Effects of heat stress on serum progesterone in cyclic ewes and on progesterone and cortisol response to ACTH in ovariectomized ewes

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Summary. The daily mean of serum progesterone in cyclic ewes ($N = 5$) as well as the profile characteristics of progesterone and cortisol in response to an acute single dose ($5 \text{ i.u./kg liveweight}^{0.75}$) of adrenocorticotrophic hormone (ACTH) into ovariectomized ewes ($N = 4$) was investigated during exposure to a constant thermoneutral temperature of $18 \pm 1^\circ\text{C}$ or to a daily cyclic heat stress temperature of 18°C – 35°C – 18°C , in an environmental chamber. Serum collected daily from the cyclic ewes was assayed for progesterone, while serum collected more frequently for 10 h, on the 14th day of exposure to the respective temperature, from the ovariectomized ewes was assayed for progesterone and cortisol by RIAs.

In cyclic ewes, heat stress increased the area under the daily progesterone curve ($P < 0.09$) but had no effect on progesterone concentration after the regression of the CL. In ovariectomized ewes, ACTH significantly elevated the response of both cortisol and progesterone ($r = 0.75$, $P < 0.001$) within 10–15 min of injection. In the ovariectomized ewes and during heat stress, the responses of progesterone and cortisol to ACTH were characterized by an initial acute rise, a transient drop, a steep elevation and a gradual but prolonged decline. During thermoneutral temperatures, this biphasic response pattern was not observed. Since progesterone concentration in the ovariectomized ewes in response to heat stress or the ACTH injection were within the range of basal progesterone observed during the preovulatory period in cyclic ewes, our results give no evidence of a direct role for adrenal progesterone in depressing pituitary function in ewes exposed to a daily cyclic heat stress.

Keywords: heat stress; ewes; ACTH; progesterone; cortisol

Introduction

Exposure of cycling cows to chronic heat stress has been shown to be associated with elevated serum progesterone concentration (Abilay *et al.*, 1975; Roussel *et al.*, 1977) and depressed adrenal glucocorticoid secretion (Christison & Johnson, 1972; Alvarez & Johnson, 1973). Elevated progesterone values in sheep and cattle have been associated with depressed gonadotrophin release (Bjersing *et al.*, 1972; Pant *et al.*, 1977; Rahe *et al.*, 1980). Therefore, the depressed luteinizing hormone surge in ewes exposed to chronic heat stress (Sheikheldin, 1987) could be due to elevated progesterone concentration. The literature is not clear regarding the source of the elevated progesterone with both the corpus luteum (CL) and the adrenal cortex being possible sources. The reports of increased progesterone secretion after the injection of exogenous ACTH into ovariectomized (Benhaj & Cooke, 1985) and ovariectomized-hypophysectomized (De Silva *et al.*, 1983) ewes

demonstrate the potential for adrenal secretion of progesterone. In support of the CL being the source of the elevated progesterone during heat stress, Wheeler & Blackshaw (1986) have reported the possible involvement of the increased release of catecholamines during heat stress in the direct enhancement of progesterone secretion from the CL.

The present investigation was initiated (1) to determine the effect of daily cyclic heat stress on serum progesterone concentration in cycling ewes, (2) to elucidate the source of elevated progesterone in heat-stressed ewes, and (3) to determine whether heat stress modifies the adrenal response to an acute exogenous dose of adrenocorticotrophic hormone (ACTH).

Materials and Methods

Experiment 1

Animals. Five adult (liveweight = 65.2 ± 1.9 kg, mean $\pm$ s.e.m.) cycling Suffolk ewes were used during the breeding season (October–December). Ewes were shorn (to fleece length of <0.5 cm) and exposed to a thermoneutral temperature of $18 \pm 1^\circ\text{C}$ or to a daily cyclic heat stress temperature of 18°C – 35°C – 18°C , by means of an environmental chamber, $2.54\text{ m} \times 1.88\text{ m} \times 2.54\text{ m}$ (Coldstream, Fleming-Pedlar Ltd, Winnipeg, MB, Canada). The relative humidity ($55 \pm 2\%$) and photoperiod (10 h light:14 h dark) were kept constant. Each ewe received a daily ration composed of 1.0 kg alfalfa pellets, 900 g barley and 1.0 kg hay. This daily allowance was given in two equal morning (07:00 h) and afternoon (15:00 h) portions. The animals were left loose in the chamber and were fed in a common feeding trough. The oestrous cycle was synchronized before exposure with two injections of prostaglandin F-2 α (cloprostenol: ICI Pharma, Mississauga, Ontario, Canada) given 10 days apart. The ewes were studied for two consecutive cycles. The first oestrous cycle was at Thermoneutral temperature and was followed by the second at heat stress. The switch of the chamber temperature from 18 to 35°C was done at 12:00 h on Day 17 of the oestrous cycle.

