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Studies of hormone patterns during the oestrous cycle of beef cows

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Summary. Hormone patterns in blood plasma were examined in relation to the bovine oestrous cycle in two experiments. During the luteal phase LH pulse frequency was low (2-3 pulses per 8 h). There were no consistent correlations between LH, FSH and progesterone concentrations. During luteolysis, falling progesterone concentrations were accompanied by an increase in oestradiol-17 β concentrations, LH concentrations and LH pulse frequency, the latter being almost one pulse per hour on the day before the preovulatory surge.

Introduction.

An understanding of the natural hormone events during the ovarian cycle is a pre-requisite for the ability to control it accurately by pharmacological means.

Plasma LH concentrations are low for most of the bovine oestrous cycle with a peak or surge of secretion occurring at about the time of oestrus (Swanson and Hafs, 1971 ; Hansel, Concannon and Lukaszewska, 1973 ; Christensen, Hopwood and Wiltbank, 1974). Although further smaller peaks in plasma LH concentrations during the luteal phase have been reported (Schams and Karg, 1969 ; Henricks, Dickey and Niswender, 1970 ; Snook, Saatman and Hansel, 1971), more detailed observations have since shown that, as in other species, episodes of LH secretion occur regularly throughout the cycle and that their frequency and amplitude appear to be dependent on the stage of the cycle (Rahe *et al.*, 1980). A peak in plasma FSH concentrations also occurs at oestrus coinciding with the LH surge (Akbar *et al.*, 1974 ; Schams and Schallenberger, 1976) with a secondary FSH peak of smaller magnitude occurring some 24 h later (Dobson, 1978). Plasma concentrations of oestradiol-17β rise over the four days before oestrus, with a peak occurring on the day before or on the day of oestrus behaviour (Glencross

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and Pope, 1981 ; Glencross *et al.*, 1981a). Progesterone concentrations begin to fall from high luteal levels on about day 17 of the cycle and remain low until days 4 or 5 of the next cycle, at the time of formation of a new corpus luteum (Robertson, 1972).

The final stages of follicle maturation are considered to be under the control of the gonadotrophins, but until recently, the relationship between LH, FSH, oestradiol-17β and progesterone have not been described in detail for the cow. The present studies were undertaken to examine in more detail than previously, changes in plasma concentrations of these hormones during the luteal and follicular phases of the ovarian cycle.

Materials and Methods.

Ten Hereford × Friesian single-suckling beef cows each undergoing normal oestrous cycles were used for the study.

1. *Experiment one.* — Blood samples (2 ml for LH assay) were taken from four cows on day 10 after oestrus (mid-luteal phase), at 10-min intervals for a 24-h period. In addition, commencing on days 17, 18, 19 and 20 after oestrus (follicular phase) blood samples (2 ml), were taken at 10-min intervals for 8-h periods (see table 1). During these intensive sampling periods, larger samples (10 ml) were taken hourly to allow for assay of FSH and progesterone. Consecutive pairs of hourly samples were then pooled to assay for oestradiol-17β. Subsequently, blood samples (2 ml) were taken at 6-hourly intervals for a further 48 h to determine the timing of the preovulatory LH surge.

2. *Experiment two.* — Blood samples were taken from six cows on day 5 (n = 2 ; early luteal phase) or day 9 (n = 4 ; mid-luteal phase) of the oestrous cycle at 15-min intervals, for an 8-h period (see table 2). Plasma was assayed for LH, FSH and progesterone.

Hormone assays. — Plasma progesterone and LH concentrations were assayed as described by Webb *et al.* (1977) and plasma FSH was assayed as described by Webb *et al.* (1980). The assay reliability criteria did not differ significantly from those previously published. Plasma concentrations of oestradiol-17β were measured using the original method of Glencross and Pope (1981) with the modifications described by Glencross *et al.* (1981b) and Peters (1984). The sensitivity, intraassay coefficient of variation (CV) and interassay CV were 1.60 pg/ml, 17.90 % and 22.80 % respectively.

Analysis of results. — Average hormone concentrations were calculated for each frequent sampling period to produce a representative hormone concentration for that occasion. Relationships between the different hormones were analysed by linear regression analysis.

