

## FSH and LH variations in beef cows during the postpartum period

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**Summary.** To study the plasma gonadotrophin profiles of 9 cows after parturition, blood samples were obtained every 20 min for 12 hrs on three occasions between 5 and 50 days postpartum and analysed by RIA techniques.

The time of the first ovulation, as judged by plasma progesterone levels, varied from 30 to more than 60 days postpartum. Variations in mean levels of FSH and LH were not significantly correlated with the postpartum interval. However, the mean levels of plasma FSH and number of LH pulses were lower in females which had not ovulated than in those which had. The cows could be classified into four groups : group 1 with less than 4 LH pulses in 12 hrs and a mean plasma FSH level < 138 ng/ml ; group 2 with more than 4 LH pulses in 12 hrs and varying plasma FSH levels ; group 3 with less than 4 LH pulses in 12 hrs and a mean plasma FSH level > 138 ng/ml ; group 4 which had ovulated. This classification indicated that the LH and FSH levels progressed significantly (2.46 to 3.56 ng/ml,  $P < 0.05$  ; 120 to 159 ng/ml,  $P < 0.01$ , respectively) from groups 1 to 3, and that they decreased in the females which had ovulated (group 4).

Since the time of the first ovulation after parturition varied, it was not possible to demonstrate any relationship between that interval and the mean plasma gonadotrophin profiles. However, when ovulation was considered as time zero there was a clear increase in plasma gonadotrophin before ovulation.

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### Introduction.

The mean level of plasma LH has been reported to increase during the first 15 days postpartum in the cow and not thereafter (Erb *et al.*, 1971 ; Ingalls, Convey and Hafs, 1973 ; Echternkamp and Hansel, 1973 ; Edgerton and Hafs, 1973). However, Arije, Wiltbank and Hopwood (1974) found episodic elevated plasma LH levels after 15 postpartum days ; these results were confirmed by Goodale *et al.* (1978) in a study using hourly sampling over a 10-hour period.

The mean plasma FSH level, on the other hand did not vary significantly during the postpartum period (Dobson, 1978 ; Schams *et al.*, 1978).

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Since the time-lag between parturition and the first ovulation is extremely variable (from 16 to more than 120 days ; Baker, 1969), it does not seem to be the right criterion to use for a study of endocrine changes after parturition.

The aim of the present study was to measure the number of plasma LH pulses and the mean LH and FSH levels during the postpartum period and to relate them to the onset of the first ovulation rather than to the time of parturition.

### Material and methods.

Multiparous cows of the Charolais (4), Salers (3) and Aubrac (2) breeds were housed in a cattle shed during the later stages of pregnancy. After calving, all cows were allowed to suckle one calf twice a day.

Blood samples were collected from each cow during three 12-h sampling periods between 5 and 50 days postpartum as shown in table 1. Within each period, blood samples were taken every 20 min via a jugular catheter inserted the day before. The plasmas were kept frozen at  $-15^{\circ}\text{C}$  until LH and FSH assays. In addition, blood samples taken once every 10 days from parturition to 60 days postpartum were used to determine progesterone and hence the onset of cyclicity ; a cow with a progesterone level higher than 0.5 ng/ml was considered to have ovulated (Thimonier, 1978) since in the postpartum period, a luteinized follicle is rarely a source of progesterone (Castenson *et al.*, 1976).

TABLE 1

*Time-lag between parturition and blood sampling in beef cows*

Cow and Breed	Days postpartum			
	5	15	30	50
1 Charolais	-	+	+	+
2 Charolais	+	+	+	-
3 Charolais	+	+	+	-
4 Charolais	-	+	+	+
5 Salers	+	+	+	-
6 Salers	-	+	+	+
7 Salers	-	+	+	+
8 Aubrac	+	+	+	-
9 Aubrac	-	+	+	+
No- of cows	4	9	9	5

+ : blood sampled ; - : blood not sampled.

Plasma LH levels were determined by specific radioimmunoassay (Pelletier, 1972), using a bovine LH (1 ng of LH BCB2 was immunologically equivalent to 2,1 ng of NIH LH S1) as a standard. The plasma progesterone levels were determined according to Terqui and Thimonier (1974). FSH levels were measured

according to the technique of Blanc and Poirier (1979), slightly modified for bovine FSH by using a bovine preparation (YAF FSH 22-2, immunologically equivalent to 4.7 ng/ml NIH FSH B1) as a standard rather than an ovine preparation. In these conditions, the RIA curves for dilution of steer plasma were parallel to the standard curve and the assay was highly specific (cross-reaction with LH : 0.02 p. 100).