Sampling procedures. Daily jugular blood (7.0 ml) was collected by vacutainers and the serum was stored at -20°C until the estimation of progesterone using radioimmunoassay (RIA) procedures.

Experiment 2

Animals. Four long-term (>5 months) ovariectomized (liveweight = 85.2 ± 2.2 kg, mean $\pm$ s.e.m.) ewes of the Suffolk breed were used. The animals were also shorn and exposed to temperature, relative humidity and photoperiod setting identical to those in Exp. 1, using the same environmental chamber. Each ewe received the same daily ration as did the ewes in Exp. 1, but was fed individually in metabolism crates. Ewes were exposed to alternate 2-week periods of thermoneutral temperature and cyclic heat stress and in each temperature 2 ewes received an acute single dose (5 i.u. per kg liveweight^{0.75}) of adrenocorticotrophic hormone (ACTH) (porcine-ACTH1-39: Sigma Chemical Co., St Louis, MO, U.S.A.) prepared in 5 ml saline (0.154 M-NaCl), while the remaining 2 ewes were given saline only. There was a 15-day rest period before each temperature exposure. During the rest periods, the chamber temperature was kept at $18 \pm 1^\circ\text{C}$.

Sampling procedures. Ewes were subjected to intensive blood sampling sessions between 07:00 and 17:00 h on Day 14 of each treatment period, using jugular catheters inserted on Day 13. Starting at 07:00 h, samples were taken at 15-min intervals for 2 h before the injection. At 09:00 h ACTH or saline was given as a single dose through the jugular catheter (total volume = 5 ml). Further samples were collected at 0, 5, 10, 15, 20, 25 and 30 min after the injection and thereafter at 15-min intervals until 17:00 h. Serum was harvested and stored at -20°C until the estimation of progesterone and cortisol using RIA procedures.

Hormone assays

Chemicals and reagents used, unless specified, were all of analytical grade. Buffers were prepared and used fresh, or kept at 4.0°C and changed every 10 days. The hormone standards and the unknowns were run in triplicate and duplicate, respectively. All samples from an individual ewe were estimated in the same run. Assay tubes were counted for 4.0 min in a liquid scintillation counter (Rackbeta #1217: LKB, Wallac OY, Finland), fitted with a microcomputer that was programmed to calculate unknown concentrations from the standard curves using spline-functions (Wold, 1974).

Progesterone. Progesterone was estimated according to the assay originally described by Abraham *et al.* (1971) and modified by Yuthasastrakosol *et al.* (1974), with the following exceptions: (1) both the standard and the unknowns were simultaneously extracted; (2) during the separation step an ordinary charcoal solution (375 mg

washed charcoal/100 ml assay buffer) was used instead of the dextran-coated charcoal; and (3) after the addition of charcoal the assay tubes were incubated at 4°C for 10 min, rather than 20 min.

The progesterone standard (4-pregnen-3,20-dione: Steraloid Inc., Wilton, NH, USA) was prepared in charcoal-stripped serum from an ovariectomized ewe which contained $<43.2 \pm 1.9$ pg progesterone/ml (20 assays). Labelled progesterone ($[^3\text{H}]$ progesterone: New England Nuclear, Boston, MA, U.S.A.) was prepared in the assay buffer to yield 34 000–37 000 c.p.m. per 100 μl per tube. Progesterone antibody provided by Dr N. C. Rawlings (Department of Veterinary Physiological Sciences, W.C.V.M., University of Saskatchewan, Saskatoon, Saskatchewan, Canada), cross-reacted at 0.47%, 0.04%, 0.01% and 0.01% with cholesterol, testosterone, hydrocortisone and oestradiol-17 β , respectively. The antibody was used at a dilution of 1:5000.

Samples were assayed at volumes of 0.5–1.0 ml per tube. The efficiency of progesterone extraction was 78.0% (26 assays). Total binding of the antibody was 41.7% and the non-specific binding was 2.2% (Rodbard *et al.*, 1970; Abraham, 1974). The sensitivity of the assay at 95% binding was 15.4 pg per tube (Eikins, 1974). When ewe serum pools containing 0.15, 2.50 and 11.20 ng/ml were assayed repeatedly, the intra-assay coefficients of variation (CVs) (Rodbard *et al.*, 1968) were 7.4%, 3.3% and 6.0%, respectively. The corresponding values for the inter-assay CVs were 15.8%, 4.6% and 6.4%, respectively.