In experiment two, cross-correlation analyses were carried out between the plasma concentrations of LH, FSH and progesterone using a variable lag between sample pairs. For example [LH] in sample 1 v. [FSH] in sample 1, [LH] in sample 1 v. [FSH] in sample 2, [LH] in sample 2 v. [FSH] in sample 3 etc. A hormone pulse was defined as an increase of at least 50 per cent from the adjacent baseline value with at least two 10 or 15-min sample points on the downward limb (Riley *et al.*, 1981).

Results.

Experiment one. — Evidence of overt oestrus and preovulatory-type LH surges (plasma concentrations of 12-18 ng/ml) as determined by the 6-hourly sampling, occurred on day 21 after the first oestrus in Cows, A, C and D and on day 22 in Cow B. For convenience, hormone data for the follicular phase were standardised to the day on which the subsequent LH surge occurred (day 0). The mean plasma hormone concentrations and LH pulse amplitudes and frequencies for the five intensive sampling periods are shown in table 1. The decrease in mean plasma progesterone concentrations during the four follicular phase sampling periods (see table 1) was negatively correlated with rising plasma LH concentrations ($r = -0.99$; $P < 0.001$), LH pulse frequency ($r = -0.99$; $P < 0.001$) and plasma oestradiol-17β concentrations ($r = -0.957$; $P < 0.001$). The increasing plasma concentrations of LH and oestradiol-17β were also highly correlated ($r = 0.927$; $P < 0.001$). Neither LH pulse amplitude nor mean FSH concentrations changed significantly between any of the sampling periods (see table 1), except that there was a significant decrease in mean FSH concentrations between days 4 and 3 before the LH surge ($P < 0.05$).

The series of hormone profiles for Cow D is shown in figure 1. In all four mid-luteal phase LH profiles, the baseline fluctuated markedly with an irregular pulse pattern compared to those taken in the follicular phase. However approximately six pulses were observed in each cow's profile during the 24-hour sampling period (see table 1). Hourly plasma FSH and oestradiol-17β concentrations fluctuated markedly during the mid-luteal sampling period but did not show any consistent pattern.

Experiment two. — Mean values for LH, FSH and progesterone concentrations are shown in table 2. LH concentrations fluctuated in a pulsatile manner. The LH pulse frequency of cows 26 and 36 on day 5 of the cycle (early luteal phase) was higher (6 to 7 pulses per 8 h) than in the four cows sampled on day 9 (2 to 3 pulses per 8 h ; mid-luteal phase) except in one cow (no. 38) which still exhibited 6 pulses per 8 h (see table 2 and fig. 2). No attempt was made to quantify FSH and progesterone pulses (see Discussion). Results of cross correlation analyses between concentrations of the three hormones are shown in table 3, but showed no consistently significant relationships.

TABLE 1
Plasma hormones during the ovarian cycle (mean ± s.e.m.).

Phase of cycle	Hormone	Concentrations			LH pulse frequency	LH pulse amplitude
	Progesterone ng/ml	Oestradiol-17β pg/ml	FSH ng/ml	LH ng/ml	Pulses/8 h	ng/ml
Mid-luteal	11.7 ± 1.7	2.88 ± 0.12	45.8 ± 7.3	1.80 ± 0.05	2.0 ± 0.18	1.91 ± 0.23
Early follicular (days before LH surge)						
4	7.2 ± 0.9	2.89 ± 0.19	59.6 ± 7.7	1.20 ± 0.30	5.0 ± 0.6	1.34 ± 0.18
3	5.4 ± 1.2	4.05 ± 0.55	41.2 ± 2.9	1.56 ± 0.38	5.6 ± 0.9	1.78 ± 0.18
2	2.5 ± 0.9	5.66 ± 1.19	41.6 ± 4.3	1.99 ± 0.40	7.1 ± 0.5	1.69 ± 0.08
1	0.8 ± 0.15	8.05 ± 1.86	46.2 ± 4.9	2.46 ± 0.64	7.7 ± 0.7	1.87 ± 0.09

