Short-term variations in plasma LH and testosterone in bull calves from birth to 1 year of age

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Summary. LH and testosterone levels in bull calves were studied in the plasma samples collected sequentially at 15-min intervals every month during the first year of life. An episodic pattern of LH release occurred after birth and the frequency and magnitude of the LH peaks increased up to 4 months of age and decreased thereafter. A testicular response was not observed before this age. It is suggested that this episodic LH activity is responsible for the testicular development which then initiates puberty.

Introduction

The variations of plasma LH concentrations in bull calves during the first year of life are a subject of controversy: LH is said not to vary (Odell, Hescox & Kiddy, 1970; Karg *et al.*, 1976), to increase during the first months after birth (Rawlings, Hafs & Swanson, 1972; Mori, Masaki, Wakabayashi, Endo & Hosoda, 1974) or to increase later (Gombe, Hall, McEntee, Hansel & Pickett, 1973). Lacroix, Garnier & Pelletier (1977) reported that plasma LH in 10 Charolais bull calves increased shortly after birth, fluctuated widely from the 5th to the 20th week and finally decreased and remained relatively steady up to 1 year of age. It is generally accepted that the testosterone concentrations are very low during the first months after birth and then increase (Rawlings *et al.*, 1972; Secchiari, Martorana, Pellegrini & Luisi, 1976; Lacroix *et al.*, 1977). The large differences in plasma hormone concentrations in weekly samples suggested to us that there might be very short-term fluctuations in release, particularly for LH, during the first months of age and that the differences in the results reported might be due to inadequacies of timing and frequency of blood sampling. The present study was therefore undertaken to examine this possibility and plasma testosterone was also measured to determine precisely the time at which LH control occurred.

Materials and Methods

The 4 Charolais bull calves used were born between 8 January and 14 February. Sequential blood samples were withdrawn by jugular venepuncture at 15-min intervals from 09:00 to 15:00 h on 1 day each month from 1 to 8 months of age and then at 10 and 12 months. Plasma samples were kept frozen at -15°C before assay for LH and testosterone. LH was measured in duplicate samples by a specific homologous double-antibody radioimmunoassay (Pelletier, 1972) which has a sensitivity of 0.2 ng/ml and an intra-assay coefficient of variation of 1.76%. The results are expressed in terms of bovine LH LER 1072–2 which has a potency of twice that of NIH-LH-S1. The radioimmunoassay for testosterone was performed after steroid extraction in 0.5 or 1 ml plasma with an ethylacetate–cyclohexane mixture (1:1 v/v) (Garnier, Cotta & Terqui, 1978). The antibody, obtained by immunization of rabbits, cross-reacted considerably (47%) only with 5 α -dihydrotestosterone (DHT). However, chromatography on celite columns

to isolate testosterone from DHT indicated that the latter was present in only negligible amounts in calf plasma. The mean sensitivity of the assay was estimated to be 0.05 ng testosterone/ml and the intra-assay coefficient of variation was 10%.

Q test analysis (Cochran, 1953) was used to determine the presence of LH peaks and analysis of variance for the number of LH peaks/6 h/animal, and the mean LH and testosterone levels.

Results

LH

A typical pattern of LH levels throughout the year is shown in Text-fig. 1. The pulsatile character of LH release during the first months of life is quite clear. If the results are expressed as a mean of the 25 samples (Table 1) collected during the 6-h period of bleeding, the mean LH release differed significantly between months ($P < 0.05$), the higher values being from 2 to 5 months of age. Basal levels were approximately 1 ng/ml and did not vary throughout the study. It was assumed that a plasma LH value which was more than twice the mean basal level in each animal and on each occasion represented a 'peak'. The number of animals having at least one such peak during the 6-h bleeding period varied significantly ($P < 0.001$): during the 2–5 month period all animals exhibited at least one peak but no peaks were observed during the 8th, 10th and 12th months. The frequency of the peaks also varied, the highest value at 3 and 4 months of age being significantly different ($P < 0.05$) from all other values (Table 1). The magnitude of the peak did not appear to vary with age, most ranging from 3 to 5 ng/ml but higher values were occasionally observed (maximum of 12.5 ng/ml).