We considered an LH value as a pulse when it was greater than the mean (+ 2 SEM) level encountered during the 12-h sampling period, two pulses being separated by at least two samples.

## Results.

1) *Ovulation*. — The number of females ovulating as a function of the postpartum interval is shown in table 2. The first cow ovulated at 30 days postpartum. At 60 days, six of the nine cows had ovulated.

TABLE 2

*Cumulated number of cows showing ovulation according to postpartum time-lag (n = 9)*

Days postpartum .....	10	20	30	40	50	60
	0	0	1	3	3	6

2) *Plasma LH concentration*. — Mean LH levels varied non-significantly from 2.2 ng/ml at 5 days postpartum to 2.76 ng/ml at 15 days postpartum and remained steady thereafter. The mean plasma LH levels at 30 days and 50 days did not differ significantly when the data on ovulating females were not included in the computation (table 3).

TABLE 3

*Variation in plasma LH and FSH levels during the postpartum period (m ± SD)*

Days postpartum		5	15	30	50
No. of cows	a	4	9	9	5
	b	0	0	8	2
LH (ng/ml)	a	2.20 ± 0.45	2.76 ± 0.79	2.74 ± 0.56	2.76 ± 0.74
	b			2.65 ± 0.53	3.35 ± 0.80
No. of LH pulses in 12 hrs	a	2.50 ± 1.7	1.89 ± 1.54	3.22 ± 1.5	2.40 ± 0.55
	b			3.50 ± 1.31	3.00 ± 1.40
FSH (ng/ml)	a	128 ± 22	132 ± 21	132 ± 22	141 ± 12
	b			130 ± 22	139 ± 22

a : all females included for calculation ; b : females with ovulation not included in the calculation.

The mean LH levels at a given postpartum interval varied between cows. For example, levels 1.88 to 4.01 ng/ml were found at 15 days postpartum. However these variations were not distributed at random. When the cows were ranked for a given postpartum day according to the magnitude of the mean plasma LH levels, a highly significant correlation coefficient ( $r = 0.97$  ;  $P < 0.01$ ) was found between the values of ranks obtained at 15 days and those obtained at 30 days postpartum. The analysis of variance at 15 and 30 days postpartum indicated further that a breed influence on plasma LH levels was nearly significant ( $P < 0.056$ ) with mean LH levels for a given postpartum day being ranked in the following descending order : Salers, Charolais, Aubrac.

Although there was no significant relationship between the number of pulses and the postpartum interval (table 3), at 30 days postpartum five out of nine cows showed more than four pulses in 12 hours and the maximum number of LH pulses observed was significantly greater in ovulating than in non-ovulating females (Mann Whitney test,  $P < 0.02$ ). Furthermore, females which did not show more than four pulses in any of the three sampling periods did not ovulate.

3) *Plasma FSH concentrations.* — Mean plasma FSH levels after parturition varied from 128 ng/ml to 141 ng/ml from 5 to 50 days postpartum, but wide variations (111 to 172 ng/ml at 15 days postpartum) were observed between animals. The mean FSH level was higher in cows which were cyclic at 60 days than in the animals still in anoestrus at that time (138 ng/ml vs 117 ng/ml, respectively ;  $P < 0.02$ ). As in the case of LH, disregarding data from ovulating cows did not change the mean FSH levels at 30 and 50 days postpartum (table 3).

4) *Distribution of cows into groups according to hormonal status.* — On the basis of the number of LH pulses in 12 h and the mean plasma FSH and progesterone levels, the animals were classified in the following groups for a given sampling period :

*Group 1* : number of LH pulses :  $< 4$  in 12 h ; FSH level :  $< 138$  ng/ml ; progesterone :  $< 0.5$  ng/ml.

TABLE 4

*Distribution of cows into groups 1 to 4 according to postpartum time-lag*

Cow	Days postpartum			
	5	15	30	50
1		Grp. 2	Grp. 2	Grp. 4
2	Grp. 1	Grp. 1	Grp. 1	
3	Grp. 1	Grp. 1	Grp. 1	
4		Grp. 1	Grp. 2	Grp. 4
5	Grp. 2	Grp. 3	Grp. 4	
6		Grp. 1	Grp. 1	Grp. 1
7		Grp. 1	Grp. 3	Grp. 4
8	Grp. 1	Grp. 1	Grp. 2	
9		Grp. 1	Grp. 2	Grp. 4

*Group 2* : number of LH pulses : > 4 ; FSH level : variable ; progesterone : < 0.5 ng/ml.

