BIOLOGY OF REPRODUCTION 19, 1108–1112 (1978)

Plasma Luteinizing Hormone (LH) and Testosterone Levels during Sexual Maturation in Beef Bull Calves[1]

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ABSTRACT

Blood samples were taken by jugular venipuncture from bull calves every other week from 1 to 40 weeks of age. Samples were also drawn by jugular cannula, every 15 min for 2 h, from a different group of 4–5 calves at 10 week intervals, from 1 to 40 weeks of age. Following the period of intensive blood collection each group of calves was castrated. Based upon histological examination of the testis, spermatogenesis was initiated after 10 weeks of age, with mature spermatozoa being present in the testis by 40 weeks of age. Body weight and epididymal weight increased linearly with age, while testicular weight increased in a curvilinear pattern, with the most dramatic increase occurring from 30 to 40 weeks of age.

Plasma concentrations of LH were low prior to 8 weeks of age, between 10 and 20 weeks of age episodic fluctuations were observed, with a return to low and stable concentrations being seen before a final gradual increase to adult levels at 40 weeks of age. Plasma concentrations of testosterone were variable, but appeared to rise markedly after 28 weeks of age, reaching adult levels by 40 weeks of age. The mode of variation of plasma concentrations of testosterone appeared to resemble that of the adult in 3 of 5 bull calves at 20 weeks of age and all 5 bull calves at 40 weeks of age; more transient peaks were observed at 10 weeks of age.

INTRODUCTION

Donovan and van der Werff ten Bosch (1965) defined puberty as the entire period when the gonads secrete hormones in amounts sufficient to cause accelerated growth of the genital organs and the appearance of secondary sexual characteristics. The onset of puberty in the dairy bull, as determined by the presence of sperm in the seminiferous tubules, appears to take place between 5 and 8 months (Macmillan and Hafs, 1968) and termination, as determined by first ejaculation of motile spermatozoa, occurs about 44 ± 1 weeks of age (Flipse and Almquist, 1961). In beef breeds, the maturational processes lag behind the dairy breeds by some 6 to 12 weeks (Wolf Almquist and Hale, 1965).

The temporal pattern of changes of testosterone concentrations in testicular tissue (Lindner, 1959; Lindner and Mann, 1960; Rawlings et al., 1972) and of testosterone and LH in plasma, have been investigated during the process of sexual maturation in the dairy bull calf (Odell et al., 1969; Rawlings et al., 1972; Secchiari et al., 1976). Secchiari et al. (1976) took blood samples every 2 weeks, but no more intensive study of plasma hormone concentrations has been made. Rawlings et al. (1972) observed a marked variability in plasma testosterone and LH levels in samples collected once a month at slaughter. This variability could have been contributed to by the infrequency and stress of sample collection. However, in the adult LH and testosterone are released in an episodic manner (Katangole et al., 1971; Thibier, 1976). The purpose of the present study was to evaluate the temporal pattern of plasma concentrations of LH and testosterone in beef bull calves, from birth to 40 weeks of age, using sensitive radioimmunoassay techniques and a less stressful, more intensive sampling regime.

MATERIALS AND METHODS

The 23 Angus × Hereford bull calves used in this study were born in April (mean birth date April 19th,

Accepted July 6, 1978.
Received April 9, 1978.

[1] Technical Contribution No. 1563 of the South Carolina Agricultural Experiment Station published with the approval of the Director.

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range 13th–29th). Calves were nursed at pasture until weaned (7 months of age). After weaning they received coastal bermudagrass hay *ad libitum* and 15 kg/head/day of a concentrate supplement (9.5 parts corn, 6.0 parts cottonseed meal, 5.0 parts citrus pulp and 9.5 parts barley).

Every other week from April until the following January, the calves were bled by jugular venipuncture, weighed and their heart girth and wither height recorded. At 10 week intervals, from 1 to 40 weeks of age, blood samples were taken from a different group of 4–5 calves every 15 min for 2 h. Indwelling jugular cannulae (Clay Adams P. E. 160, I. D. 1.4 mm, O. D. 1.9 mm) were inserted 24 h before the latter sampling. Following the period of intensive blood collection, each group of calves was castrated. The testicles and epididymides were weighed and a section of testis was examined histologically, to assess the stage of reproductive development of each group of calves. Therefore, the number of calves sampled on the biweekly schedule decreased by 4 or 5 every 10 weeks. All blood samplings were made between 0900 h and 1200 h, but the periods of frequent sampling were not performed on the same day as a biweekly blood collection. Blood samples were chilled, centrifuged and plasma stored at −20°C until assayed.

