Serum profiles of androstenedione, testosterone and LH from birth through puberty in buffalo bull calves

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Summary. Blood samples were taken once per week for 4–7 weeks from 59 buffalo calves in 14 age groups, 1–2 months apart. Hormones were quantified by validated radioimmunoassays. Values of androstenedione and testosterone were low at birth (141.3 ± 33.5 pg/ml and 18.0 ± 2.9 pg/ml, respectively; mean $\pm$ s.d.). Serum androstenedione concentrations gradually increased from birth until 8 months of age and declined ($P < 0.05$) thereafter, whereas mean testosterone values were low up to 8 months and then significantly ($P < 0.05$) increased as age advanced. LH concentrations averaged 2.12 ± 0.47 ng/ml at birth. Thereafter, a decline in LH values was followed by an increase between 6 and 15 months of age. We conclude that, in buffalo bull calves, the pubertal period occurs from about 8 to 15 months of age. For pubertal buffalo bulls 15–17 months of age, serum concentrations of androstenedione, testosterone and LH were 156.9 ± 54.6 pg/ml, 208.4 ± 93.8 pg/ml and 2.10 ± 0.70 ng/ml, respectively.

Introduction

Despite the great number of studies devoted to androstenedione, testosterone and LH concentrations in peripheral blood in bull calves (*Bos taurus*) (MacMillan & Hafs, 1968; Rawlings, Hafs & Swanson, 1972; Schams & Butz, 1972; Karg *et al.*, 1976; Secchiari, Martorana, Pellegrini & Luisi, 1976; Lacroix, Garnier & Pelletier, 1977; Rawlings, Fletcher, Henricks & Hill, 1978; Lacroix & Pelletier, 1979; McCarthy, Hafs & Convey, 1979; Sundby & Velle, 1980; Amann & Walker, 1983), similar studies on hormonal changes in buffalo bull calves (*Bubalus bubalis*) are still lacking.

Puberty in Egyptian buffaloes (defined as the age when an ejaculate is obtained containing 10×10^6 spermatozoa/ml at which 10% or more are motile: Wolf, Almquist & Hale, 1965) takes place at a mean age of 17 months (Hemeida & Badawy, 1981). For dairy and beef bulls, puberty occurs at the age of 8–11 and 11–12 months respectively, while Brahman bulls reach puberty at 16 months of age (Almquist & Cunningham, 1967; Lunstra, Ford & Echterkamp, 1978; Fields, Hentges & Cornelisse, 1982). In most species, LH and testosterone are critical regulators of male sexual maturation. As yet no reports have been published on LH and testosterone secretory patterns in pubertal buffalo bulls.

The present attempt was, therefore, undertaken to study the changes in peripheral androstenedione, testosterone and LH concentrations in buffalo bull calves from birth through puberty.

Materials and Methods

Animals

The 59 buffalo bull calves, ranging in age from birth to 19 months, were allotted to 14 age groups, 1–2 months apart, with each group including 3–6 animals. All buffaloes were born on a farm near Cairo where they had been kept under the same conditions. They were fed according to conventional feeding standards (Chalmers, 1974).

Collection of samples

Blood samples were collected by venepuncture from the jugular vein once per week for 4–7 weeks from each animal. All collections were made at 08:00 h over 3 months from March to May. The buffalo calves were accustomed to handling and the samples could be taken with minimal restraint. Blood samples were allowed to clot at 4°C, then serum was harvested by centrifugation at 2000 *g* for 30 min. Serum was stored at –20°C until assayed.

Radioimmunoassays

Testosterone and androstenedione. Antisera against testosterone and androstenedione were kindly supplied by Dr B. Hoffmann, Institute of Physiology, Munich University, West Germany. Testosterone concentrations were determined on toluene:petroleum ether (2:5 v/v) extracts of buffalo serum following the procedure of Karg *et al.* (1976). The samples were assayed in duplicate. The antibody used to assay testosterone cross-reacted considerably (54%) only with 5 α -dihydro-testosterone, but it was assumed that the DHT concentration in buffalo serum was negligible because no differences were observed in plasma testosterone concentrations of developing bull calves before or after separation of DHT by thin-layer chromatography, although the DHT cross-reacted strongly with the antibody used (Karg *et al.*, 1976; Lacroix *et al.*, 1977). The sensitivity of the assay, the smallest concentration significantly distinguishable from zero, was 0.3 pg/tube. The intra-assay coefficient of variation averaged 8.6% and the inter-assay variation was 13.6%.