Table 1. Summary of the changes in plasma LH and testosterone concentrations in 4 bull calves bled every 15 min for 6 h at different ages

Age (months)	No. of bulls with peaks*	No. of LH peaks/animal	Mean $\pm$ s.e.m. LH conc. (ng/ml)†	Mean $\pm$ s.e.m. testosterone conc. (ng/ml)‡
1	3	1.0	1.17 $\pm$ 0.25	0.31 $\pm$ 0.03
2	4	1.50 $\pm$ 0.29†	1.40 $\pm$ 0.19	0.26 $\pm$ 0.02
3	4	3.25 $\pm$ 0.48†	1.59 $\pm$ 0.20	0.43 $\pm$ 0.12
4	4	2.75 $\pm$ 0.25†	1.64 $\pm$ 0.22	0.91 $\pm$ 0.25
5	4	1.75 $\pm$ 0.25†	1.61 $\pm$ 0.11	2.32 $\pm$ 0.71
6	2	0.50	1.03 $\pm$ 0.12	1.19 $\pm$ 0.19
7	2	0.50	1.07 $\pm$ 0.19	—
8	0	0	0.94 $\pm$ 0.10	0.47 $\pm$ 0.13
10	0	0	1.03 $\pm$ 0.12	0.70 $\pm$ 0.29
12	0	0	1.05 $\pm$ 0.08	0.62 $\pm$ 0.22

* A peak is defined as a value at least twice the basal level for each animal on each occasion.

† Mean $\pm$ s.e.m. when all animals had at least one LH peak.

‡ For 100 samples, i.e. 25 samples/calf.

Testosterone

Mean plasma concentrations changed markedly during the year ($P < 0.001$) (Table 1). During the 3 first months after birth, the testosterone values were low and no or only small peaks occurred after the LH pulses. From 4 to 6 months, large testosterone peaks usually followed the LH peaks but the correlations between the hormones were not easy to demonstrate (Text-fig. 1). No major testosterone release was observed at 8, 10 and 12 months of age but minor fluctuations occurred, in contrast to the low regular levels seen in the 1- to 3-month period.

Text-fig. 1. Plasma LH (—) and testosterone (shaded area) concentrations in one representative bull calf sampled every 15 min for 6 h at different ages.

Discussion

It is evident that the frequency of plasma LH pulses during a 6-h sampling period each month is high between 2 and 5 months after birth. This period corresponds exactly with that when elevated LH values were found in Charolais calves bled at weekly intervals (Lacroix *et al.*, 1977) and in calves of four other breeds (A. Lacroix, F. Menissier & J. Pelletier, unpublished results). In these latter studies, involving 42 calves born over a period of 5 months, the occurrence of peaks at a particular age after birth suggests that such peaks are not related to photoperiodic changes. Similar pulsatile patterns of LH release have been shown in prepubertal male lambs (Foster, 1974), pigs (Colenbrander & Van Straaten, 1977) and children (Weitzman, Boyar, Kapen & Hellman, 1975).

After 5 months of age, the number of LH peaks undoubtedly decreases but cannot be considered as entirely absent although no peaks were observed at 8, 10 and 12 months in the present study. In fact, some high plasma LH values which must be related to 'peaks' have been observed in 6–12-month-old Charolais (Lacroix *et al.*, 1977), Brown Swiss (Karg *et al.*, 1976) and Friesian (Thibier, 1975) calves. These unfrequent LH peaks have been shown, however, to produce a large testosterone release. In the present study the absence of LH peaks after 8 months is presumably responsible for the low testosterone level observed. In 6-year-old bulls a high frequency of LH peaks has been reported (Katongole, Naftolin & Short, 1971) and this suggests a difference in intensity of pulsatile pattern in prepubertal calves and adult bulls.

The present study confirms previous observations (Secchiari *et al.*, 1976; Karg *et al.*, 1976; Lacroix *et al.*, 1977) that testosterone release is low from birth until 3 months of age. Such a low testosterone level after birth has also been shown in the male lamb (Crim & Geschwind, 1972; Courot, de Reviere & Pelletier, 1975) but not in the male piglet in which there are high testosterone concentrations in the early post-natal period (Meusy-Desolle, 1975). From 4 months of age plasma testosterone levels in bulls increase and fluctuate widely, especially after an LH peak.

The testosterone response after an LH peak occurred at the time of onset of the increase in testicular weight which takes place when the animals reach about 150 kg body weight (Attal & Courot, 1963), i.e. between 3 and 4 months of age in Charolais bulls. We suggest that this increase in testicular activity is at first related to the preceding LH peaks and is then finally responsible for the decline in the number of such peaks. This endocrine relationship may indicate the occurrence of puberty in the calf. The present data are not in agreement with the hypothesis of 'gonadostat' (Ramirez & McCann, 1963; Grumbach, Roth, Kaplan & Kelch, 1974) since a pulsatile pattern of LH is observed without a testicular response for several months. Therefore, the most probable hypothesis is that which relates puberty to a change in the hypothalamic sensitivity to inhibition by the central nervous system (Levasseur, 1977) as suggested for man (Boyar *et al.*, 1974; Weitzman *et al.*, 1975) or rhesus monkeys (Dierschke *et al.*, 1974).

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