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Release of prostaglandin F-2α and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths*

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Summary. Ewes (N = 32) were bled every 2 h from 5 days before expected oestrus until the end of oestrus. Plasma concentrations were determined for progesterone to monitor luteal activity and for the prostaglandin F-2α (PGF-2α) metabolites, 15-keto-13,14-dihydro-PGF-2α and 11-ketotetranor-PGF to determine uterine synthesis and release of PGF-2α. Most of the variation in cycle length was associated with the time of onset of luteolysis, the timing of events after luteolysis being constant and not related to cycle length. The time of occurrence of the first PGF-2α pulse and the interval between this pulse and the start of luteolysis were the two main determinants responsible for oestrous cycle length. Several PGF-2α pulses with interpulse intervals of 15·9 h occurred before the onset of functional luteolysis compared with 7·7 h for pulses associated with luteolysis. The numbers of PGF-2α pulses and interpulse intervals were similar for oestrous cycles of different lengths. While a gradual decline in progesterone concentrations was observed before functional luteolysis in the ewes with longer cycles, this did not appear to be an integral part of the stimulus which initiates the pulse frequency of PGF-2α required for luteolysis. We therefore suggest that differences in oestrous cycle length in the ewe are determined by the time of the onset of PGF-2α pulsatile release, and especially by the time of increased pulse frequency.

Keywords: PGF-2α; luteolysis; sheep; oestrous cycle

Introduction

The length of the oestrous cycle in the ewe is known to vary among individuals and according to breed of ewe. Average cycle lengths from 16·5 to 17·5 days are usually reported (Quirke *et al.*, 1979; Bindon *et al.*, 1979; Baird & McNeilly, 1981); in our own studies we have found most cycles to range between 15 and 18 days with occasional cycles as long as 21 days being observed. The length of the follicular phase of the ewe is relatively constant (Bindon *et al.*, 1979; Cahill *et al.*, 1981; Baird *et al.*, 1981) and therefore the variation in the length of the oestrous cycle appears to be mainly the result of variation in the time of onset of luteolysis. Luteolysis is initiated in the ewe by the synthesis and release of prostaglandin (PG) F-2α by the uterus (McCracken *et al.*, 1972). Differences in the

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timing of luteolysis must arise from differences in one or more of the following measures: time of the start of PGF-2α release; interval between start of PGF-2α release and onset of functional luteolysis, and interval from start to end of luteolysis. Currently there is no information about the contribution of each of these three components to the total length of the oestrous cycle of the ewe.

In this study we have examined the timing of events associated with luteolysis in ewes with different oestrous cycle lengths. The hormonal profiles of these animals were evaluated in detail and we also studied ewes from several breeds to evaluate the possibility that, even for ewes having the same cycle length, the timing of events associated with luteolysis might vary as a function of breed.

Materials and Methods

Animals. Ewes of the following breeds were used: Dorset (N = 6), Finnish-Landrace (N = 7), Rambouillet (N = 6), Suffolk (N = 7) and Finnish-Landrace × Rambouillet (N = 6). The animals and breeds were selected in order to have representative numbers of animals with cycles of 15–19 days in duration. They were maintained in an outdoor pen with access to an indoor area and were fed alfalfa cubes. They were teased daily with vasectomized rams from May to September and twice daily subsequently. The first day of oestrus was designated Day 0 of the oestrous cycle. Before the study, the occurrence of oestrus was recorded for 5 complete oestrous cycles for each ewe and cycle lengths were calculated. The repeatability of oestrous cycle length was calculated as the intra-class correlation from the variance components among and within ewes (Turner & Young, 1969).

Blood samples were obtained daily by jugular venepuncture starting on the 1st day of the 6th oestrous cycle for each ewe. We found a high repeatability of oestrous cycle lengths (0·62, $P < 0·01$, 160 cycles) which enabled us to predict the day of expected onset of the next oestrus and the time of onset of the intensive bleeding schedule. Blood samples were obtained at 2-h intervals, starting 5 days before the expected onset of the 7th oestrus, until the end of oestrus for each animal. The sampling interval was selected on the basis that previous work including our own has shown PGF-2α pulses to have a duration of several hours and an interval time of 6–8 h (Thorburn *et al.*, 1972, 1973; McCracken *et al.*, 1981, 1984; Zarco *et al.*, 1984). The samples were immediately centrifuged, and the plasma was kept in three aliquants at −20°C until assayed. Ewes were checked for oestrus with vasectomized rams every 2 h during the intensive bleeding regimen.