LH was quantitated by an established double antibody radioimmunoassay procedure, utilizing an antiserum (B225) proven specific for bovine LH (Niswender et al., 1968; Niswender et al., 1969). Highly purified bovine LH (LER 1072-2, LH potency 1.66 NIH-LH-S1 units/mg) was used for reference standards and for iodination. The linear range of the assay standard curves fell between 0.05 and 6.1 ng per assay tube. The sensitivity of the assay, the smallest amount of hormone significantly different from 0, was 0.25 ng/ml plasma. The intraassay coefficient of variation was 9% and the interassay variation was 15%.

Testosterone was quantitated by radioimmunoassay, after extraction from plasma with ether and using an antiserum specific to testosterone (Bosch et al., 1974). All samples were analyzed in duplicate and all estimations were corrected for procedural losses. The linear range of the standard curves fell between 5 and 950 pg/assay tube. The sensitivity of the assay was 50 pg/ml plasma. The intraassay coefficient of variation was 6.6% and the interassay variation was 13.6%. Data were analyzed by regression, analysis of variance and correlation procedures. Comparisons among means were carried out using Duncan's New Multiple Range test.

RESULTS

A linear increase in weight, wither height and heart girth was observed for the bull calves over the period of study (Fig. 1). The relationship between body weight of bull calves (Y) and their ages in weeks (X) is described by the regression equation Y = 29.42 + 3.36X. Average daily gain in weight during the 40 week experimental period was 0.49 kg/day.

Increases in testicular weight followed a curvilinear pattern from 1 to 40 weeks of age; a slow phase of growth was observed from 1 to 30 weeks of age and an accelerated phase from 30 to 40 weeks of age (Fig. 1) Gonocytes were observed in the seminiferous tubules of bull calves of 1 and 10 weeks of age, secondary spermatocytes at 30 weeks of age and at 40 weeks mature spermatozoa, testes from 20-week-old calves were not processed. Epididymal weight increased linearly with age (Fig. 1).

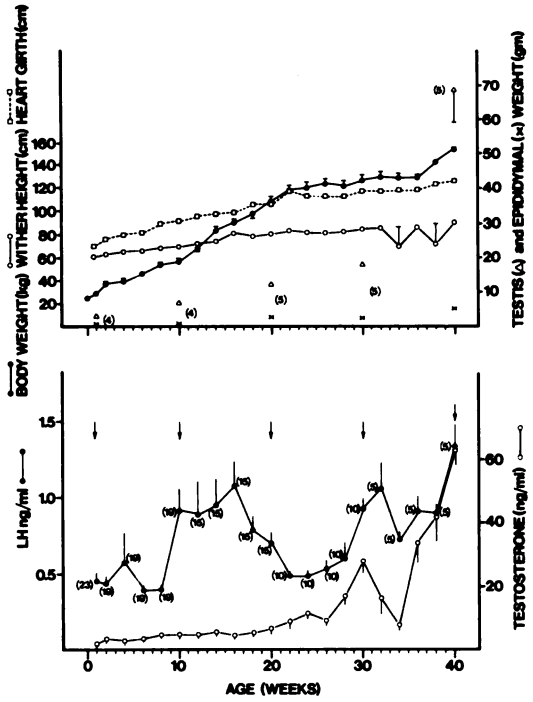

Fig. 1. Mean body weight, wither height, heart girth, testes weight, epididymal weight, plasma concentrations of LH and testosterone ($\bar{X}$ ± SEM. SEM included only where appreciable) in intact bull calves sampled biweekly from 1 to 40 weeks of age. Arrows mark times at which 4 or 5 calves were intensively bled and castrated. Figures in parentheses indicate the number of observations at each point, for data collected on a biweekly basis or every 10 weeks at castration.

The temporal pattern of jugular plasma concentrations of LH and testosterone ($\bar{X}$ ± SEM), in samples collected at 1 and 2 weeks of age and thereafter every 2 weeks, until 40 weeks of age, are depicted in Fig. 1. Plasma LH concentrations were low from 1 to 8 weeks of age, rose to 16 weeks of age (1.04 ± 0.17 ng/ml) and fell to a nadir at 24 weeks of age (0.45 ± 0.05 ng/ml). Subsequently, plasma concentrations of LH rose to 1.30 ± 0.15 ng/ml at 40 weeks of age. The peaks in plasma LH concentrations at 16 and 40 weeks of age were significantly different ($P<0.05$) from the

plasma LH concentrations observed at 8 or 24 weeks of age. Plasma concentrations of testosterone were low from 1 to 4 weeks of age, increasing gradually to 28 weeks of age (1.63 ± 0.43 ng/ml). Subsequently, although more variable, concentrations rose to a peak at 40 weeks of age (6.4 ± 0.75 ng/ml). Testosterone concentrations in the plasma from 28 to 40 weeks of age, except for that at 34 weeks of age, were significantly ($P<0.05$) different from concentrations at 1 to 26 weeks of age.

