and the shape of the GnRH-induced FSH and LH curves (treatment by time interaction) were significantly lower in non-cooled versus cooled controls. The GnRH-induced increase in LH secretion was unaffected by chronic heat stress in cows with high concentrations of oestradiol in plasma. In winter, acute heat stress depressed the mean concentration of FSH in plasma and decreased the GnRH-induced release of FSH in cases with low but not with high concentrations of oestradiol in plasma. The peak of the GnRH-induced surge of LH in all acutely heat stressed cows was significantly lower in winter than in control cows, irrespective of concentrations of oestradiol in plasma. These results show that heat stress affects the secretion of gonadotrophins more in cows with low concentrations of oestradiol than in those with high concentrations of oestradiol in plasma.

Introduction

The low fertility of dairy cattle in summer in subtropical climates is well known. Conception rates in Israel decrease from 52% in winter to 24% in summer (Ron *et al.*, 1984), and similar values have been recorded in southern USA (Monty and Wolf, 1974). Fertility has been found to be sensitive to heat stress during the 12 days before oestrus, especially on day 2 before oestrus (Ingraham *et al.*, 1976), the day after oestrus (Badinga *et al.*, 1985), and days 1–7 after artificial insemination (Putney *et al.*, 1989). Cooling cows from days 0 to 8 after oestrus was found to increase conception rates in one study (Stott and Wiersma, 1976), but not in another (Her *et al.*, 1988). Relieving heat stress in summer, from day of calving until 150 days of lactation, increased conception rates to 59%, compared with 17% for non-cooled controls (Wolfenson *et al.*, 1988).

The role of gonadotrophins in the regulation of functions of follicles and the corpus luteum in cattle is well documented (Ireland, 1987). At the beginning of the follicular phase, gonadotrophin secretion increases in a pulsatile pattern and follicular development accelerates until the preovulatory surges of LH and FSH and ovulation. (Ireland and Roche, 1982; Dielman *et al.*, 1986). Disturbances in gonadotrophin secretion during the follicular phase may impair follicular development and ovulation, and the function of the subsequent corpus luteum (McNatty *et al.*, 1981; Quirk and Fortune, 1986).

The effect of heat stress on LH secretion during the follicular phase of the oestrous cycle in cattle is unclear. Concentrations of LH in plasma have been reported to be unchanged (Gwazdauskas *et al.*, 1981; Gauthier, 1986), increased (Roman-Ponce *et al.*, 1981) or decreased (Madan and Johnson, 1973) by heat stress. These discrepancies may be associated with differences in sampling frequency, which varied from once a day to once every 3 h, and depended on whether heat stress was acute or chronic. To the best of our knowledge, the effects of heat stress

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on FSH secretion have not been reported for cows or other mammals.

The work reported here examined the effects of acute and chronic heat stress on tonic and GnRH-stimulated gonadotrophin secretion in lactating cows.

Materials and Methods

Animals

The study was carried out on 40 multiparous Israeli-Holstein cyclic dairy cows over 200 days in lactation in the dairy herd of Kibbutz Ketura, located in the desert, south of the Dead Sea.

Experimental protocol

The oestrous cycles of the cows were synchronized by two injections of 25 mg PGF_{2α} (Lutalyse: Upjohn, Puurs) administered 14 days apart. On day 11 ± 1 of the cycle (day 0 = oestrus), the presence of a corpus luteum was determined by rectal palpation and a blood sample was taken to determine concentrations of plasma progesterone. PGF_{2α} was administered at 10:00 h to induce regression of the corpus luteum, and a heparinized catheter (Abbocath, 16-gauge, 14 cm, Abbott Hospitals, Chicago, IL) was inserted into the jugular vein. Three out of 43 cows were excluded from the experiment because concentrations of progesterone in their plasma were below 0.5 ng ml⁻¹ on day 11, indicating absence of active luteal tissue.