Hormonal assays. All samples were analysed for progesterone, 15-keto-13,14-dihydro-PGF-2α and 11-ketotetranor-PGF. The analysis of progesterone was done by an enzyme immunoassay (Munro & Stabenfeldt, 1984). The sensitivity of the assay was 75 pg/ml. The inter-assay coefficient of variation ranged from 10·9 to 14·5% at different concentrations of hormone, and the intra-assay coefficient of variation from 4·9 to 10·5%.

The release of PGF-2α in pulsatile mode was determined by the measurement of the 15-keto-13,14-dihydro-PGF-2α and 11-ketotetranor metabolites of PGF-2α. We have previously found that the identification of PGF-2α pulses is more accurate when both metabolites are determined in plasma samples collected at 2-h intervals (Zarco *et al.*, 1984). The initial main metabolite of PGF-2α, 15-keto-13,14-dihydro-PGF-2α, reaches peak concentrations within minutes after PGF-2α administration with a $t_{\frac{1}{2}}$ of about 10 min (Granström & Kindahl, 1982a) while the 11-ketotetranor metabolites take longer to reach peak concentrations and have a half life of about 2 h in the ewe (Granström & Kindahl, 1982b, c). Metabolites of PGF-2α were analysed because of much longer half lives as compared to PGF-2α and because metabolite concentrations are stable in blood samples (require tissues such as the lungs for formation) whereas PGF-2α can be synthesized by components of blood *in vitro* unless rigidly controlled.

The radioimmunoassay for the initial main plasma metabolite of PGF-2α, 15-keto-13,14-dihydro-PGF-2α, has been previously described (Granström & Kindahl, 1982a). The sensitivity of the assay was 10 pg/ml. Inter-assay coefficient of variation was 14%, and intra-assay coefficient of variation ranged from 6·6 to 11·7%. The radioimmunoassay for 11-ketotetranor-PGF metabolites has been described (Granström & Kindahl, 1982b, c). The sensitivity of the assay is 4 pg and the intra- and inter-assay coefficients of variation are 6·3 to 10·1% and 12%, respectively (Granström & Kindahl, 1976; Basu *et al.*, 1987). The antibody did not differentiate between the different 11-ketotetranor-PGF compounds.

Definitions. Significant pulses of each PGF-2α metabolite were identified in each animal by an iterative method of analysis (Zarco *et al.*, 1984). A pulse of PGF-2α release was considered to have occurred when a pulse of one or both of the PGF-2α metabolites was identified. Interpulse intervals were estimated as the time elapsed between the peak values of two consecutive pulses.

Functional luteolysis was considered to have begun at a certain sampling time if the mean progesterone value of the subsequent 4 samples was less than the mean minus 2 standard deviations of all previous samples for that particular ewe. The time of completion of luteolysis was defined as the time when progesterone declined to $< 0·25$ ng/ml. The time of onset of oestrus was defined as the time when the ewe first permitted mounting by a vasectomized ram.

Data analysis. The following values were recorded for each ewe: (1) time of first pulse of PGF-2α; (2) time of onset of functional luteolysis; (3) time of completion of luteolysis; (4) time of onset of oestrus; (5) interval from the first detected pulse of PGF-2α to the onset of oestrus and the components of this interval, namely the interval from the first

PGF-2α pulse to start of functional luteolysis, the interval from start to end of functional luteolysis, and the interval from completion of luteolysis to start of oestrus; (6) total number of pulses of PGF-2α and the number of pulses occurring before and after the start of functional luteolysis; (7) interpulse intervals for all pulses and for pulses occurring before the onset of and during luteolysis. The effects of breed on these measures were studied by least squares analysis of variance procedures. The relationship of the values to the length of the cycle was determined by regression analysis.