Plasma concentrations of LH and testosterone in samples taken every 15 min for 2 h at 1, 10, 20, 30 and 40 weeks of age are shown in Fig. 2. Figures for representative animals are shown at each age. The patterns of plasma concentration of LH and testosterone were similar within groups at any age, but when comparing individuals the various fluctuations were not synchronized. At 1 week of age, plasma concentrations of LH and testosterone were low and stable (< 0.30 ng/ml and < 0.06 ng/ml, respectively). At 10 weeks of age, plasma LH concentrations were less than 0.35 ng/ml. Plasma concentrations of testosterone at this time showed episodic fluctuations, with 1 to 2 peaks of 0.5 ng/ml to 9 ng/ml occurring during the 2 h sampling periods. This includes partial peaks observed at either end of the period of blood collection. The intervening basal concentrations were similar to those seen in bull calves 1 week of age.

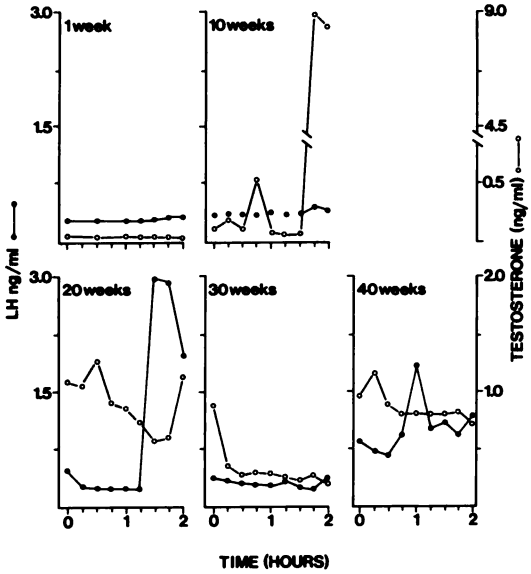

FIG. 2. Plasma concentrations of LH and testosterone in representative bull calves bled every 15 min. for 2 h at 1, 10, 20, 30 and 40 weeks of age each.

Basal plasma concentrations of LH at 20 weeks of age were less than 0.35 ng/ml. However, in each calf sampled at this time, from 1 to 3 peaks of LH were noted, with peak values ranging from 0.60 ng/ml to 3.0 ng/ml. Of the 5 bull calves sampled intensively at 20 weeks of age, plasma concentrations of testosterone were low in 2 (< 0.22 ng/ml), while in 3, concentrations varied in the range of less than 0.25 ng/ml to 1.72 ng/ml (Fig. 2).

In all bull calves sampled intensively at 30 weeks of age, plasma concentrations of LH were low and stable (< 0.35 ng/ml). Plasma testosterone concentrations remained below 0.35 ng/ml, except for one initial value for 1 bull calf (Fig. 2). Elevated basal plasma concentrations of LH were observed in all animals intensively sampled at 40 weeks of age, with plasma LH concentrations fluctuating between 0.75 ng/ml and 1.50 ng/ml. Plasma concentrations of testosterone were elevated for all, or part, of the 2 h blood collection period, with values ranging from 0.20 ng/ml to 2.00 ng/ml.

DISCUSSION

The temporal pattern of plasma concentrations of testosterone in bull calves, in samples taken every other week from birth to 40 weeks of age, resembles those observed by Rawlings et al. (1972) and Secchiari et al. (1976). Concentrations observed at 40 weeks of age resemble those of Hereford bulls at 1 year of age (Swanson et al., 1971), but were lower than values obtained for mature dairy bulls (Katongole et al., 1971; Thibier, 1976).

The transient peaks of plasma concentrations of testosterone revealed at 10 weeks of age by the more intensive blood sampling regimen, were not observed at any other time and were presumably not detected by the less intensive sampling regimen. In the mature bull, plasma testosterone concentrations vary as a series of peaks and troughs (Katongole et al., 1971; Thibier, 1976). Three to 7 peaks are observed in a 24 h period, with peaks lasting approximately 70 min. Thibier (1976) bled a group of mature dairy bulls every 10 min for 2 h and observed a plasma testosterone pattern temporarily, very similar to that observed in this study in 3 of 5 and 5 of 5 bull calves of 20 and 40 weeks of age, respectively. It is evident that the temporal pattern of plasma concentrations of testosterone resembles that observed in the adult as early as 20 weeks of age.