Experiments started the following morning at 06:00 h (on day 12 ± 1 of the cycle), 20 h after the PGF_{2α} injection, to ensure low progesterone concentrations well in advance of the endogenous surge of LH. Blood was withdrawn at intervals of 15 min for 7 h to determine the pattern of basal LH secretion. After this, 10 µg of a GnRH analogue (Buserelin: Receptal, Hoechst, Munich) was injected i.m. to induce a gonadotrophin surge. Blood was then withdrawn at intervals of 20 min for a second period of 7 h. Food and water were supplied *ad libitum* throughout the day. Blood samples were stored on ice for up to 30 min before centrifugation (2000 g, 20 min) and plasma was stored at -20°C. Body temperatures were frequently recorded throughout that day.

Thermal treatments

Experiments were conducted over two years. Each thermal treatment within a year/season was compared with matched contemporary controls (treatments 1 versus 2, 3 versus 4, and 5 versus 6, as described below). Cows were randomly assigned to treatments in each season according to days in lactation, number of lactations and milk production. Treatment 1 (control, *n* = 11) consisted of cows kept under the mild winter conditions of the area; maximum and minimum air temperatures were 25.3 and 15.1°C, respectively, and outdoor relative humidity at peak air temperature was 35%. Treatment 2 (acute heat stress, *n* = 8) consisted of animals exposed to winter conditions as described previously, but acutely heat stressed on day 12 at 40°C and 60% relative humidity for 16 h in a climate chamber. Treatment 3 (summer cooled, *n* = 5) comprised cows

exposed to summer weather: mean maximum and minimum air temperatures and relative humidity were 39°C, 23°C and 16%, respectively, under shade. Cows were cooled five times a day for 20 min; this consisted of 1 min sprinkling and 4 min forced ventilation cycles. They could also cool themselves voluntarily under sprinklers operating along the manger from 08:00 to 20:00 h daily. The cooling systems and their impact on performance of cows were described in detail in Flamenbaum *et al.* (1986) and Her *et al.* (1988). In treatment 4 (summer shaded, *n* = 5), cows were exposed to similar summer conditions but were offered shade as the only means for heat stress relief. Treatment 5 (summer cooled, *n* = 4) was identical to treatment 3, but was carried out the following year to serve as concomitant control for treatment 6 (summer heat stress, *n* = 7). In the latter, cows were not provided with any means for heat stress relief and were also deprived of shade from 07:00 to 15:00 h. This group was thus exposed to harsher conditions than that in treatment 4. All summer treatments were applied for 4 weeks before the day of the experiment. Nutrition and management was similar throughout the two-year experiment.

Radioimmunoassay

LH was determined by double-antibody radioimmunoassay. Plasma samples (0.1 ml) were diluted in 0.4 ml of assay buffer (0.05 mol phosphate-buffered saline l⁻¹, PBS) pH 7.4 and 0.2% EDTA. Bovine LH-B7 (NIDDK, National Institute of Diabetes, Digestive and Kidney Diseases, Baltimore, MD) was iodinated using the Iodogen method (Fracker and Speck, 1978). Briefly, 20 µg LH was incubated for 4 min with 1 µCi ¹²⁵I-Na in an Iodogen-coated tube. Iodinated LH was separated from free iodine by anion exchange AG 2*8 (BioRad, Richmond, CA). Iodinated LH (100 µl, 18 000 c.p.m.), diluted in PBS containing 1% BSA, was added to the samples (100 µl) and then anti-ovine-LH-B23 (NIDDK), diluted 1:450 000 in 200 µl PBS containing 1% normal rabbit serum, was added. At this dilution, a mean of 30% maximal binding was obtained. Samples were incubated overnight at 22°C, after which 200 µl of anti-rabbit serum IgG raised in goat was added and the mixture was incubated overnight at 4°C. Bound and free LH were separated by centrifugation at 1500 g for 15 min, and the supernatant was decanted and radioactivity in the pellet was measured in a gamma counter. The standard curves ranged from 0.078 to 40 ng per tube. Mean ± SEM of measured concentrations of two known quantities of LH (34.7 and 17.3 ng ml⁻¹) in plasma samples of dry pregnant cows were 36.6 ± 2.7 and 19.5 ± 1.2, respectively, which is 5.5 and 12.4% above the expected values, respectively. Assay sensitivity was 0.1 ng and intra- and interassay coefficients of variation were 3.7 and 6.7%, respectively.