Cortisol. The cortisol RIA was a modification of the competitive protein-binding assay previously described by Rawlings & Ward (1978). The assay buffer (phosphate buffer saline, PBS, containing 5.38 g monobasic sodium phosphate, 8.66 g anhydrous dibasic sodium phosphate, 9.0 g sodium chloride, 1.0 g sodium azide and 1.0 g gelatin in 1.0 litre deionized distilled water, pH = 7.4) was identical to the one used in the progesterone assay (Yuthasastrakosol *et al.*, 1974). Cortisol standard (hydrocortisone: Steraloid Inc.) was prepared in charcoal-stripped serum from an ovariectomized ewe and contained $<106 \pm 11$ pg cortisol/ml (16 assays). Labelled cortisol ($[^3\text{H}]$ hydrocortisone; New England Nuclear), was prepared in the assay buffer to give 28 000 d.p.m. (6000 c.p.m.) in a total volume of 200 μl (~ 60 pg per tube).

The antibody provided by Dr N. C. Rawlings (W.C.V.M., University of Saskatchewan) cross-reacted at 10% with progesterone, but at $<1.0\%$ with dehydroepiandrosterone, pregnenolone, testosterone, oestradiol-17 β , oestradiol-17 α or cholesterol. The antibody was prepared in the assay buffer and used at a dilution of 1:2500.

Since the adrenal glands of the ovariectomized ewe secrete moderate quantities of progesterone, progesterone was extracted from all samples with petroleum ether (Grade #20–40°C, J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.) before cortisol assay. All the extraction steps were performed at 4°C and 1.0 ml petroleum ether was added to (12 $\times$ 75) culture tubes containing 200 μl of the standard (triplicates) or the unknowns (duplicates). The tubes were stoppered, vortexed gently (20 sec) and kept at -20°C for 1.0 h. The supernatant (containing progesterone) was then aspirated. Subsequently, 1.5 ml absolute alcohol were added to all tubes. After vortexing (60 sec) and centrifugation (at 1000 g) for 10 min, the supernatant layer was decanted into a new set of (12 $\times$ 75) tubes and alcohol was evaporated in a water bath (at 60°C) under a gentle flow of nitrogen. After this final extraction, 200 μl cortisol antibody were pipetted to all tubes, except the tubes for non-specific binding into which an equivalent volume of buffer was pipetted. Then 200 μl of the cortisol trace were added to all tubes, including the NSB tubes. The subsequent steps of the assay were as described for the progesterone assay.

The efficiency of progesterone removal by petroleum ether was estimated to be 85.5% (6 assays). Total binding of the antibody was 24.0% ($n = 23$) with non-specific binding of 0.8%. The sensitivity of the assay at 95% binding was 46.5 pg per tube. The intra-assay CVs of ewe serum pools containing 10.7, 20.5 and 32.3 ng cortisol/ml were 9.3%, 6.2% and 5.7%, respectively. The corresponding values for the inter-assay CVs were 11.1%, 7.6% and 11.7%, respectively.

Statistical analysis

All estimates of hormone concentrations made on frequently collected samples were initially subjected to analysis by the Pulsar programme (Merriam & Wachter, 1982), which identifies baselines, amplitudes, peaks, frequencies, duration of peaks and interpeak intervals. This programme eliminates circadian and long-term trends by using Robust Locally Weighted Regression Analysis (Cleveland, 1979). Hormone peaks were identified using the multiples of the intra-assay standard deviations (s.d.) by which a pulse should rise above the preceding nadir point to be identified as a peak (defined in the Program as 'G Values'). These values were selected as 5.5, 4.5, 4.0, 3.5 and 3.0 times the s.d. when a peak is comprised of 1, 2, 3, 4 and 5 points, respectively. Subsequently, the data were subjected to analyses of variance (ANOVA) using computer programs according to the software packages provided by Statistical Analysis Systems (SAS Institute, Inc. 1982). All hormone data were transformed (log base 10) before the analysis. Progesterone data from cyclic ewes were analysed by arbitrarily dividing the oestrous cycle into follicular (Days 1–4), early luteal (Days 5–8), mid-luteal (Days 9–12) and late luteal (Days 13–16) phases. In addition, the area under the daily progesterone curve was calculated as described by Howland *et al.* (1978). Comparisons between treatments and temperatures were made using $2 \times 2 \times 4$ factorial design (Snedecor & Cochran, 1967). The two factors were treatment (ACTH vs saline) and temperature (18 vs 35°C); ewes were considered as blocks. Duncan's multiple range test was used to compare differences in progesterone values between phases of the oestrous cycle. Whenever necessary, simple correlation coefficients were calculated according to Snedecor & Cochran (1967). A simple *t* test was used to determine differences between hormone means before and after the injection of saline or ACTH.