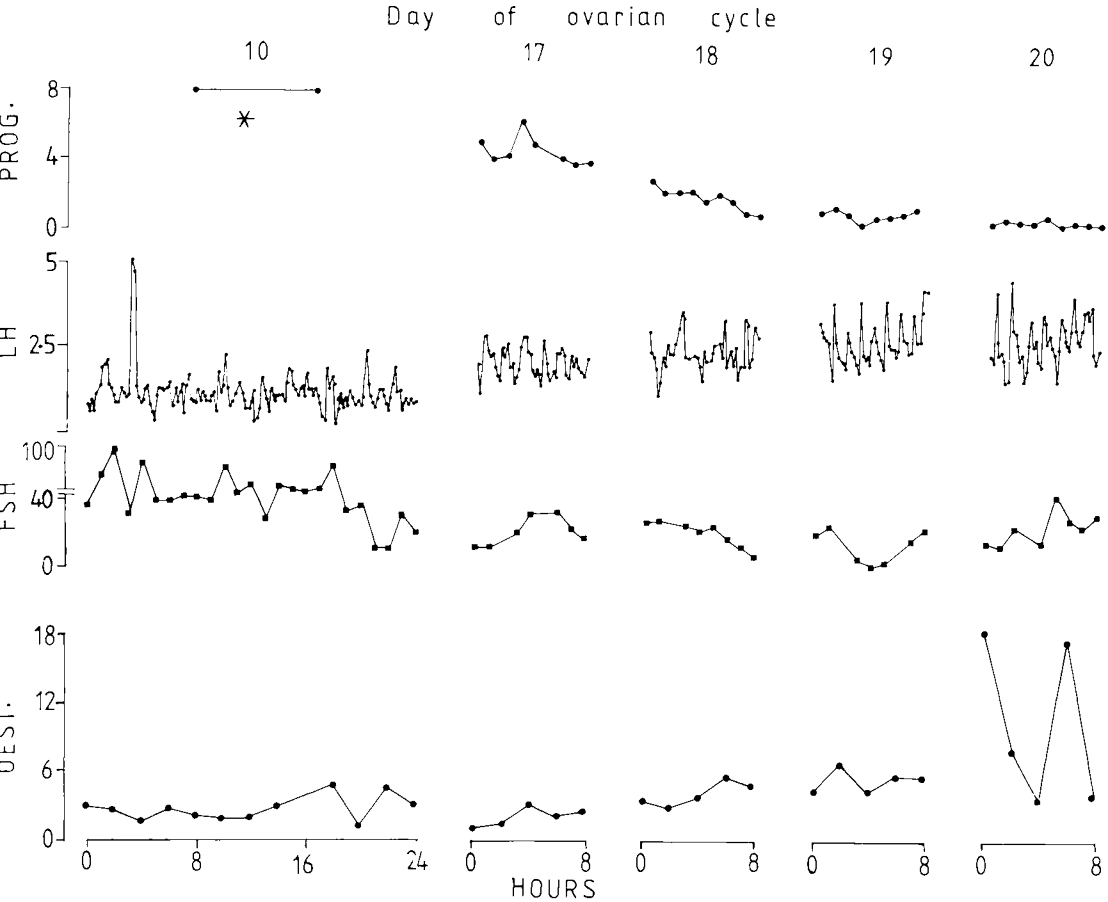

FIG. 1. — *Plasma concentrations of progesterone, LH, FSH* (ng/ml) *and oestradiol-17β* (pg/ml) *in cow D measured during the mid-luteal phase and on days 17, 18, 19 and 20 of the ovarian cycle.* Note the progressive fall in progesterone concentrations to basal levels by day 20. The LH baseline fluctuates markedly during the mid-luteal phase but a pulsatile pattern becomes more distinct between days 17 and 20 as the baseline rises. Plasma FSH concentrations fluctuate markedly but show no consistent change. Oestradiol-17β concentrations rise from low levels to peak concentrations on day 20.
* Single pooled sample only measured for progesterone on day 10.

