Plasma progesterone levels throughout the oestrous cycle and release of LH at oestrus in sheep with different ovulation rates

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Summary. The concentrations of progesterone in the peripheral plasma throughout the oestrous cycle and the preovulatory LH discharge were examined in Finnish Landrace, Galway and Fingalway (Finnish Landrace $\times$ Galway) ewes. Progesterone levels were significantly higher in Finnish Landrace ewes during the luteal phase of the cycle (Days 10–13) than in Galways or Fingalways in which the concentrations were similar. Luteal-phase progesterone levels were almost 50% higher during December than during October in all three breeds. The relationship between the number of CL and plasma progesterone was not a simple linear function. All aspects of the preovulatory LH discharge were similar in the three breeds with the exception of the timing of the LH release in relation to the onset of oestrus. This occurred earliest in the Galway and latest in the Finnish Landrace while the Fingalway was intermediate.

Introduction

The pattern of progesterone concentration in the peripheral plasma during the ovine oestrous cycle has been investigated by numerous authors (Stabenfeldt, Holt & Ewing, 1969; Thorburn, Bassett & Smith, 1969; McNatty, Revfeim & Young, 1973; Sarda, Robertson & Smeaton, 1973). There is, however, a paucity of comparative data on the levels of progesterone in the peripheral plasma of ewes which differ widely in natural ovulation rate and on the relationship between the number of corpora lutea and blood levels of progesterone. Finnish Landrace, Fingalway and Galway ewes are known to differ widely in genetic potential for ovulation rate (Hanrahan & Quirke, 1975). The experiment reported in the present paper was undertaken to obtain information on blood levels of progesterone throughout the oestrous cycle and also to characterize the preovulatory discharge of LH at oestrus for these 3 breeds. A preliminary report on part of this work has already been published (Quirke & Gosling, 1976).

Materials and Methods

The 56 ewes used were 5–6 years old and were of the Finnish Landrace (19 ewes), Fingalway (Finnish Landrace σ $\times$ Galway ϕ , 18 ewes) and Galway (19 ewes) breeds. They were assembled into a single flock in August 1974 and run at pasture until the end of the experiment. The animals were assigned at random (but balanced for breed) to 3 groups to facilitate the conduct of the experiment. The dates of oestrus and the hormonal measurements taken are summarized in Table 1.

Table 1. Experimental plan

	No. of ewes	Dates of		Hormone determinations	
		Sponge removal	2nd oestrus after sponge removal	Progesterone	LH
Group 1	15	17/9/1974	6–7 October	+	—
Group 2	15	23/9/1974	11–14 October	+	+
Group 3	26	11/11/1974	29 Nov.–2 Dec.	+	+

Oestrus and ovulation

Oestrus was synchronized by the use of progestagen-impregnated sponges (Veramix: Upjohn Co. Ltd, Crawley, Sussex) which were inserted into the anterior vagina for 14 days. The onset of first oestrus was established by using painted, vasectomized rams and inspecting the ewes twice daily for raddle marks. The onset of the second oestrus after sponge withdrawal was determined for the ewes in Group 1 by changing the raddle colour on the teaser rams and inspecting the flock daily for fresh marks while the animals were at pasture. The ewes in Groups 2 and 3 were housed on the 17th day after sponge removal and checked individually for oestrus every 4 h. The onset of oestrus was assumed to have occurred 2 h before the first positive test. The duration of oestrus was determined by testing the ewes individually every 2 h after the first positive test until the end of oestrus. Ewes were only associated with rams during the periodic checks for the presence of oestrus. At the end of oestrus the animals were released to pasture with teaser rams and observed once daily for the onset of the third oestrous period after sponge removal.

The number of ova shed at the second oestrus after treatment was assessed by counting the number of corpora lutea in the ovaries at laparoscopy between Days 7 and 11 of the cycle (day of onset of oestrus = Day 0).

Blood collection

To avoid any effect of progestagen treatment on hormone levels, blood samples for progesterone assay were taken daily (between 09:00 and 11:00 h) for the duration of the 2nd oestrous cycle after sponge removal. Blood samples for LH determination were collected from the ewes in Groups 2 and 3 close to the expected time of onset of and during the 2nd oestrus after treatment. These samples were collected in conjunction with the tests which established the onset and duration of this oestrus and were taken 1 h after each test.

The blood samples were taken from a jugular vein into evacuated tubes containing EDTA (Becton and Dickinson). All samples were centrifuged within 45 min of collection and blood awaiting centrifugation was kept in a refrigerator. The plasma was stored at -20°C until assay.