Results

Experiment 1

Ambient temperature (T_{ambient}). There were minimal fluctuations in the 24-h T_{ambient} during exposure to thermoneutral temperature ($18.13 \pm 0.10^{\circ}\text{C}$). However, during exposure to cyclic heat stress, T_{ambient} rose progressively from $21.3 \pm 0.2^{\circ}\text{C}$ at 07:00 h to $26.4 \pm 0.3^{\circ}\text{C}$ at 09:00 h, to $30.2 \pm 0.2^{\circ}\text{C}$ at 11:00 h and reached a peak of $33.8 \pm 0.2^{\circ}\text{C}$ at 14:00 h. After this peak at 14:00 h, T_{ambient} was stable until 15:00 h and then fell steeply to $<22^{\circ}\text{C}$ within 2 h and remained at this temperature until the early morning hours.

Progesterone concentration in cycling ewes. Progesterone concentration during the oestrous cycle is depicted in Fig. 1. Mean progesterone values tended to be elevated between Days 2 and 11 of the cycle during cyclic heat stress compared to thermoneutral temperature. Analysis of variance of the area under the daily progesterone curve showed greater area during heat stress ($P < 0.09$). The removal of one ewe which had lower progesterone during heat stress, resulted in a significant

Fig. 1. Changes in mean ($\pm$ s.e.m.) concentration of daily serum progesterone in cycling ewes ($N = 5$) during exposure to thermoneutral temperature ($\bullet$) or during daily cyclic heat stress ($\square$).

Table 1. Progesterone concentrations (ng/ml) in the follicular (Days 1–4), early luteal (Days 5–8), mid-luteal (Days 9–12) and late luteal (Days 13–16) phases of the oestrous cycle in ewes exposed to a thermoneutral temperature or to a daily cyclic heat stress

Phase of cycle	Temperature	
	Thermoneutral	Heat stress
Follicular	0.92 $\pm$ 0.13 ^a	1.15 $\pm$ 0.17 ^b
Early luteal	2.52 $\pm$ 0.17 ^c	3.15 $\pm$ 0.15 ^d
Mid-luteal	3.33 $\pm$ 0.14 ^{d,e}	3.64 $\pm$ 0.16 ^e
Late luteal	1.43 $\pm$ 0.29 ^b	1.10 $\pm$ 0.26 ^{a,b}

Values are mean $\pm$ s.e.m. for 5 ewes.
^{a–e}Means having the same superscript do not differ ($P > 0.05$).

Fig. 2. Changes in mean ($\pm$ s.e.m.) concentrations of serum progesterone (a, b) and cortisol (c, d) in ovariectomized ewes ($N = 4$) in response to injection of ACTH (a, c) or saline (b, d) (at time 0) during exposure to thermoneutral temperature (●) or during daily cyclic heat stress (□).

difference between temperatures ($P < 0.01$). Analysis of variance of the oestrous cycle by phase revealed significant differences between phases ($P < 0.001$) and also temperature $\times$ phase interaction ($P < 0.01$). Duncan's multiple range test showed that progesterone was elevated in the follicular and early-luteal phases during heat stress compared to the corresponding phases during

thermoneutral temperature ($P < 0.05$) (Table 1). Progesterone values after regression of the CL (late luteal phase) were comparable between the two temperatures.

Experiment 2

Ambient temperature. The T_{ambient} during exposures to thermoneutral or high temperature was similar to that in Exp. 1.

Progesterone response to the injections of saline or ACTH. The progesterone profiles before and after the injections are shown in Fig. 2. Progesterone concentrations in the intervals before and after saline were comparable (Fig. 2b). However, mean progesterone concentration tended to be more variable during exposure to thermoneutral temperature.