To predict the timing of luteolytic events as a function of the duration of the oestrous cycle, regression equations in the general form $y = a + bx$ were derived, where y is the event to be predicted, a is a constant, b the pooled within breed regression coefficient and x the length of the cycle in question. From a physiological point of view, cycle length should be the dependent variable if there is a cause–effect relationship between any of these traits and cycle length. However, cycle length was used as the independent variable because it is an easily observed trait from which the other, more difficult to observe, traits can be predicted.

Results

Three ewes developed spontaneous persistence of luteal function and were eliminated from the study. Data in relation to these 3 animals have been presented elsewhere (Zarco *et al.*, 1984).

As shown in Table 1, there were breed differences in the mean length of the oestrous cycle studied intensively ($P < 0.025$). Table 1 also shows, on a breed basis, the timing of events associated with luteolysis and the intervals between such events. Only the time of start and time of completion of luteolysis were different for different breeds ($P < 0.01$). In all cases, the variation associated with breed was greatly reduced (as indicated by lower F values) when the ANOVA was recalculated using cycle length as a concomitant variable (analysis of covariance; Neter & Wasserman, 1974), differences between breeds being no longer significant for any value. For this reason, data from all breeds were pooled, and all subsequent regression analyses were done using cycle length as the sole independent variable.

Table 1. Time of occurrence of several events associated with luteolysis in sheep (onset of oestrus = Day 1)

	Breed						
	Suffolk (N = 6)	Dorset (N = 6)	Finn (N = 6)	Rambouillet (N = 5)	F × R (N = 6)	ANOVA*	Covariance analysis†
Cycle length (days)	15·5	16·5	16·9	17·5	17·3	F = 3·71 $P < 0.025$	
Day of:							
First PGF-2α pulse	12·9	13·1	14·1	14·1	14·0	F = 2·02 N.S.	F < 1 N.S.
Start of luteolysis	13·2	14·5	15·3	15·7	15·4	F = 5·84 $P < 0.01$	F = 2·25 N.S.
Completion of luteolysis	14·4	15·5	15·9	16·3	16·3	F = 4·12 $P < 0.01$	F = 0·65 N.S.
Interval (h) for:							
First pulse to start of luteolysis	9·3	32·7	30·0	37·6	33·0	F = 2·21 N.S.	F < 1 N.S.
Start to end of luteolysis	28·0	24·0	13·3	15·6	20·7	F = 2·54 N.S.	F = 1·87 N.S.
End of luteolysis to oestrus	26·0	25·3	24·3	28·0	24·3	F < 1 N.S.	F < 1 N.S.

*The F values represent variation due to breed. The error term for the calculation of F is the variation within breeds plus random error.

†The F values represent the variation among breeds after the sum of squares and their associated degrees of freedom have been adjusted for cycle length.