Hormone estimations

Plasma progesterone concentrations were determined using a conventional radioimmunoassay system with extraction into petroleum ether, $40\text{--}60^{\circ}\text{C}$ boiling range (BDH 10178), a tritiated label ($[1,2,6,7\text{-}^3\text{H}]\text{progesterone}$: Radiochemical Centre, Amersham) and a charcoal/Dextran separation step, as described by Gosling, Parker & Fottrell (1975). The extraction step had an efficiency of $88.7 \pm 0.6\%$ (s.e.m., $n = 67$), and the solvent blank was negligible as only selected batches of solvent were used. The antiserum (Y2O/2) was kindly supplied by Dr Brian Cook. Specificity was investigated by determining the concentrations of a wide range of steroids needed to give 50% inhibition of binding. Apart from steroids substituted in the 11 position (10% of progesterone, without extraction) only deoxycorticosterone cross-reacted to

any significant extent (8%). Analysis of plasma containing low levels of progesterone gave recoveries of 105.6 and 99.9% when 200 and 1000 pg progesterone respectively were added. The sensitivity, calculated from the error in the blank (twice the s.d.) and the slope for each assay, was in the range 20–50 pg/ml. For a plasma pool containing 2.40 ng progesterone/ml the within- and between-assay coefficients of variation were 6.7 and 9.2% respectively.

Plasma LH concentrations were determined using a liquid-phase, double-antibody radioimmunoassay (Hanrahan, Quirke & Gosling, 1977). The antiserum (D.B. 5/5), was kindly supplied by Dr Graham Jenkin. The sensitivity, calculated as described above, was 0.5 ng/ml. For a pooled sample containing about 40 ng LH/ml, the within- and between-assay coefficients of variation were 13 and 17% respectively. The results were expressed in terms of NIH-LH-S18.

Samples were assigned to assay runs, for both hormones, so that all genetic groups were represented in each run. The samples from individual animals were included in a single run as far as possible. Hormone determinations were carried out in duplicate and the mean value was used in all subsequent analyses.

Results

One Fingalway ewe which died in the course of the experiment and 2 Finnish Landrace ewes and one Galway, which had missed cycles, have been excluded from the results. It was not possible to count the number of CL in the ovaries of one Finn ewe at laparoscopy and this ewe was killed and the reproductive organs recovered on Day 12 of the 2nd cycle after treatment; this animal has been excluded from the results relating to plasma progesterone concentrations and cycle length but has been included elsewhere.

Plasma progesterone concentrations

The least squares means for bodyweight, ovulation rate and cycle length are given in Table 2. Breed means for luteal-phase progesterone (sum of the daily values of Days 10–13) and total progesterone (sum of daily values throughout the cycle) concentrations are also given. Breed differences for bodyweight and ovulation rate were all significant ($P < 0.05$). Cycle length was significantly shorter for the Finnish Landrace than for the Galway ($P < 0.01$); the Fingalway was intermediate. Values for luteal-phase and total progesterone were highest in the Finnish Landrace breed. For the purebred ewes, only the values for luteal-phase progesterone were different ($t = 2.52$, $P < 0.05$). Tests for heterosis were not significant for any trait. The mean daily plasma progesterone concentrations throughout the oestrous cycle are shown for the 3 breeds in Text-fig. 1, and for the three groups in Text-fig. 2. The data for the 3 breeds within a group were combined because there was no evidence of any breed $\times$ group interactions. Progesterone levels for ewes in Groups 1 and 2, whose cycles occurred in October, were lower

Table 2. Least squares means ($\pm$ s.e.m.) for various characteristics in the 3 breeds of sheep

	Finnish Landrace	Fingalway	Galway
No. of ewes	16	17	18
Bodyweight (kg)	46.1 $\pm$ 1.8	63.5 $\pm$ 1.6	72.4 $\pm$ 1.6
Ovulation rate	3.41 $\pm$ 0.19	2.05 $\pm$ 0.17	1.53 $\pm$ 0.17
Cycle length (days)	16.4 $\pm$ 0.20	16.7 $\pm$ 0.20	17.2 $\pm$ 0.20
Luteal-phase progesterone conc. (ng/ml)*	8.0 $\pm$ 0.75	7.3 $\pm$ 0.69	6.1 $\pm$ 0.68
Total progesterone conc. (ng/ml)†	19.2 $\pm$ 1.20	16.8 $\pm$ 1.10	16.2 $\pm$ 1.08

* Sum of values on Days 10–13.