*Group 3* : number of LH pulses : < 4 ; FSH level : > 138 ng/ml ; progesterone : < 0.5 ng/ml.

*Group 4* : progesterone level : > 0.5 ng/ml.

Using this classification, animals tended to be distributed successively into groups 1 to 4 as the postpartum interval increased. Conversely, they never moved from one group to another which had a lower numerical classification (table 4).

The plasma level of gonadotrophins in progressing from group 1 to group 3 increased from 2.46 to 3.56 ng/ml ( $P < 0.05$ ) and from 120 to 159 ng/ml ( $P < 0.01$ ) for LH and FSH, respectively (table 5). Plasma LH and FSH levels were lower in cows in group 4 compared to animals in group 3 ( $P < 0.05$ ), although the number of LH pulses was not different (table 5). It is noteworthy that : (i) the number of these pulses per 12 h in groups 1 and 3 ( $1.79 \pm 0.9$  and  $1.5 \pm 0.7$ , respectively) was very different from the frequency in group 2 (4 pulses) ; (ii) the FSH levels for group 2 were intermediate between groups 1 and 3 although no criterion was fixed for the levels of that hormone in this group.

TABLE 5

*Variation of plasma LH and FSH levels in groups 1 to 4 (m  $\pm$  SD)*

Group	Group 1 n = 14	Group 2 n = 6	Group 3 n = 2	Group 4 n = 5
LH (ng/ml)	2.46 $\pm$ 0.62 <sup>(a)</sup>	2.68 $\pm$ 0.40 <sup>(a)</sup>	3.56 $\pm$ 0.75 <sup>(b)</sup>	2.89 $\pm$ 0.8 <sup>(a)</sup>
No. of LH pulses in 12 hrs	1.79 $\pm$ 0.9 <sup>(a)</sup>	4.67 $\pm$ 0.8 <sup>(b)</sup>	1.50 $\pm$ 0.7 <sup>(a)</sup>	2.20 $\pm$ 0.8 <sup>(a)</sup>
FSH (ng/ml)	120 $\pm$ 14 <sup>(a)</sup>	141 $\pm$ 6 <sup>(b)</sup>	159 $\pm$ 7 <sup>(c)</sup>	147 $\pm$ 7.0 <sup>(b)</sup>

Values with same superscript are not significantly different at  $P < 0.05$ .

## Discussion.

No authors considering parturition as time zero when studying postpartum hormone profiles in cattle have found any clear change 2 weeks later (Erb *et al.*, 1971 ; Ingalls, Convey and Hafs, 1973 ; Echterkamp and Hansel, 1973 ; Edgerton and Hafs, 1973). In our study, the cows which had not ovulated by 60 days showed the lowest FSH levels and never displayed four LH pulses per 12 h. Conversely, all the ovulating females showed the highest FSH levels and four or more LH pulses in at least one 12-h period.

Furthermore the distribution into groups indicated that the cows tended to fall successively into groups 1 to 3 before ovulation. Ovulating females were distributed in at least two different groups, while those which had not ovulated by 60 days postpartum remained in group 1. This would indicate that the physiological status varied with the postpartum time-lag which is also the case in ewes (Gayerie, Cognie and Locatelli, 1980).

The idea that the time-course of preovulatory endocrine events might vary according to the individual suggested that inter-group comparison was a better approach to the study of postpartum hormonal variations than that of grouping the animals together at a fixed date after calving. Comparisons of inter-group hormone levels indicated that the differences were significant for both FSH ( $P < 0.01$ ) and LH ( $P < 0.05$ ).

Postpartum gonadotrophin variations in the cow may be summarized as follows (fig. 1) :

- 1) low plasma LH and FSH levels and weak LH pulsatility may be due to depressed hypophyseal LRH sensitivity (Kesler *et al.*, 1978 ; Fernandes *et al.*, 1978) ;
- 2) plasma LH and FSH levels and LH pulsatility then increase concomitantly with mean LH hypophyseal sensitivity to LRH (Fernandes *et al.*, 1978 ; Kesler *et al.*, 1978) ;
- 3) thereafter, plasma LH and FSH levels continue to increase in spite of a decrease in LH pulsatility ;
- 4) when the cows have ovulated, the increased progesterone levels could cause the gonadotrophin levels to decrease (Pelletier and Thimonier, 1975).

A similar pattern for the number of LH pulses (increasing then decreasing) has been shown by Rawlings *et al.* (1980) when the females are pooled at a fixed date before the day of ovulation.