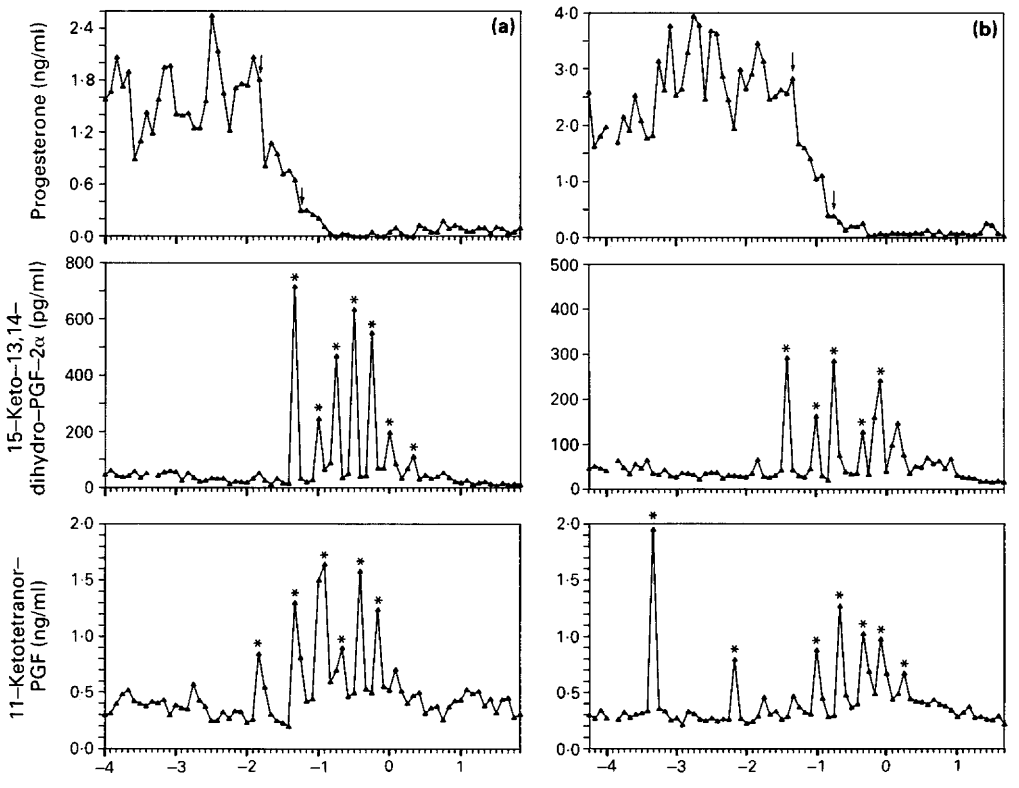

Fig. 1(a) and (b).

Figure 1 (a,b,c,d) shows the hormonal profiles of 4 ewes with different cycle lengths. The results pertaining to the relation between oestrous cycle length and the events associated with luteolysis are summarized in Table 2. Most of the variation in cycle length was associated with variation in the time of completion of luteolysis (A in Table 2), while the interval from completion of luteolysis to the onset of oestrus (B in Table 2) was not related to cycle length. Most of the variation in the time of completion of luteolysis was associated with variation in the time of the start of luteolysis (1 in Table 2), which was closely correlated to cycle length. There was no relationship between the duration of the luteolytic process (2 in Table 2) and cycle length.

The time of start of luteolysis was further divided into two components: (1) the time of the first pulse of PGF-2α secretion, and (2) the interval between this pulse and the onset of luteolysis (a and b in Table 2). Both components were correlated to cycle length and the contribution of each to the variation in cycle length was similar. Graphic representations of the main correlations to cycle length are presented in Fig. 2.

The mean (±s.e.m.) number of pulses of PGF-2α detected in each ewe was 8·3 ± 0·3 and the mean interval between pulses was 10·1 ± 0·4 h. It was found that 2·1 ± 0·2 pulses occurred before the onset of functional luteolysis and 6·3 ± 0·3 were temporally associated with luteolysis including those observed after luteolysis. The pulses which occurred before the onset of luteolysis had a lower frequency (15·9 ± 1·1 h intervals) than did the pulses associated with luteolysis (7·7 ± 0·2 h intervals).

Neither the total number of pulses nor the number of pulses occurring during luteolysis were significantly related to cycle length. The number of pulses occurring before luteolysis tended to increase as cycle length increased, i.e. Suffolks with 15·5-day cycle intervals tended to have one

