Variation in peripheral progesterone concentrations with environmental temperature in ewes on Days 7 and 11 of the oestrous cycle

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Summary. Progesterone concentrations in peripheral plasma samples on Days 7 and 11 of oestrous cycles throughout the breeding season showed a negative relationship to the mean minimum environmental temperature for the Days 0–7 and 0–11 respectively of that cycle during which the samples were collected.

Introduction

Peripheral progesterone concentrations in sheep in the northern hemisphere are reported to be significantly higher in cyclic ewes in November, when the temperature would be expected to be lower, than in July (Lamond, Gaddy & Kennedy, 1972) and in December than in October (Quirke, Hanrahan & Gosling, 1979), and similarly in early pregnant ewes in December than in March (Rhind, Chesworth & Robinson, 1978). Such a relationship has also been observed in cold seasons compared to hot seasons in dairy cows (Rosenberg *et al.*, 1982) and Murrah buffalo cows (Rao & Pandey, 1982). However, in none of these experiments was the environmental temperature actually measured. We have therefore analysed data on progesterone concentrations in peripheral venous samples collected throughout the year and the corresponding environmental temperatures for such a relationship.

Other aspects of the progesterone concentration data have been presented and discussed elsewhere (Wheeler & Land, 1977; Wheeler, 1978).

Materials and Methods

The 15 Merino, 21 Finnish Landrace (Landrace) and 16 Scottish Blackface (Blackface) ewes were observed from June 1972 (before the onset of the 1972–73 breeding season) until autumn 1973 (after the onset of the 1973–74 breeding season). All the ewes were obtained from established flocks and were maintained outside at the ABRO Dryden Field Laboratory. Ewes in oestrus were detected with raddled, vasectomized rams.

The animals were subjected to laparoscopic examination of the ovaries during the first 3 oestrous cycles of the breeding season, during every subsequent oestrous cycle for the Merino ewes

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and during alternate oestrous cycles for the other animals, and at 14–21-day intervals during the non-breeding seasons.

Jugular venous blood samples were collected between 08:00 and 11:00 h into heparinized, evacuated tubes on Days 7 and 11 of the oestrous cycles during which the ovaries were observed (first day of oestrus: Day 0). The plasma was stored at -20°C until assayed for progesterone using the radioimmunoassay described by Scaramuzzi, Corker, Young & Baird (1975). The antiserum (91920/9) had cross-reactions of 100% with progesterone, 121% with 11α -hydroxyprogesterone, 78% with 11β -hydroxyprogesterone and 82% with 11 -ketoprogesterone. The mean ($\pm$ s.e.m.) extraction efficiency for 200 μl plasma was $62.8 \pm 0.28\%$ ($n = 1528$). The repeated assay of aliquants of a plasma pool obtained from a ewe on Day 10 of the oestrous cycle gave a mean ($\pm$ s.e.m.) progesterone concentration of 5.29 ± 0.40 ng/ml (13 assays). The blank values for 200 μl samples taken through the method were 0.438 ± 0.091 ng/ml ($n = 19$) for plasma from an anoestrous ewe and 0.323 ± 0.092 ng/ml ($n = 19$) for buffer. The mean ($\pm$ s.e.m.) minimum detectable dose was 0.214 ± 0.059 ng/ml ($n = 25$). The inter-assay coefficient of variation was 14.2%.

The minimum daily temperatures and other meteorological data were recorded at the adjacent A.R.C. Poultry Research Centre each day.

The data were first analysed by calculating the regression of the progesterone concentration (following transformation to logarithms; Wheeler & Land, 1977) on the minimum temperature on Day 1 of that oestrous cycle (the day of ovulation), and on the day on which the plasma sample was collected. The data were further analysed by examining the distributions of the mean minimum temperature for Days 0–7 and 0–11 of the oestrous cycle during which the plasma samples were collected relative to the progesterone concentrations in samples collected on Days 7 and 11 of the oestrous cycle respectively.

Results

The minimum daily temperatures and the progesterone concentrations are shown in Text-fig. 1.

Preliminary regression analysis revealed that differences existed between individual ewes with respect to average progesterone concentrations. Subsequent regression models to investigate the presence or absence of temperature effects incorporated terms to allow for these ewe differences. In these regression models, minimum daily temperature had a small but consistent negative association with the progesterone concentration. These effects were statistically significant for Merino and Landrace ewes ($P < 0.05$) but were not significant for Blackface ewes ($P > 0.05$), progesterone concentrations increasing for the three breeds by between 2 and 4% for each degree centigrade reduction in minimum temperature on the day on which the sample was collected. Similar, but not such consistent, associations were found with the minimum temperature on Day 1 of the oestrous cycle.