FSH was determined by radioimmunoassay carried out at Colorado State University, Fort Collins, CO, as described and validated by L'Hermite *et al.* (1972) and Akbar *et al.* (1974). Samples (pooled at intervals of 1 h) obtained after GnRH administration were used, and samples from only half the cows were used from treatment 1. Assay sensitivity was 2 ng per tube, and intra- and interassay coefficients of variation were 5.7 and 16.1%, respectively. Concentrations of plasma progesterone were determined by radioimmunoassay after petrol ether extraction (Bauminger *et al.*, 1974; Meidan *et al.*, 1990). Mean

assay sensitivity was 10 pg per tube, and intra- and interassay coefficients of variation were 5.1 and 5.6%, respectively. Oestradiol was determined by radioimmunoassay, in one assay, after di-ethyl ether extraction at the Agricultural Research Organization (Volcani Center), Rehovot (Rosenberg *et al.*, 1987). Intra-assay coefficient of variation was 13.0%. Assay sensitivity was 1.0 pg per tube.

Statistical analysis

Statistical analyses were performed separately within each season (winter or summer), owing to the different nature of the thermal treatments (short-term in winter versus long-term in summer), as well as differences in other seasonal characteristics such as photoperiod. For LH, the Pulsar Algorithmic Procedure (Merriam and Wachter, 1982) was used to calculate the overall mean, baseline (mean excluding peak values), pulse amplitude and pulse frequency before GnRH injection. Parameters were then analysed by the General Linear Model (GLM) procedures of the Statistical Analysis System (SAS User's Guide, 1985). The repeated measures routine was used to analyse LH and FSH following GnRH injection. Thermal treatment was taken as the error term. For time effect and treatment by time interaction, residual variance was used as the error term. Peak LH and FSH surges after GnRH injection were examined by one-way analysis of variance. The effects of year and interactions with treatments and time were examined to determine whether LH and FSH data from similar thermal treatments for both summers could be pooled.

Results

Body temperature

The mean maximal body temperatures of winter heat stressed, and first summer and second summer heat stressed cows were significantly higher than those of respective controls ($39.5 \pm 0.1^\circ\text{C}$ versus $38.3 \pm 0.1^\circ\text{C}$; $39.1 \pm 0.1^\circ\text{C}$ versus $38.7 \pm 0.2^\circ\text{C}$; $39.4 \pm 0.1^\circ\text{C}$ versus $38.4 \pm 0.2^\circ\text{C}$ respectively, $P < 0.01$). Body temperature of the summer heat stressed shaded group (first summer) was lower than that of the summer chronically heat stressed group (second summer).

Steroid concentrations in plasma

Plasma progesterone concentrations (means $\pm$ SEM) before PGF_{2a} administration (day 11 ± 1 of the oestrous cycle), at 07:00 and 13:00 h on the day of the experiment (day 12 ± 1), and at 09:00 on the next day were 6.1 ± 0.7 , 0.9 ± 0.1 , 0.6 ± 0.05 and 0.2 ± 0.01 ng ml⁻¹, respectively. These data clearly indicated corpus luteum regression following PGF_{2a} injection. Progesterone values on the day of the experiment (day 12) were below the concentrations reported to inhibit gonadotrophin secretion (Schallenberger *et al.*, 1984). The concentrations of oestradiol in plasma samples taken on the day of the experiment during the first 2 h of collection of blood samples, before GnRH challenge, averaged 4.6 ± 0.7 pg ml⁻¹

Table 1. Mean and peak concentrations of plasma LH and FSH (ng ml⁻¹) after an i.m. injection of GnRH in cows with high and low concentrations of plasma oestradiol

Parameter	Low oestradiol (< 3.3 pg ml ⁻¹ ; $n = 18$)	High oestradiol (> 3.4 pg ml ⁻¹ ; $n = 22$)
LH		
Mean	16.5 ± 1.9	18.6 ± 1.5
Peak	37.7 ± 5.6	$51.9 \pm 4.5^*$
FSH		
Mean	30.7 ± 1.5	$35.6 \pm 1.3^{**}$
Peak	44.4 ± 2.5	$56.2 \pm 2.2^{**}$

Values are means $\pm$ SEM.