Serum progesterone profiles before the injection of ACTH were comparable to those observed in the same interval before saline injection (Fig. 2a). After the injection of ACTH during thermoneutral temperature, progesterone rose acutely to values significantly higher ($P < 0.05$) than the preinjection concentration within 15 min (0.48 ± 0.05 ng/ml) and further increased to a peak of 0.62 ± 0.05 ng/ml at 30 min after injection. Values continued to fluctuate reaching a maximum at 75 min after ACTH and then declined gradually over the remainder of the sampling period. Mean progesterone value at 75 min after ACTH during thermoneutral temperature was higher than the preinjection concentration ($P < 0.05$), but was comparable to the peak at 30 min after injection.

Progesterone secretion after ACTH during heat stress revealed a biphasic response. Heat stress enhanced adrenal progesterone, resulting in a peak at 15 min after ACTH injection. Then, instead of the elevated progesterone values which were observed during the corresponding period at 18°C , concentration declined during the next 30 min to a nadir value at 45 min after ACTH. After these low concentrations progesterone rose to a second peak which was comparable in magnitude to the first. This second peak was followed by a gradual but a prolonged declining trend to the end of the sampling period.

Analysis of variance of progesterone concentration by hourly periods ($n = 9$) after the injection of saline or ACTH revealed significant differences between treatments ($P < 0.05$) and periods ($P < 0.01$) as well as treatment $\times$ period ($P < 0.01$) and temperature $\times$ period ($P < 0.05$) interactions.

Analysis of variance of the Pulsar data for the profile characteristics of progesterone in response to saline or ACTH showed no differences between treatments or temperatures and no interactions in the characteristics measured, except for the mean basal progesterone (Table 2), which was significantly elevated by ACTH ($P < 0.05$).

Cortisol response to the injections of saline or ACTH. The cortisol profiles were analysed in a fashion similar to those of progesterone. Profiles before and after saline injection were similar between temperatures (Fig. 2d). Irrespective of the temperature, an abrupt rise in cortisol followed the injection of saline. However, values returned to preinjection concentration within 60 min.

The cortisol means before ACTH injection were not significantly different between temperatures (Fig. 2c). After the injection an acute increase ($P < 0.05$) in cortisol was detectable within 10 min, irrespective of the temperature. During thermoneutral temperature exposure there was an acute steady rise reaching a peak at 75 min after injection. The concentrations after the peak showed some fluctuation in amplitudes with a tendency to fall gradually over time in fashion similar to that of progesterone during the same temperature ($r = 0.75$, $P < 0.001$).

During heat stress, cortisol was elevated in response to ACTH at 10–25 min after the injection. However, this was followed by a transient decline, 10–25 min after ACTH. This drop was similar to the one observed for progesterone after the injection of ACTH. Thereafter, cortisol concentration rose to values seen during the thermoneutral temperature. Cortisol response to ACTH during heat stress therefore also showed a biphasic trend which was similar to that seen for progesterone ($r = 0.50$, $P < 0.001$).

Table 2. Profile characteristics of progesterone and cortisol in ovariectomized ewes treated with saline or ACTH during exposure to thermoneutral temperature or daily cyclic heat stress

	Treatment			
	Saline		ACTH	
	Thermoneutral temperature	Heat stress	Thermoneutral temperature	Heat stress
Progesterone				
Mean conc.* (ng/ml)	0.27 ± 0.02	0.31 ± 0.01	0.37 ± 0.02	0.38 ± 0.03
Pulse amplitude (ng/ml)	0.12 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.13 ± 0.01
Pulse frequency (per min)	0.025 ± 0.001	0.022 ± 0.002	0.021 ± 0.001	0.021 ± 0.001
Duration of pulse peak (min)	25.93 ± 2.02	31.52 ± 4.72	33.09 ± 2.90	32.67 ± 2.74
Interpeak interval (min)	41.77 ± 2.81	47.35 ± 4.12	48.00 ± 2.64	49.81 ± 3.04
Cortisol				
Mean conc.† (ng/ml)	8.78 ± 0.31	7.68 ± 1.44	77.20 ± 7.84	86.15 ± 10.05
Pulse amplitude* (ng/ml)	19.53 ± 2.24	10.40 ± 1.42	57.98 ± 15.15	59.98 ± 11.41
Pulse frequency (per min)	0.017 ± 0.002	0.020 ± 0.001	0.022 ± 0.001	0.022 ± 0.002
Duration of pulse peak (min)	41.20 ± 4.72	32.25 ± 1.47	29.18 ± 1.63	30.60 ± 2.35
Interpeak interval (min)	62.35 ± 5.90	51.83 ± 3.40	48.00 ± 1.95	48.73 ± 4.01

Values are mean ± s.e.m. for 4 ewes.