TABLE 2
Mean (± s.e.m.) *hormone concentrations* (ng/ml) *during the luteal phase of the ovarian cycle.*

Cow no	Day of cycle	LH	FSH	Progesterone
26	5	1.24 ± 0.06 (6)	61.06 ± 4.83	5.30 ± 0.25
36	5	1.97 ± 0.12 (7)	69.35 ± 3.93	5.75 ± 0.31
25	9	2.01 ± 0.12 (2)	76.02 ± 5.46	11.45 ± 0.35
31	9	1.82 ± 0.15 (3)	83.58 ± 4.09	8.69 ± 0.24
37	9	1.59 ± 0.09 (2)	121.66 ± 7.07	11.64 ± 0.51
38	9	1.91 ± 0.12 (6)	119.60 ± 6.09	11.78 ± 0.47

Values in parentheses represent the number of hormone pulses during the 8-hour sampling period.

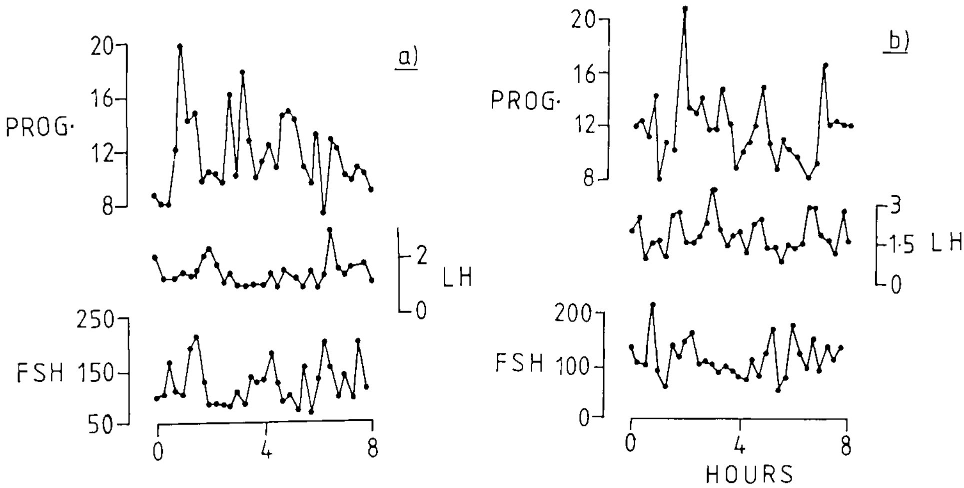

FIG. 2. — *Plasma concentrations of progesterone, FSH and LH* (ng/ml) *in* a) *cow 37 and* b) *cow 38, both on day 9 of the ovarian cycle* (mid-luteal phase).

TABLE 3
Cross correlation coefficients between hormone concentrations in cows during the luteal phase of the ovarian cycle.

	LH : FSH		FSH : LH		LH : Prog		Prog : LH		FSH : Prog		Prog : FSH	
Cow No	(1)r	(2)lag	r	lag	r	lag	r	lag	r	lag	r	lag
26	− 0.24	2	0.26	5	− 0.40	4	—	—	− 0.20	2	− 0.39*	3
36	− 0.36*	5	0.23	5	0.35*	5	0.40*	8	0.25	6	0.28	7
25	0.35*	4	0.23	7	− 0.31	8	0.28	2	− 0.31	2	0.43*	5
31	0.31	1	0.41*	7	—	—	0.50*	12	0.19	2	0.22	2
37	—	—	0.40*	2	0.27	5	—	—	—	—	0.24	2
38	0.30	3	0.22	4	0.43*	2	0.25	6	0.46*	5	0.28	2

* r values > 0.35 are significant at the 5 % level.
(1)r = correlation coefficient.
(2)lag = shift in sample no between the two hormone values.

Discussion.

Experiment one. — All cows underwent a 21-day cycle except Cow B which underwent the preovulatory LH surge approximately 24 h later than cows A, C and D. The timing of the LH surge has previously been shown to coincide approximately with behavioural oestrus (Schams *et al.,* 1977) and the present data support this relationship. Plasma LH and oestradiol-17β concentrations increased during the follicular phase, in association with the fall in progesterone concentrations. It is generally accepted that the withdrawal of negative feedback due to decreasing progesterone concentrations following luteolysis, is followed by an increase in tonic LH release, occurring as a result of increasing LH pulse frequency (Schallenberger *et al.,* 1984). Increasing LH concentrations are then considered to stimulate follicular oestradiol secretion.