† Sum of the daily values throughout the cycle.

Text-fig. 1. Mean daily plasma progesterone concentrations throughout the oestrous cycle in Galway (—), Fingalway (.....) and Finnish Landrace (---) ewes.

between the 6th and 13th days after the onset of oestrus than for ewes in Group 3 (cycles mainly in early December) and the difference was highly significant ($P < 0.01$) for total progesterone and luteal-phase concentrations. Ovulation rate, however, was similar for the three groups, the mean values being 2.27 ± 0.18 , 2.50 ± 0.21 and 2.22 ± 0.13 for Groups 1, 2 and 3 respectively.

Relationship between plasma progesterone and ovulation rate

This relationship was examined at two distinct phases of the oestrous cycle. The progesterone variables were (i) early progesterone which was the sum of the level on Days 1–4 and (ii) luteal progesterone which was the sum of the level on Days 10–13. The regressions of these variables on the number of CL and its log value were computed for each breed and the results were examined for heterogeneity among breeds. The analyses are summarized in Table 3

Text-fig. 2. Mean daily plasma progesterone concentrations during the second oestrous cycle after removal of progestagen-impregnated sponges on three dates during the breeding season: 17 September (Group 1, ---); 23 September (Group 2,); 11 November (Group 3, —).

Table 3. Analysis of the relationship between number of corpora lutea and plasma progesterone concentrations early in the cycle (Days 1–4 inclusive) and during the luteal phase (Days 10–13)

	Early progesterone		Luteal-phase progesterone	
	No. of CL	Log (no. of CL)	No. of CL	Log (no. of CL)
<i>Regression coefficients</i>				
Finnish Landrace	-0.26**	-1.83*	-0.26	-1.70
Fingalway	0.68**	2.54**	1.46	6.00
Galway	0.45*	1.49*	2.56**	8.49**
Source	d.f.	Mean Squares		
Heterogeneity	2	1.94**	1.32**	15.23*
Error	42	0.19	0.20	3.04

* $P < 0.05$.** $P < 0.01$.

and show significant heterogeneity among breeds in all cases except the regression of luteal progesterone on log (no. of CL). When breed differences in luteal progesterone were examined they just failed to reach formal significance ($F = 3.18$, critical $F = 3.23$). With log (no. of CL) as covariate the F ratio for breed differences was reduced to 0.014 and there was no significant difference between the mean luteal progesterone values for Finn and Galway breeds (14.6 versus 14.8 ng/ml). There was no evidence for breed differences in early progesterone levels.

The regression of log (luteal progesterone) on log (no. of CL) was computed for each breed and the resulting values were -0.072 ± 0.217 , 0.322 ± 0.203 and 0.570 ± 0.166 for Finnish Landrace, Fingalway and Galway ewes, respectively. All these regression coefficients are significantly smaller than unity, showing that a given relative increase in number of CL is accompanied by a smaller relative increase in plasma progesterone level in the luteal phase of the cycle. The regression coefficient for Galways was larger than that for Finnish Landrace ewes ($P < 0.05$).

LH release and duration of oestrus

The release of LH before ovulation was defined in terms of the following variables, having assumed an arbitrary threshold value of 6 ng/ml; (a) the interval between the onset of oestrus and beginning of the LH release, (b) the duration of the LH release, (c) the maximum LH concentration observed, and (d) the integral of the concentration of LH in excess of 6 ng/ml over the duration of the release. The breed means for these variables and the ovulation rates and durations of oestrus for the animals involved with this part of the study are given in Table 4.

Table 4. Least squares means $\pm$ s.e.m. for various characteristics related to the preovulatory LH discharge in the 3 breeds of sheep

	Finnish Landrace	Fingalway	Galway
No. of ewes	11	13	11
Ovulation rate	3.55 ± 0.24	2.00 ± 0.22	1.55 ± 0.24
Duration of oestrus (h)	41.1 ± 1.7	32.2 ± 1.6	28.0 ± 1.7
Interval from onset of oestrus to LH release (h)	$+8.9 \pm 0.9$	$+3.9 \pm 0.8$	-0.6 ± 0.9
Duration of the LH release (h)	11.8 ± 0.7	10.7 ± 0.6	12.2 ± 0.7
Maximum LH level observed (ng/ml)	89.5 ± 10.7	65.8 ± 9.9	85.7 ± 10.7
Total LH released*	270.4 ± 29.7	194.7 ± 27.3	239.2 ± 29.7

* Integral of LH concentration over the duration of LH release.