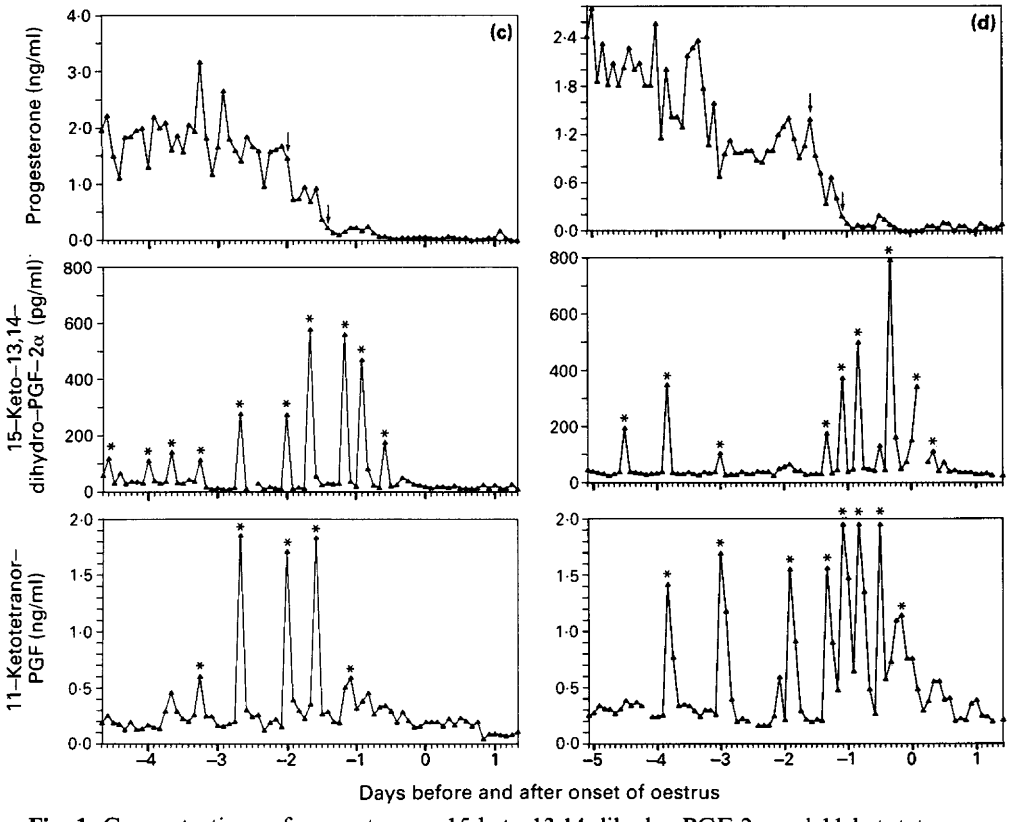

Fig. 1. Concentrations of progesterone, 15-keto-13,14-dihydro-PGF-2α and 11-ketotetranor-PGF in ewes with oestrous cycles of (a) 15·0 days, (b) 16·2 days, (c) 17·6 days and (d) 18·1 days. Values identified as significant pulses of either PGF-2α metabolite are indicated by an asterisk. The times of initiation and completion of functional luteolysis are indicated by arrows. The time scale is normalized to the onset of oestrus (0).

Table 2. Relationship of the oestrous cycle length with the time of occurrence of events associated with luteolysis in sheep

Oestrous cycle component	Mean ± s.e.m. (days) (N = 29)	Regression on cycle length	Correlation with cycle length
A. Time from previous oestrus to completion of luteolysis	15·67 ± 0·20	$y = -0{\cdot}28 + 0{\cdot}95x$ $P < 0{\cdot}01$	0·98
1. Time from previous oestrus to start of luteolysis	14·82 ± 0·22	$y = -2{\cdot}89 + 1{\cdot}06x$ $P < 0{\cdot}01$	0·93
(a) time of first PGF-2α pulse	13·63 ± 0·16	$y = 3{\cdot}39 \pm 0{\cdot}61x$ $P < 0{\cdot}01$	0·65
(b) interval from first pulse to start of luteolysis	1·19 ± 0·15	$y = -6{\cdot}17 + 0{\cdot}44x$ $P < 0{\cdot}01$	0·61
2. Interval from start to end of luteolysis	0·85 ± 0·08	$y = 2{\cdot}54 - 0{\cdot}10x$ N.S.	0·27
B. Interval from end of luteolysis to oestrus	1·07 ± 0·04	$y = 0{\cdot}26 + 0{\cdot}05x$ N.S.	0·24