Significant differences between groups (* $P < 0.06$; ** $P < 0.01$).

(range 0.5–10.2 pg ml⁻¹). Progesterone and oestradiol concentrations did not vary with thermal treatments (data not shown).

Cows were classified into two groups on the basis of the concentration of oestradiol in their blood: a 'low' oestradiol group, in which the oestradiol concentration was below 3.3 pg ml⁻¹ (1.9 ± 0.2 pg ml⁻¹, $n = 18$), and a 'high' oestradiol group, in which oestradiol values were above 3.4 pg ml⁻¹ (6.3 ± 0.5 pg ml⁻¹, $n = 22$). In the high oestradiol group, GnRH-induced LH and FSH peak values and the mean FSH concentration were significantly higher than those in the low oestradiol group (Table 1). In addition, their interaction with time after administration of GnRH was statistically significant ($P < 0.01$).

Examination of the pooled LH data for basal concentrations and GnRH-induced release showed no significant difference, or a non-significant trend between the same thermal treatments carried out in the two consecutive summers. The LH data were therefore pooled to increase the number of animals in each treatment group. A year effect was detected ($P < 0.06$) for FSH concentrations in plasma and these were therefore analysed separately for the first and second summers.

Pattern of LH secretion before administration of GnRH

The effect of acute heat stress in winter on the pulsatile pattern of LH secretion was not assessed, as blood samples were not collected sufficiently frequently. In summer, the mean tonic concentrations of LH in plasma as well as LH baseline and pulse frequency were not affected by chronic heat stress. However, LH pulse amplitude was 30% smaller in non-cooled than in cooled cows ($P < 0.05$, Table 2). In the cows with high concentrations of oestradiol in plasma, chronic heat stress had no effect on tonic LH secretion (Table 2). In the group with low concentrations of oestradiol in plasma, LH pulse amplitude in chronically heat stressed cows was 50% lower ($P < 0.05$) than in control cows. The mean and baseline concentrations of plasma LH in the group with low concentrations of plasma oestradiol were also lower in chronically heat stressed than in control cows (Table 2). Chronic heat stress did not modify LH pulse frequency (Table 2).

Table 2. Concentrations of mean and baseline plasma LH and LH pulse amplitude and frequency in cows subjected to chronic heat stress in summer

Plasma LH	Overall		Low oestradiol		High oestradiol	
	Control	Heat stressed	Control	Heat stressed	Control	Heat stressed
<i>n</i>	9	12	6	6	3	6
Mean (ng ml ⁻¹)	2.7 ± 0.3	2.2 ± 0.5	3.1 ± 0.3	1.9 ± 0.6†	1.9 ± 0.3	2.0 ± 0.3
Baseline (ng ml ⁻¹) ^a	1.9 ± 0.2	1.6 ± 0.4	2.1 ± 0.2	1.4 ± 0.4	1.6 ± 0.3	1.3 ± 0.3
Amplitude (ng ml ⁻¹)	4.2 ± 0.5	2.9 ± 0.4*	4.8 ± 0.5	2.5 ± 0.6*	2.5 ± 0.5	2.7 ± 0.4
Frequency (h ⁻¹)	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	0.7 ± 0.3	0.8 ± 0.3

Values are means ± SEM.
Significant differences between treatments (**P* < 0.05; †*P* < 0.07).
^aMean, excluding pulses.
n: number of cows.

Fig. 1. Mean (±SEM) plasma gonadotrophin concentrations, after GnRH challenge in cows in summer at the beginning of the follicular phase, with low concentrations of plasma oestradiol and subjected to heat stress (non-cooled). (a) LH of cooled (○) and non-cooled (●) cows; (b) FSH of cooled (○) and non-cooled (●) cows.