There were significant breed differences in ovulation rate and duration of oestrus ($P < 0.05$) and in the interval between the onset of oestrus and the start of the discharge of LH ($P < 0.01$). The other variables were similar for all three breeds.

The start of the preovulatory LH surge preceded oestrus by more than $\frac{1}{2}$ h in the Galway breed and occurred almost 9 h after the onset of oestrus in the Finnish Landrace breed. The crossbred Fingalway lay between the parental breed values. The pooled within-breed correlations between ovulation rate and (1) duration of oestrus and (2) interval between the onset of oestrus and beginning of the preovulatory LH discharge were 0.07 and 0.32, respectively.

Discussion

The pattern of progesterone levels observed during the oestrous cycle was similar to that previously reported for ewes (Stabenfeldt *et al.*, 1969; Thorburn *et al.*, 1969; Sarda *et al.*, 1973; McNatty *et al.*, 1973). While breed differences in ovulation rate were reflected in the levels of plasma progesterone in the luteal phase of the cycle the relationship between number of CL and plasma progesterone was not a simple linear function. The negative relationship between luteal progesterone and number of CL in the Finnish Landrace breed was not significantly different from zero, suggesting that progesterone level was at a plateau in this breed due to its high ovulation rate. It was evident from the results in the other breeds that successive increments in ovulation rate were accompanied by disproportionately small increases in progesterone level. Overall breed differences in the relationship between log (luteal-phase progesterone) and log (no. of CL) were not significant, suggesting that a common function could describe the dependence of plasma progesterone level in the luteal phase of the cycle on the number of CL. However, the regression coefficients for Finnish Landrace and Galway ewes were significantly different. Because there was little overlap between these breeds in the number of CL the present data do not allow an adequate test of breed differences in the relationship between number of CL and luteal-phase progesterone levels.

The regression of luteal-phase progesterone on the number of CL was largest in the Galway breed but the value of 2.56 is only a little larger than the standard deviation about the regression line, i.e. 1.74. This means that luteal-phase progesterone levels are of little value in distinguishing between ewes with 1 or 2 CL. This agrees with the findings of numerous other workers (Edgar & Ronaldson, 1958; Bindon, Ch'ang & Turner, 1971; Robertson & Sarda, 1971; Lamond & Gaddy, 1972; Lamond, Gaddy & Kennedy, 1972) and, together with the evidence for an asymptotic relationship between luteal-phase progesterone level and CL number found in the present study, would suggest that under normal physiological conditions progesterone levels are maintained within well defined limits.

A positive association between the number of CL in the ovaries after stimulation with PMSG and plasma progesterone has been noted in the ewe (Short, 1960; Thorburn *et al.*, 1969; Bindon *et al.*, 1971; Eastwood, Payne, Fairclough & McDonald, 1976), cow (Lamond & Gaddy, 1972) and pig (Webel, Reimers & Dziuk, 1975) during the oestrous cycle and in early pregnancy. Progesterone levels in gonadotrophin-treated heifers, however, were not found (Rajamhendren, Lague & Baker, 1976) to be correlated with the number of CL despite a very wide range in ovulation rate (1–17). The apparent disparity between these findings may, perhaps, be explained by the results of Plotka, Erb & Harrington (1970) who reported that the progesterone content of ovine luteal tissue is mainly related to the weight of the CL rather than to the number of CL or days after oestrus. There is clearly a need for information on the weight of CL in ewes with different ovulation rates, both natural and after gonadotrophin administration.

Luteal-phase progesterone levels are almost 50% higher in December than in October. This increase in progesterone concentration as the season advances is consistent with the results of Lamond *et al.* (1972) and Wheeler & Land (1977). It is difficult to find an explanation for this pattern but it may be associated with seasonal variation in plasma prolactin levels. An inverse

relationship between progesterone and prolactin levels during early pregnancy has been shown for ewes in December and March (Rhind, Chesworth & Robinson, 1978) with progesterone levels being higher in December than in March.

The present study shows that the maximum LH concentration, duration of the preovulatory LH discharge and the integral of LH concentration over the duration of discharge are not related to ovulation rate and are in accord with those of Thimonier & Pelletier (1971) and Land, Pelletier, Thimonier & Mauleon (1973). The results appear to confirm the generality of the findings in both of these reports that the interval between the onset of oestrus and the start of the discharge of LH increases with the number of ova shed. Data are not yet available to confirm or refute the hypothesis put forward by Land *et al.* (1973) that this relationship arises because of different sensitivities to oestrogen of the hypothalamic centres responsible for the release of LH.

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