In the present study, it is not clear why plasma concentrations of testosterone are so low during the 2 h bleeding period at 30 weeks of age. It is unlikely that peaks of plasma testosterone were missed in all animals. In general, at 30 and 40 weeks of age plasma concentrations of testosterone were lower in plasma obtained during the 2 h bleeding period than in parallel samples taken biweekly. It seems possible that plasma hormone concentrations in the older bull calves could have been elevated by the stress of handling and venipuncture.

Stressful stimuli have been shown to cause growth hormone and prolactin release in cattle (Eaton et al., 1968; Johke, 1970), but the effects of stress on LH and testosterone release are not clear. In the present study the calves were very quiet during blood collection using jugular cannulae, but considerable stress was involved with handling for jugular venipuncture. Secchiari et al. (1976) described the pattern of concentrations of testosterone in the dairy bull calf sampled every 2 weeks through sexual maturation. Testosterone levels rose slowly until 6.5 months of age and then varied as a series of peaks and troughs to 14 months of age. In the present study, although there is an obvious disparity in the temporal pattern of plasma concentrations of testosterone, depending on the mode of sampling, both sets of data indicate the presence of a trough in plasma concentrations of testosterone during the period 30 to 35 weeks of age.

Rawlings et al. (1972) observed a biphasic pattern in plasma concentrations of LH during sexual maturation in Holstein bull calves. A similar pattern is indicated by the results of the present experiment in beef bull calves, bled biweekly from 1 to 40 weeks of age. The initial prepuberal increase in plasma concentrations of LH, observed in the present study, appears to be produced by frequent elevations of a relatively short duration (< 45 min). Very little variation was seen in plasma LH concentrations in the 5 bulls bled for 2 h at 10 weeks of age. At 20 weeks of age, all 5 bulls bled for 2 h exhibited a marked episodic fluctuation in plasma concentrations of LH. If these data are taken on an individual basis, with the data from biweekly bleedings, it appears that each bull calf experienced this mode of variation in plasma LH levels for a period of 3–6 weeks. Some variation in the timing of this phase exists between bulls, as the rate of sexual maturation probably varies between animals (MacMillan and Hafs, 1969). Again, the discrepancy in plasma concentrations of LH between the 2 sampling regimens at 30 weeks of age may reflect the influence of stress on blood hormone levels in the older bull calf. Plasma concentrations of LH observed at 40 weeks of age resemble those in yearling Hereford and Holstein bulls (Swanson et al., 1971; Rawlings et al., 1972). Levels were lower than those in adult mature bulls (Katongole et al., 1971) and in contrast to testosterone, the pattern of change in concentration did not resemble that of the adult as closely.

In the present study, no consistent correlation between plasma concentrations of LH and testosterone was observed. This was especially noted at 10 weeks of age and even at 20 and 40 weeks of age, where concentrations of testosterone and LH in plasma were elevated but in some cases, no clear interrelationship was seen. In the male rat, a prepuberal surge in plasma concentrations of LH is observed (Ojeda and Ramirez, 1972), at a time when plasma testosterone concentrations are low (Resko et al., 1968). Several 5α reduced androgens are produced by the testis of the rat (Lacroix et al., 1975) and these products may be involved in the control of gonadotropin secretion (Zanisi et al., 1973).

In the present study, the early prepuberal rise in plasma concentration of LH probably coincides with the onset of spermatogenesis. The appearance of mature spermatozoa in the testis occurred, following a period of gradually rising plasma concentrations of LH and testosterone. It is interesting to note that the most rapid phase of testicular and epididymal growth occurred during this latter phase of development. In the male rat, plasma concentrations of FSH (Goldman et al., 1971; Ojeda and Ramierz, 1972) and prolactin (Negro-Vilar et al., 1977) change markedly during sexual maturation. FSH is probably involved in the initiation of spermatogenesis (Steinberger, 1975) and prolactin may play a role in the development of the male sex accessory glands (Hafiez et al., 1971).

ACKNOWLEDGMENTS

One of us (N. C. R.) acknowledges the receipt of a postdoctoral fellowship from the South Carolina Agricultural Experiment Station. It is a pleasure to thank the graduate students at the Departments of Animal Science, Food Science and Dairy Science for

their help with blood collection. The assistance of Mr. B. Kennedy and Mr. D. Hudson is also acknowledged.

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RECOMMENDED REVIEWS

McCann, S. M., Ojeda, S. and Negro-Vilar, A. (1974). Sex steroids, pituitary and hypothalamic hormones during puberty in experimental animals. In: The Control of the Onset of Puberty. (M. M. Grumbach, G. D. Grave and F. E. Meyer, eds.). Wiley, New York. pp. 1–19.

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