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PLASMA TESTOSTERONE IN BULLS SEASONAL VARIATION*

By

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SUNDBY, A. and R. TOLLMAN: *Plasma testosterone in bulls. Seasonal variation.* Acta vet. scand. 1978, 19, 263—268. — Levels of peripheral plasma testosterone and LH were studied in 4 bulls hourly during a 12 hr. sampling period at 5 times of the year. The average plasma testosterone levels were significantly lower in October (1.8 ng/ml, $P < 0.001$) and December (2.5 ng/ml, $P < 0.05$) than in February, June and August (3.5, 3.7 and 3.7 ng/ml respectively). LH showed a slight fluctuation during the day, with values ranging between 0.8 and 3.8 ng/ml, but underwent no significant seasonal variation. A significant increase in average plasma testosterone was observed 1 hr. after the LH peaks ($P < 0.001$).

plasma testosterone; seasonal variation; bulls.

Seasonal variation in the levels of plasma testosterone has been demonstrated in the male of many species. The phenomenon has been described in seasonal breeders e.g. mink (*Nieschlag & Bieniek 1975*), rams (*Katongole et al. 1974*, *Purvis et al. 1974* and *Schanbacher & Lunstra 1976*), reindeer and caribou (*Whitehead & McEvan 1973*) and red deer (*Lincoln et al. 1970*). A similar phenomenon is seen in continuous breeders such as rats (*Mock & Frankel 1974*), rabbits (*Moor & Youglai 1975*), dogs (*Eik-Nes 1971*) and monkeys (*Plant et al. 1974*). Thus, before making comparisons of plasma levels of testosterone between bulls based on samples taken at any time throughout the year, it would be of value to investigate a possible seasonal variation in plasma testosterone.

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The previously demonstrated extensive variations in plasma testosterone levels in bulls in samples taken at different times during the day (*Katongole et al.* 1971, *Sanwal et al.* 1974) indicate the necessity of using mean values from numerous blood samples as the basis for comparison of hormone levels at different times of the year.

A close relationship between LH secretion and testosterone release has been demonstrated in bulls (*Katongole et al.* 1971, *Smith et al.* 1973 and *Thibier* 1975) and rams (*Sanford et al.* 1974). The existence of this LH-testosterone relationship as well as possibilities of finding seasonal variation in plasma LH made it of interest to include LH in the study.

MATERIAL AND METHODS

Seasonal variations of plasma testosterone were studied in 4 healthy, fertile bulls belonging to the Norwegian Red Breed, and ranging in age from 14 to 23 months. The bulls were used as semen donors twice a week during the whole year, and kept at Stensby Artificial Insemination Station, Hamar.

Blood was collected every hour during a 12 hr. period starting at 10 a.m., using vacutainers (Becton, Dickinson Ltd., France). Dates of sampling were June 22nd, August 27th, October 24th, December 19th 1973, and February 20th 1974.

Testosterone was measured by the RIA method of *Sanwal et al.* (1974) as modified by *Sundby et al.* (1975). All samples were analyzed in duplicate. The intraassay variation of the method was ± 0.37 ng/ml corresponding to a coefficient of variation of 11.9 %.

The measurement of plasma LH was based on the method of *Sand & Torjesen* (1973) as modified by *Flatlandsmo* (in prep.) with the additional modification of adding plasma instead of serum to the standard curve. The intraassay variation of the method was ± 0.43 ng/ml, corresponding to a coefficient of variation of 26 %.

RESULTS

Table 1 shows the mean values and ranges of plasma testosterone for each of the 4 bulls at the 5 different times of the year. The concentrations measured ranged between 0.2 and 15.8 ng/ml. The average plasma testosterone levels for the 4 bulls, based on hourly sampling during a 12 hr. period, were significantly

Table 1. Means and ranges of plasma testosterone (ng/ml) of 4 1—2 year old bulls for 5 12 hr. sampling periods, each representing 12 blood samples.