Further examination of the data revealed that the temperature effects were mainly due to a disproportionately large number of very high progesterone readings occurring at times of below average minimum temperatures. This is illustrated in Table 1 which shows the number of concentrations on Days 7 and 11 of the oestrous cycle above and below 7.0 ng/ml when the mean minimum temperatures for Days 0–7 and 0–11 respectively of the same oestrous cycle were low and high (below and above 3°C). For all 3 breeds the distributions suggest an association between high progesterone and low temperatures. Taking the 3 breeds together the statistical evidence for this association is quite strong; $\chi_1^2 = 12.9$ ($P < 0.001$) for concentrations on Day 7 and $\chi_1^2 = 8.4$ ($P < 0.005$) for concentrations on Day 11.

Progesterone concentrations were also examined for association with wind velocity and with a chill factor based on wind speed and temperature (Siple & Passel, 1945). Although some association was found it was not as strong as the minimum temperature effect.

Text-fig. 1. Variation in the daily minimum temperature and peripheral progesterone concentrations on Days 7 and 11 of the oestrous cycle in Merino (+), Finnish Landrace (x) and Scottish Blackface (●) ewes.

Table 1. Distributions of high and low (above and below 7 ng/ml) peripheral plasma progesterone concentrations in samples collected on Days 7 and 11 of the oestrous cycle with respect to the distribution of the associated low and high (below and above 3°C) mean minimum temperature for the Days 0–7 and 0–11 of the oestrous cycle during which the plasma samples were collected

Breed	Progesterone conc.	Days 0–6		Days 0–11	
		<3°C	>3°C	<3°C	>3°C
Merino	>7 ng/ml	6	1	4	2
	<7 ng/ml	45	56	50	52
Blackface	>7 ng/ml	2	1	6	3
	<7 ng/ml	36	56	34	53
Landrace	>7 ng/ml	9	2	10	3
	<7 ng/ml	43	70	45	66
All breeds	>7 ng/ml	17	4	20	8
	<7 ng/ml	124	182	129	171

Discussion

This is the first description of a direct relationship between the peripheral progesterone concentration in ewes and the environmental temperature. Although these associations are statistically significant they are not strong associations, and their detection in the present study is to some extent a consequence of the large number of observations. The temperature effect on the progesterone concentration is small compared to the overall variation in the data. The associations are too weak to be useful in predicting with confidence that low temperatures necessarily imply high progesterone concentrations.

The associations between high progesterone concentrations and low temperatures were marginally stronger for the temperature on the day that the blood sample was collected than for the temperature on the day of ovulation and corpus luteum formation (Day 1 of the oestrous cycle). Such similarity between these two associations suggests that the variation in the temperature did not influence the size of the corpus luteum at its establishment to a greater degree than the activity of the existing corpus luteum, although these two temperatures would be expected to be correlated.

Environmental temperature follows a distinct seasonal variation which might be expected to have a direct, parallel variation in the nutritional status of the sheep in the study. This would be a serious complication as undernutrition is known to stimulate increased progesterone concentrations (Cumming *et al.*, 1971; Lamond *et al.*, 1972; Shevah, Black, Carr & Land, 1975). However, this is known not to be the case in the present study as the plasma non-esterified fatty acid concentrations in these sheep and their mean body weights did not present any indication of a seasonal variation that might be linked to the environmental temperature (Wheeler & Land, 1977).

This effect of temperature on progesterone concentration might be mediated by prolactin, which is increased in the rat by a variety of stress stimuli (Krulich, Hefco, Illner & Read, 1974), varies directly in sheep with the environmental temperature (Schillo, Alliston & Malven, 1978; Hooley, Findlay & Stephenson, 1979), varies inversely with the peripheral progesterone concentration in sheep (Rhind *et al.*, 1978), and suppresses progesterone production by human granulosa cells *in vitro* (McNatty, Sawers & McNeilly, 1974).

Alternatively, as with other forms of stress, exposure to heat and cold results in a re-adjustment of the tonic discharge of the sympathetic and parasympathetic nervous systems (Cannon, 1929). It is well established that the sheep ovary is richly innervated by the autonomic nervous system (Stefenson, Owman, Sjöberg, Sporrang & Walles, 1981), and adrenergic agonists stimulate progesterone secretion by sheep luteal tissue *in vitro* (Jordan, Caffrey & Niswender, 1978). The effect of the low temperature may therefore be mediated by an increased sympathetic tone.

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