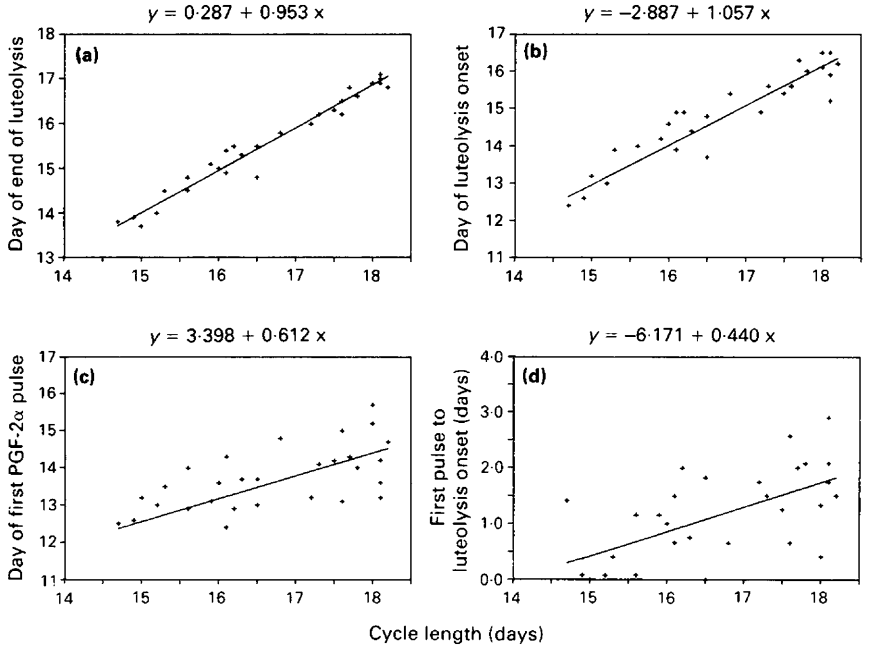

Fig. 2. Regression curves of oestrous cycle length against: (a) day of end of luteolysis; (b) day of onset of luteolysis; (c) day of first pulse of PGF-2α; (d) interval from the first PGF-2α pulse to start of functional luteolysis. The regression equations are shown on top of each panel, the units of the equations are in days. The regression coefficients are statistically significant in all cases ($P < 0{\cdot}01$).

pulse before luteolysis whereas other breeds with longer cycle intervals had closer to two pulses. There was no relationship between cycle length and the frequency of all pulses, pulses occurring before the onset of luteolysis, or pulses associated with luteolysis.

The use of two PGF-2α metabolite assay systems allowed a more accurate assessment to be made of pulsatile synthesis and release of PGF-2α. The 11-ketotetranor-PGF assay allowed 17/135 PGF-2α pulses to be identified in the absence of any significant change in 15-keto-13,14-dihydro-PGF-2α concentrations and the 15-keto-13,14-dihydro-PGF-2α assay identified 27/135 pulses in the absence of any significant change in 11-ketotetranor-PGF concentrations.

Progesterone concentrations associated with PGF-2α pulses occurring before the onset of luteolysis were studied to assess the effect of such pulses on luteal function. For each pulse, the average progesterone concentration of the 4 samples preceding the pulse were compared to the mean values of samples 1–3 and 4–6 after the pulse. In 29 of 36 cases the mean progesterone concentrations of samples 1–3 after the pulse were lower than the mean levels of the 4 samples before the pulse. In 23 of the 29 cases the mean progesterone values were higher in samples 4–6 than in samples 1–3 after the pulse. The decline and subsequent recovery were statistically significant ($P < 0{\cdot}025$) when studied by ANOVA for repeated measures.

In an analysis of the timing of the onset of luteolysis, oestrous cycles were divided into 4 groups according to cycle length (Group I, 15·0–15·9 days; Group II, 16·0–16·9 days; Group III, 17·0–17·9 days; and Group IV, 18·0–18·9 days). For each of these groups progesterone concentrations in animals from Day 4 before oestrus (Day −4) to the time of completion of luteolysis were analysed by fitting a regression model that included the linear and quadratic effects of time on progesterone concentrations. Both the linear and quadratic coefficients were significant in all 4 groups ($P < 0{\cdot}01$). The quadratic effect was assumed to be due to the increased rate of progesterone