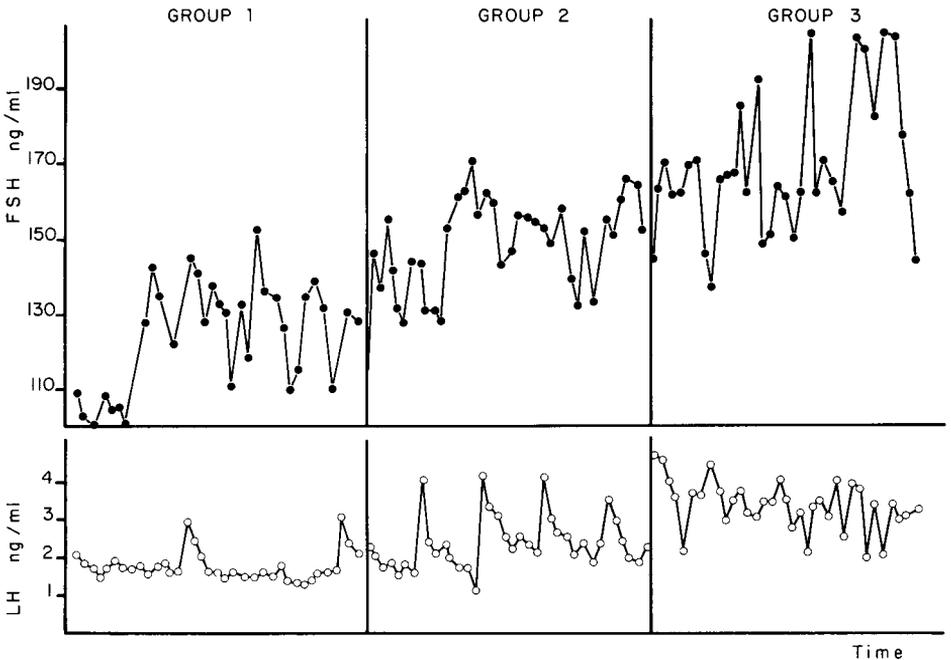


FIG. 1. — Plasma FSH and LH typical profiles in 3 different postpartum cows of group 1, 2 and 3 sampled every 20 min for 12 hrs on each occasion.

The first three stages are similar to those described before puberty in the calf (Lacroix and Pelletier, 1979) or in short-day reinitiation of activity in the ram (Lincoln, Peet and Cunningham, 1977). They suggest that the mechanisms involved in these processes correspond to those observed in the postpartum physiology of this study.

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**Résumé.** Pour étudier l'évolution des gonadotropines plasmatiques après parturition, 9 vaches ont subi des prélèvements de sang toutes les 20 min. pendant 12 h, 3 fois entre 5 et 50 jours postpartum. Les dosages hormonaux ont été effectués par radioimmunologie.

Le moment de la première ovulation, obtenu à partir de l'analyse du niveau de progesterone plasmatique, varie de 30 à plus de 60 jours postpartum. Les niveaux moyens de FSH et de LH ne varient pas de façon significative en fonction de l'intervalle postpartum. Il en est cependant différemment si pour un jour postpartum donné les animaux sont répartis en 4 groupes définis de la façon suivante : groupe 1, tout animal ayant moins de 4 pulses de LH en 12 h et une moyenne de FSH plasmatique  $< 138$  ng/ml ; groupe 2, tout animal ayant plus de 4 pulses de LH en 12 h, FSH indifférent ; groupe 3, tout animal ayant moins de 4 pulses de LH en 12 h et une moyenne de FSH plasmatique  $> 138$  ng/ml ; groupe 4, tout animal ayant ovulé. Dans ces conditions, on observe une augmentation régulière des niveaux moyens de LH (2.46 à 3.56 ng/ml ;  $P < 0.05$ ) et de FSH (120 à 159 ng/ml ;  $P < 0.01$ ) du groupe 1 au groupe 3. Pour les animaux ayant ovulé (groupe 4) les niveaux diminuent. Les animaux changeant de groupe passent uniquement dans un groupe d'ordre supérieur mais jamais l'inverse. L'ovulation a lieu au plus tard 20 jours après l'entrée dans le groupe 2.

Ainsi, du fait de la variation du moment de la première ovulation après le part, l'intervalle postpartum ne permet pas de mettre en évidence une évolution hormonale moyenne alors que la répartition en groupes d'après des critères endocriniens montre bien un enchaînement d'événements conduisant à la première ovulation.

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