Time of the year	Bull 1	Bull 2	Bull 3	Bull 4	Mean \pm s Bulls 1, 2, 3, 4
June 22nd	3.7 (0.8—5.5)	2.6 (0.8—5.5)	3.9 (0.8—7.9)	4.6 (0.7—7.2)	3.7 \pm 2.3
Aug. 27th	5.9 (1.2—15.8)	2.2 (0.3—4.7)	5.1 (1.6—15.8)	1.7 (0.8—3.5)	3.7 \pm 3.7
Oct. 24th	2.3 (1.1—6.0)	1.5 (0.4—5.8)	2.0 (0.8—6.0)	1.4 (0.7—3.2)	1.8 \pm 1.3
Dec. 19th	3.0 (1.1—6.4)	2.3 (0.8—4.2)	3.0 (1.1—7.1)	1.8 (0.2—4.8)	2.5 \pm 1.6
Feb. 20th	4.9 (2.4—7.2)	2.9 (1.6—5.4)	2.9 (0.5—6.0)	3.4 (0.3—5.0)	3.5 \pm 1.7

lower in October ($P < 0.001$) and December ($P < 0.05$) than in June, August and February.

Plasma LH showed a slight diurnal fluctuation with values ranging between 0.8 and 3.8 ng/ml, but underwent no significant seasonal variation.

When LH levels were matched to testosterone levels, a systematic pattern was, however, observed. At the time of the occurrence of the LH peak the average testosterone level was 2.8 ± 1.9 ng/ml. One hour later the average value had increased to 4.9 ± 2.8 ng/ml. This increase is statistically highly significant ($P < 0.001$).

Not shown in Table 1 is the observation that all the animals had low testosterone levels at about 4 p.m., independent of the season of the year. In 3 of the bulls, consistent plasma testosterone pattern was observed on the different sampling days throughout the year. For each of these bulls a significant difference was found ($P < 0.02$) between the lowest and the highest values. In the fourth bull no consistent variation could be found. When the mean values for each sampling during the 12 hr. periods were calculated, including all bulls and all times of the year, a consistent diurnal variation pattern with peak at 12 a.m. and 8 p.m. and low value at 4 p.m. emerged. The differences between the mean values at 12 a.m. and 4 p.m., and between 4 p.m. and 8 p.m. were statistically significant ($P < 0.02$ and $P < 0.01$ respectively).

DISCUSSION

The plasma testosterone values recorded were in close agreement with the findings of other authors (Sanwal *et al.* 1974,

Smith *et al.* 1973, and Thibier & Martin 1975), but lower than the values reported by Katongole *et al.* (1971). The mean plasma testosterone values during the 12 hr. sampling period based on samplings 5 times of the year from 4 bulls, were quite similar to the mean plasma testosterone pattern of the 4 bulls described by Sanwal *et al.*, with low values at 4 p.m. and high values at 12 a.m. and 8 p.m.. This quite synchronized testosterone pattern can support the theory of Katongole *et al.* (1971) about an inherent central rythm controlling the hypophyseal testicular axis.

Plasma LH concentrations were within the ranges described by Smith *et al.* and Thibier (1975), although slightly lower than those described by Ectors *et al.* (1974) and Gombe *et al.* (1973) in bulls of similar age. Secchiari *et al.* (1976) and Karg *et al.* (1976) concluded that there is no evidence of seasonal effect upon plasma testosterone concentration in bulls, as 6 or 4 animals, respectively, which were born at different times showed similar testosterone pattern during development. The present investigation indicates the presence of a seasonal variation of plasma testosterone in bulls. On the average the levels were at their lowest at the end of October and rose through December and February to reach their highest values in June and August. No significant seasonal variation was found in plasma LH. However, in spite of the relatively high coefficient of variation of the LH method, a significant positive relationship was found between the LH peak and succeeding plasma testosterone level.

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SAMMENDRAG

Plasma testostérone hos okser — årstidsvariasjon.

Plasma testostérone og LH nivået ble studert hos 4 okser i prøver tatt hver time i en 12 timers periode ved 5 forskjellige tider av året. Gjennomsnittlig plasma testostéron var signifikant lavere i oktober (1,8 ng/ml, $P < 0,001$) og desember (2,5 ng/ml, $P < 0,05$) enn i februar, juni og august (henholdsvis 3,5, 3,7 og 3,7 ng/ml). LH svingte litt i løpet av dagen. Plasma konsentrasjonen varierte fra 0,8 til 3,8 ng/ml, men viste ingen signifikant årstidsvariasjon. En signifikant øking i plasma testostéron ble observert en time etter LH toppene ($P < 0,001$).

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