*Treatment effect, $P < 0.05$.

†Treatment effect, $P < 0.01$.

The analysis of variance of the concentration of cortisol in response to the injection of saline or ACTH by hourly periods ($n = 9$) showed significant differences between treatments ($P < 0.001$) and periods ($P < 0.01$) as well as a significant interaction between treatment and period ($P < 0.01$).

The analysis of variance of the Pulsar characteristics of cortisol profiles also showed significant elevation in mean basal cortisol in ACTH-treated ewes ($P < 0.01$) (Table 2). In addition, the cortisol pulse amplitude was greater in ACTH-treated animals ($P < 0.05$).

Discussion

The maximum temperature treatment used in this study was similar to the maximum but constant temperature settings used by Christison & Johnson (1972) to induce heat stress in cattle. In the present study cyclic heat stress was used. Exposure of cycling ewes to cyclic heat stress has been shown to be accompanied by significant changes in rectal temperature and respiration rate and associated with dramatic changes in the release profiles of prolactin when T_{ambient} rose above 31–33°C (Sheikheldin, 1987). Therefore, the ewes in this study were assumed to be heat-stressed to the same magnitude as the ewes in that previous study. Abilay *et al.* (1975) have used a constant temperature at 33.5°C to induce the elevation of progesterone in cycling cows. However, despite the usefulness of the exposure of animals to constant temperature settings in answering specific questions related to hyperthermia, diurnal fluctuations are more representative of the natural conditions.

By adopting a daily cyclic T_{ambient} , the aim of this study was to reduce the severity of physiological and hormonal disturbances which occur when animals are exposed to constant temperatures above their thermocomfort zones.

The larger area under daily progesterone in the cycling ewes during cyclic heat stress in this investigation agrees with a previous report of increased progesterone in cyclic cows exposed to a constant heat stress of 33.5°C (Abilay *et al.*, 1975). The current results clearly indicate that the progesterone response to cyclic heat stress in cycling ewes occurred mainly in the presence of an active CL in the ovary. Progesterone concentration after regression of the CL was not affected by temperature. A similar observation was made in cows when progesterone concentration on the day of oestrus (after regression of the CL) was not different in animals kept at 33.5°C and in those kept at 18.2°C (Abilay *et al.*, 1975).

The lack of temperature effect on cortisol values in saline-treated ovariectomized ewes in this study appears to be in disagreement with the previous report in which cortisol was depressed in cycling ewes exposed to heat stress (Sheikheldin, 1987). The lack of effect of exposure to cyclic temperature on cortisol was also reported for cattle subjected to temperature settings lower than in the present investigation (Shayanfar *et al.*, 1975). The variance of the present results with the previous report might have resulted from insufficient number of samples to establish significant differences in this investigation.

In agreement with earlier findings (De Silva *et al.*, 1983; Benhaj & Cooke, 1985), the present results show that the adrenals secrete progesterone. However, in saline-injected ovariectomized ewes, progesterone was not elevated during heat stress as compared to values seen during the preovulatory period (following regression of the CL) in cycling ewes. After regression of the CL in the cycling ewes progesterone concentration was not influenced by cyclic heat stress. Therefore, although the adrenals are a second source of progesterone secretion in the ewe, they do not appear to increase their progesterone secretion in response to cyclic heat stress and stress-induced elevation in progesterone probably represents increased luteal secretion of progesterone.

The results also confirm earlier reports that a dose of exogenous ACTH, in ovariectomized ewes, sufficient to elevate cortisol significantly, will also elevate progesterone (De Silva *et al.*, 1983; Benhaj & Cooke, 1985). In addition, the present results show that the secretory profiles of both cortisol and progesterone, in response to ACTH, followed a biphasic trend during cyclic heat stress, which might have been due to a transient lack of steroidogenic precursors.

In conclusion, this study provides no evidence that cyclic heat stress elevates adrenal progesterone output. Even when the adrenal is stimulated by exogenous ACTH the peripheral concentration of progesterone achieved is relatively low, not rising above that observed during the onset of the preovulatory gonadotrophin surges in cycling ewes (Sheikheldin, 1987). Therefore, based on these findings, the involvement of adrenal progesterone secretion in inhibiting the release of gonadotrophins is doubtful during cyclic heat stress.

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