LH secretion after the administration of GnRH

The data for LH measurements after GnRH administration were analysed separately for summer (two years pool; Fig. 1a) and winter (Fig. 2a). In the summer, the overall mean concentration of LH in control (*n* = 9) and chronically heat stressed (*n* = 12) cows (18.9 ± 2.2 versus 17.9 ± 1.9 ng ml⁻¹), and the peak of the GnRH induced LH surge in control and chronically heat stressed cows (48.9 ± 5.5 versus 44.8 ± 6.3 ng ml⁻¹) did not differ. Similarly, no trend was noted between treatments in cows with high concentrations of plasma oestradiol (means: 15.7 ± 4.6 versus 17.1 ± 4.5 ng ml⁻¹; peaks: 45.3 ± 10.6 versus 43.8 ± 10.6 ng ml⁻¹, for control (*n* = 3) and

chronically heat stressed cows (*n* = 6), respectively). In cows with low concentrations of plasma oestradiol, peak LH release was 27% lower in heat stressed than in control cows (*P* < 0.10). In cows with low concentrations of plasma oestradiol, the pattern of GnRH-induced LH release differed, with concentrations of plasma LH increasing more rapidly in control than in the chronically heat stressed groups (treatment × time interaction, *P* < 0.01, Fig. 1a). There was no significant effect of heat stress on plasma LH concentrations after the GnRH-induced peak had been reached.

In the winter, the mean concentration of plasma LH in all control cows (*n* = 11) tended to be higher than that of heat-stressed (*n* = 8) cows (19.7 ± 2.1 versus 14.1 ± 2.6 ng ml⁻¹, respectively, *P* < 0.1). The peak of the GnRH-induced LH surge was 34% lower (54.2 ± 6.6 versus 36.0 ± 7.7 ng ml⁻¹, *P* < 0.05) in the heat-stressed animals. Heat stress did not affect these parameters in cows with high concentrations of plasma oestradiol (means: 20.7 ± 2.8 versus 15.8 ± 3.8 ng ml⁻¹; peaks: 59.7 ± 9.2 versus 42.9 ± 11.6 ng ml⁻¹, for control (*n* = 8) and heat-stressed cows (*n* = 5), respectively). In heat stressed animals with low concentrations of plasma oestradiol, the peak of the GnRH-induced LH surge tended to be lower (38%, *P* < 0.10) than in control cows (Fig. 2a). The GnRH-induced LH surge in the heat stressed cows also tended to be attenuated compared with that in control cows (Fig. 2a, treatment by time interaction, *P* < 0.10).

FSH after administration of GnRH

The mean concentrations of plasma FSH, and the peaks of GnRH-induced FSH surges in all cows in the first and second summers were virtually identical in control (*n* = 9) and chronically heat stressed (*n* = 12) groups (means: 31.3 ± 2.2 versus 31.0 ± 2.0 ng ml⁻¹; peaks: 51.6 ± 5.1 versus 50.1 ± 4.3 ng ml⁻¹, respectively). This indicates that, on average, heat stress had no effect on plasma FSH concentration. However, in animals with low concentrations of plasma oestradiol in the second summer, heat stress reduced the mean concentration of plasma FSH to 81% of that in the control cows (*n* = 4; *P* < 0.05; Fig. 1b) and reduced the peak of the GnRH-induced FSH surge to 75% of that of control cows (35.6 ± 2.3 versus

Fig. 2. Mean ($\pm$ SEM) plasma gonadotrophin concentrations after GnRH challenge in cows in winter at the beginning of the follicular phase, with low concentrations of plasma oestradiol and subjected to heat stress. (a) LH of control (○) and heat-stressed (●) cows; (b) FSH of control (○) and heat-stressed (●) cows.

47.4 ± 5.1 ng ml⁻¹, $P < 0.01$). The number of animals observed in the first summer was too small to classify them by concentrations of plasma oestradiol. Thermal stress altered the pattern of the GnRH-induced FSH surge in cows with low concentrations of plasma oestradiol (treatment by time interaction, $P < 0.01$), by reducing plasma FSH more than in control cows (Fig. 1b).