In the present study, the increase in mean LH concentrations during the follicular phase was reflected in an increase in LH pulse frequency to that of approximately one pulse per hour on the day before the preovulatory LH surge and oestrus (table 1). Hourly or more frequent pulses of LH are associated with this stage of the cycle in Holstein heifers (Rahe *et al.,* 1980) and in sheep (Hauger, Karsch and Foster, 1977 ; Baird, 1978). We have also suggested that in the post-partum anoestrous cow there is a gradual increase towards an hourly LH pulse frequency, before the first ovulation occurs (Lamming *et al.,* 1982). The frequency of LH pulses during the luteal phase of approximately one per four hours is in good agreement with that reported by Rahe *et al.* (1980).

In the present data, the LH pulse amplitude did not vary significantly with the stage of the cycle, in contrast to the report by Rahe *et al.* (1980) who classified LH pulses in the mid-luteal phase as high amplitude-low frequency. The fluctating LH baseline and generally less discrete pulses during the mid-luteal phase (see fig. 1), may reflect differences in breed, age, parity and/or environment between the two studies.

The rise in plasma oestradiol concentrations between days 17 and 20 of the cycle, is in agreement with the findings of Glencross *et al.* (1981a) and probably reflects an increased rate of secretion by the dominant ovarian follicle destined to ovulate (Hackett and Hafs, 1969 ; Schallenberger *et al.,* 1984). Rising oestradiol concentrations are known to induce the preovulatory gonadotropin LH surge by a positive feedback effect (Kesner, Convey and Anderson, 1981).

Experiment two. — LH concentrations fluctuated in a pulsatile manner during both the early and mid-luteal phase and there was a tendency for LH pulse frequency to be lower in the mid-luteal phase. These results are in general agreement with those in Experiment one, althought the LH concentrations and pulse frequency tended to be higher in Experiment two. Consistent discrete pulses of FSH and progesterone analogous to those of LH were not observed. However, oscillations in the concentrations of these two hormones did occur, but most did not fulfil our previous definition of a pulse (Riley, Peters and Lamming, 1981), since there was often only a single elevated point between adjacent troughs. A pulsatile pattern of FSH secretion has been described in the cow (Walters, Schams and Schallenberger, 1984 ; Schallenberger *et al.,* 1984 ; Walters and Schallenberger, 1984). The differences in FSH patterns between the two studies may reflect differences in the RIA procedures used. Walters *et al.* (1984) have also described discrete pulsatile patterns of progesterone during the luteal phase in the cow, but these were in samples taken from the posterior vena cava, therefore reflecting more directly, ovarian output of this steroid.

In that study there was a close correlation between vena cava FSH and progesterone concentrations. In the present study the measurement of these hormones in jugular blood may have precluded the finding of a similar relationship.

Based on both the present data and other studies it is concluded that :
1) LH pulse frequency is low (2 to 3 pulses per 8 hours in most cows) during the mid-luteal phase. As progesterone concentrations fall during luteolysis, the LH

pulse frequency increases, stimulating follicle maturation and oestradiol production, which eventually culminates in the preovulatory gonadotrophin surge and ovulation. As progesterone concentrations rise during the next luteal phase the LH pulse frequency is reduced.
2) FSH concentrations appear to change little throughout the cycle, except for a significant decrease during luteolysis. FSH may be a factor involved in regulating pulsatile progesterone secretion since in one study their pulse frequencies were similar and highly correlated (Walters *et al.*, 1984).

Although short-term changes were not measured in this study, others have demonstrated pulsatile patterns of oestradiol-17β albeit in the posterior vena cava. The frequency appears to be related directly to that of LH (Walters *et al.*, 1984).

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Résumé. *Etude des diagrammes hormonaux chez la vache durant le cycle œstral.*

Au cours de deux expériences, les concentrations plasmatiques de différentes hormones ont été déterminées chez la vache durant le cycle œstral. Pendant la phase lutéale, la fréquence des « pulses » de LH est faible (2 à 3 par période de 8 h). Il n'existe pas de corrélation nette entre les concentrations de LH, de FSH et de progestérone. Pendant la lutéolyse, la chute des taux de progestérone s'accompagne d'une élévation des taux d'œstradiol 17β et de LH. La fréquence des « pulses » de cette dernière hormone atteint presque 1 « pulse » par heure un jour avant la décharge préovulatoire.

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