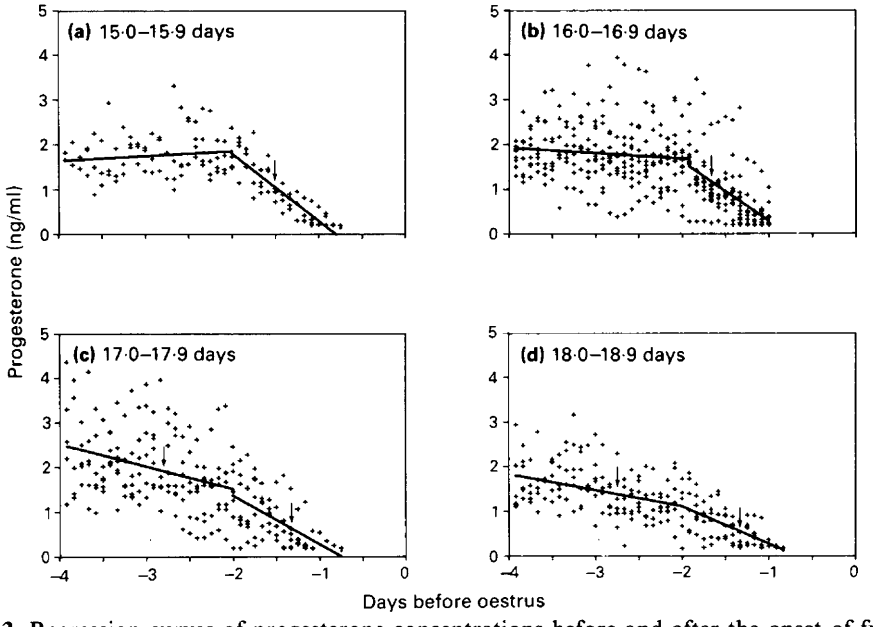

Fig. 3. Regression curves of progesterone concentrations before and after the onset of functional luteolysis in ewes with different oestrous cycle lengths. The cycle lengths of the ewes used for the regressions are shown at top of each panel. The slopes of lines indicated by an arrow are significantly different from 0 ($P < 0{\cdot}01$). The data for panels (a), (b), (c) and (d) came from 7, 9, 7 and 6 ewes, respectively.

decline that follows the onset of functional luteolysis. Therefore, progesterone concentrations of each of the 4 groups were analysed by fitting two regression lines: the first line represents the profile of progesterone before the onset of luteolysis and included data from Day −4 to the time of onset of luteolysis, and the second line represents the profile of progesterone during the process of luteolysis and includes data from the time of luteolysis onset to the end of luteolysis. The resulting regression lines are shown in Fig. 3. There was no significant change in the concentration of progesterone between Day −4 and the onset of luteolysis in Groups I and II. In contrast, there was a significant decrease in progesterone concentrations during this period in Groups III and IV ($P < 0{\cdot}01$); the 4 regression lines were different from each other ($P < 0{\cdot}01$). The progesterone concentrations showed a significant linear decrease during luteolysis in all 4 groups ($P < 0{\cdot}01$). The 4 regression lines were different ($P < 0{\cdot}01$) due to a higher starting concentration of progesterone at the time of luteolysis onset in animals with shorter cycles.

The sampling protocol had no effect on luteolysis and subsequent follicle development in that an ANOVA for repeated measures revealed that the mean length of the oestrous cycles under study was not different from the length of the previous cycles for the same ewes ($P > 0{\cdot}10$).

Discussion

Our results show that the length of the oestrous cycle in ewes is almost completely determined by the time at which functional luteolysis is initiated. The timing of the first pulse of PGF-2α release and the interval from this pulse to the start of functional luteolysis were responsible for the variability in the time of luteolysis onset. Once functional luteolysis begins, the time required for progesterone to reach baseline concentrations and the timing of events subsequent to luteolysis are constant and not related to oestrous cycle length. These results support previous reports of an

intrinsic constancy of the timing of events following the onset of luteolysis (Trounson *et al.*, 1976; Bindon *et al.*, 1979; Cahill *et al.*, 1981; Baird & McNeilly, 1981; Baird *et al.*, 1981; Tsonis *et al.*, 1984). Our finding that the natural variability in the length of the oestrous cycle of the ewe is due mainly to differences in the time at which the onset of luteolysis occurs leads one to view with caution the results of studies in which specific events related to luteal function are assumed to occur at specific days of the cycle. This caution has been previously expressed by others (Peterson *et al.*, 1976; O'Shea *et al.*, 1977).

The combined results of the ANOVA and covariance analysis of breed effects indicate that the measures studied were associated with the length of the oestrous cycle and that the differences between breeds were simply due to the differences in the mean cycle length of each breed. This indicates that, although the cycle length of a particular breed might be genetically determined, the physiological mechanism by which a given cycle length is obtained is the same for different breeds.