In winter, the overall mean concentration of FSH in control ($n = 7$) and heat-stressed cows ($n = 8$) did not differ (36.1 ± 2.1 versus 31.7 ± 1.9 ng ml⁻¹, respectively), whereas the peak of the GnRH-induced FSH surge tended to be lower in the latter (56.1 ± 4.1 versus 46.4 ± 3.6 ng ml⁻¹, respectively, $P < 0.08$), and the overall curve was lower in the heat-stressed group (treatment by time interaction, $P < 0.05$). The concentration of plasma FSH concentrations in cows with high concentrations of plasma oestradiol were similar in control ($n = 4$) and heat-stressed ($n = 6$) groups (means: 35.0 ± 2.8 versus 33.9 ± 2.5 ng ml⁻¹; GnRH-induced peaks: 54.6 ± 4.9 versus 51.8 ± 4.4 ng ml⁻¹, respectively). In contrast, in cows with low concentrations of plasma oestradiol, heat stress significantly ($P < 0.01$) reduced the mean concentration of FSH to 73%, and the peak of the GnRH-induced FSH surge to 63% of that of control animals (peaks: 59.2 ± 3.3 versus 37.3 ± 2.7 ng ml⁻¹).

Discussion

The objective of this study was to investigate the effects of hyperthermia on gonadotrophin secretion during the early fol-

licular phase when the pituitary gland is expected to be highly responsive to GnRH after the regression of the corpus luteum and removal of the negative feedback effect of progesterone (Ireland and Roche, 1982). Experiments were conducted before the preovulatory surge in gonadotrophin secretion was initiated. The wide range of concentrations of plasma oestradiol in each group of cows indicated the stage of the oestrous cycle at which PGF_{2α} was administered. During this stage (days 10–12), the first follicular wave is replaced by a second wave (Fortune *et al.*, 1991). Cows with high concentrations of plasma oestradiol probably had an oestrogen-active, dominant follicle in the first or second wave. In cows with low concentrations of plasma oestradiol, the dominant follicle in the first wave may have become atretic and the dominant follicle in the second wave may have been poorly developed. The concentrations of plasma oestradiol in the low and high oestrogen groups are within the range previously reported for early follicular phase, and with the higher concentrations found prior to the preovulatory surge of plasma oestradiol (Schallenberger *et al.*, 1984; Imakawa *et al.*, 1986).

The ratio of cows with high and low concentrations of plasma oestradiol was approximately 1:1 ($n = 18$ and 22, respectively). However, further analysis of seasonal differences (independent of the thermal treatments within season) on the ratio of cows with high and low concentrations of oestradiol indicated that (i) the number of cows with low and high concentrations of oestradiol was 6 and 13 in winter, respectively, and in summer 12 and 9 cows, respectively. Chi-square analysis indicates that these differences were not, however, statistically significant ($P < 0.10$) and suggests that chronic heat stress may affect follicular development.

Two major points emerged from an analysis of the LH data collected from cows in the summer: (i) chronic heat stress had no effect on tonic LH secretion or GnRH-induced LH release in cows with high concentrations of plasma oestradiol and (ii) chronic heat stress depressed LH and FSH concentrations in cows with low concentrations of plasma oestradiol.

The reduction in mean and baseline tonic LH concentrations before GnRH injection in chronically heat stressed cows with low concentrations of plasma oestradiol in summer are in agreement with similar effects of heat stress in ovariectomized ewes (Schillo *et al.*, 1978). Heat stress did not alter LH pulse frequency in ovariectomized ewes (Schillo *et al.*, 1978) or in intact cows (this study), whereas in sows heat stress reduced frequency of LH pulses but increased their amplitude (Barb *et al.*, 1991). In cows, LH pulse frequency is reduced by heat stress in early, but not mid-luteal phase of the oestrous cycle (Wise *et al.*, 1988). Changes in tonic LH secretion during the early follicular phase could alter ovarian function. This is suggested by the observation that the impaired LH pulse pattern in ewes may be associated with disturbed timing of ovulation, abnormal corpus luteum function and reduced conception rates (McNatty *et al.*, 1981). Furthermore, Savio *et al.* (1992) have shown that the pattern of tonic LH secretion influences the rate of growth and turnover of the dominant follicle in the ovary of cyclic cows.

In studies in which cows were not grouped according to concentrations of plasma oestradiol, heat stress during the oestrous cycle either had no effect on plasma tonic LH (Gwazdauskas *et al.*, 1981), or even increased it (Roman-Ponce *et al.*, 1981). Observations on the effects of heat stress on the

preovulatory surge of LH are similarly controversial: a reduction of endogenous LH surge by heat stress was reported in heifers (Madan and Johnson, 1973), but not in cows (Gwazdauskas *et al.*, 1981; Rosenberg *et al.*, 1982; Gauthier, 1986). However, in these studies, the sampling frequency of 3–12 h may have been inadequate to detect any effects on plasma LH secretion.