The mechanisms that control the timing of the first release of PGF-2α are not known. For the past two decades evidence has been slowly accumulating in favour of the concept that luteolysis occurs after the uterus has been exposed to progesterone for a relatively fixed period of time and that the CL is thus indirectly responsible for its own demise (Woody *et al.*, 1967, 1968; Ottobre *et al.*, 1980). If this concept is true, the length of the ovine oestrous cycle could be ultimately determined by the time elapsed between ovulation and the development of the capacity of the corpus luteum to produce physiologically significant amounts of progesterone. Variations in this interval could be responsible for the variation in the time of the onset of luteolysis as found in the present study. We studied the profiles of progesterone during the first 10 days of the oestrous cycle of each animal, but were unable to find any relationship between the time or the rate of progesterone increase and the length of the oestrous cycle. However, during the first 10 days of the oestrous cycle samples were only taken daily and so subtle differences in progesterone patterns could have been missed. A detailed study of the patterns of progesterone secretion early in the oestrous cycle, and their relation to cycle length, is needed.

Our finding that the average interpulse interval for pulses occurring before the onset of luteolysis was longer (15·9 h) than the average interpulse interval for pulses associated with luteolysis (7·7 h) supports previous suggestions that a series of pulses occurring at relatively short intervals (6–8 h) is necessary for irreversible luteolysis in sheep (Thorburn *et al.*, 1972, 1973; McCracken *et al.*, 1981, 1984; Zarco *et al.*, 1984). The finding that most low-frequency PGF-2α pulses were followed by transient declines in progesterone concentrations suggests that the CL can respond to PGF-2α beginning with the first pulse of each cycle. However, the increased frequency of the pulses associated with luteolysis is essential for irreversible luteolysis, since it allows for a new surge of PGF-2α to act upon the CL before it has recovered from the inhibitory effect of the previous pulse.

We have previously found that the analysis of 2 metabolites of PGF-2α, 15-keto-13,14-dihydro-PGF-2α and 11-ketotetranor-PGF, gave a more accurate assessment of PGF-2α pulses than did either one alone (Zarco *et al.*, 1984). The reason for this is not clear because the half-lives of the metabolites, 10 min for 15-keto-13,14-dihydro-PGF-2α (Granström & Kindahl, 1976) and about 2 h for 11-ketotetranor-PGF (Granström & Kindahl, 1982b, c), are such that, theoretically, no pulses should be missed with each assay system. The time frames wherein PGF-2α pulses were identified by only one metabolite assay were logical in terms of associated pulse rates.

It has been suggested that a decline in progesterone concentration is the trigger for the establishment of the high-frequency pulsatile PGF-2α synthesis and release observed at luteolysis (Thorburn *et al.*, 1973; for review see Rothchild, 1981). However, in animals with 15–16·9 day cycles (current study) the progesterone concentrations did not decrease before the frequency of PGF-2α release increased, suggesting that a previous decline in progesterone concentrations is not essential for the establishment of a high-frequency release pattern of PGF-2α. This is supported by the results of Fairclough *et al.* (1983) who found that the treatment of intact ewes with medroxyprogesterone acetate twice a day starting on Day 10 of the cycle did not alter the length of the oestrous cycle, with the pulsatile secretion of both PGF-2α and oxytocin occurring at the expected time.

Further, our finding that most PGF-2α pulses occurring before luteolysis caused a transient decline in progesterone concentrations, without an immediate increase in the frequency of PGF-2α secretion, suggests that a decline in progesterone concentrations is not a signal sufficient to increase the frequency of PGF-2α release. There is experimental evidence in favour of this concept in that administration of either an inhibitor of 3β-hydroxysteroid-dehydrogenase (trilostane) (Jenkin *et al.*, 1984) or non-luteolytic doses of PGF-2α (Thorburn & Nicol, 1971) during the mid-luteal phase resulted in transient declines in progesterone concentrations that were followed by recovery to pre-treatment values. Concentrations of 15-keto-13,14-dihydro-PGF-2α did not increase during or after the decline in progesterone induced by trilostane, indicating that the initial fall in progesterone was not able to initiate endogenous release of PGF-2α that would have led to completion of luteolysis.

It is concluded that the length of the oestrous cycle is determined by the time of luteolysis onset; the mechanisms which control the timing of the first PGF-2α release and its subsequent increase in frequency are not known. A decline in progesterone concentration does not appear to be essential for the increased frequency of pulsatile PGF-2α release required for luteolysis.

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