In this study heat stress affected LH secretion mainly in cows with low concentrations of oestradiol. This finding suggests that high concentrations of plasma oestradiol counteract the effects of heat stress, or alternatively, that the neuroendocrine mechanism controlling gonadotrophin secretion is more sensitive to heat stress when concentrations of plasma oestradiol are low than when they are high. This could be due to a positive feedback action of oestradiol on gonadotrophin secretion (Kesner *et al.*, 1981; Kesner and Convey, 1982; Schallenger *et al.*, 1984; Imakawa *et al.*, 1986). In dairy cows, the stimulating effect of oestrogen on LH secretion is proportional to the concentration of oestrogen in the plasma (Dielman *et al.*, 1986; Lucy and Stevenson, 1986). The apparent conflicting observations on the effect of heat stress on LH secretion may result from the fact that animals were not classified by oestradiol concentrations. The classification of data in this study by concentration of plasma oestradiol resulted in a small number of cows in some subgroups, so that generalizations made should be viewed with caution. However, the similarity of the winter and the summer results strengthens the suggestion that heat stress affects LH and FSH secretion mainly in cows with low concentrations of plasma oestradiol.

Heat stress may affect LH secretion by reducing the amount of GnRH released during each pulse, consequently reducing gonadotrophin synthesis and hence pituitary gonadotrophin stores. This would account for the decreased pituitary response to GnRH in heat-stressed animals with low concentrations of plasma oestradiol. This view is consistent with the finding of a lower LH content in the hypophysis of pigs in summer than in winter (Armstrong *et al.*, 1986).

Acute heat stress for 16 h during the winter season was imposed in this study to examine the effect of short-term heat stress on non-acclimated cows, in contrast to chronic heat stress in summer. The effects of heat stress in the two seasons, though similar in nature, were not similar for all facets of gonadotrophin secretion. In summer, overall mean concentrations of gonadotrophins and peak GnRH-induced surges of LH and FSH were not affected by chronic heat stress, whereas in winter, acute heat stress depressed and GnRH induced release of gonadotrophins. The marked effects of acute heat stress in winter may be related to the fact that cows in winter are not as well acclimated to heat as they are in summer.

The reduction in gonadotrophin secretion in response to heat stress in winter and summer suggests similar modes of action for both acute and chronic heat stress. Gonadotrophin secretion was induced in this experiment by the same dose of GnRH in all animals. The effects therefore result from a direct effect of heat stress on gonadotrophin secretion. The similarity of the responses to acute and chronic heat stress indicates that the involvement of a possible nutritional deficiency induced by chronic heat stress is not essential to produce the restriction in gonadotrophin secretion. Both secretion and storage of gonadotrophins could be affected, although acute heat stress is expected to affect mainly secretion, whereas chronic heat

stress would affect both secretion and storage. This has also been implied in a study on post-weaning primiparous sows, where LH content of the hypophysis was found to be lower in summer than in winter (Armstrong *et al.*, 1986).

The study reported here demonstrates for the first time that FSH secretion is reduced by heat stress, and that this effect is most pronounced in cows with low concentrations of plasma oestradiol. This finding could be related to the previously reported impairment of follicular development by heat stress in cows (Wolfenson *et al.*, 1992). This impairment is reflected by the emergence of fewer small antral follicles, which may be related to the suppression of FSH secretion by heat stress observed here. Reduction in FSH secretion could also affect oestradiol production by follicular granulosa cells.

In conclusion, heat stress, either in its seasonal chronic form or as an acute short-term exposure, affected gonadotrophin secretion during the early follicular phase of the oestrous cycle, mainly in cows with low concentrations of plasma oestradiol. Hyperthermia during this phase may affect tonic LH and preovulatory release of gonadotrophin secretion. The precise nature of the effect of heat stress on gonadotrophin secretion and its relationship to concentration of oestradiol or to the precise stage of the cycle require further investigation.

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