Androstenedione concentrations were assessed on toluene:petroleum ether (2:5 v/v) extracts of buffalo serum using a procedure identical to that employed for testosterone. The sensitivity of the assay, intra- and inter-assay coefficients of variation were 1.3 pg/tube, 9.0% and 14.8%, respectively.

The average recovery of testosterone (from 50 to 200 pg) added to 200 μ l samples of buffalo cow serum was $83.5 \pm 11.2\%$. After addition of androstenedione (50–200 pg) to 300 μ l buffalo cow serum, recovery of androstenedione was $85.0 \pm 9.7\%$. Parallelism was also determined by quantitating testosterone and androstenedione in different volumes of serum from a buffalo bull and an anoestrous buffalo cow. Toluene:petroleum ether (2:5 v/v) extracts of 150 to 350 μ l buffalo serum gave results parallel to the standard curves for testosterone or androstenedione.

LH. Serum LH concentrations were estimated by a double-antibody, non-equilibrium assay with rabbit 15 antibody (Niswender, Reichert, Midgley & Nalbandov, 1969). The standard employed in this assay was NIH-LH-B9. The sensitivity of the assay was 0.4 ng/tube. The intra-assay coefficient of variation averaged 8.9% and the interassay variation was 16.0%.

Further validation of the assay was accomplished by showing parallelism of different volumes of pooled buffalo serum samples to the standard curve (Text-fig. 1) and by demonstrating recovery of added NIH-LH-B9 to buffalo cow and bull serum. After addition of LH (80, 160, 320 pg) to 200 μ l serum samples of buffalo bulls and anoestrous buffalo cows, recovery of LH was $88.2 \pm 9.0\%$.

Text-fig. 1. Parallelism of different volumes of buffalo bull serum (---) to the standard curve of LH (—).

Statistical analyses

Hormone concentrations in samples obtained at birth and during 1–2-month periods were grouped together (mean $\pm$ s.d.). The effect of age on hormone concentrations was determined by one-way analysis of variance. If the F-value was significant, differences amongst means were evaluated by the Studentized Range Q method and were considered to be significant if $P < 0.05$ (Snedecor & Cochran, 1976).

Results

The mean concentrations ($\pm$ s.d.) of androstenedione, testosterone and LH at birth and for each 1–2-month period throughout the study are shown in Text-fig. 2. Age influenced both androstenedione ($P < 0.05$) and testosterone ($P < 0.01$) concentrations. Serum concentrations of androstenedione were low at birth (141.3 ± 33.5 pg/ml) but gradually increased with age. After a statistically significant drop ($P < 0.05$) at 8–9 months of age, serum androstenedione values gradually decreased as age advanced, reaching its lowest level at 17–19 months of age (106.0 ± 35.3 pg/ml).

Text-fig. 2. Serum profiles of androstenedione, testosterone and LH in buffalo bulls from birth through puberty. Values are mean $\pm$ s.d.

Testosterone concentrations were very low (18.0 ± 2.9 pg/ml) in serum of buffalo bull calves at birth and remained low up to 8 months of age (26.0 – 40.6 pg/ml). The first significant increase ($P < 0.05$) in serum testosterone occurred at 8–9 months of age. The onset of a second rise ($P < 0.05$) in testosterone concentrations at 13–15 months of age was followed by a linear and sharp increase with advancing age, reaching 376.2 ± 139.7 pg/ml serum in 17–19-month-old buffalo bulls.

The testosterone:androstenedione ratios remained low (between 0.1 to 0.2) for the first 8 months of life (Text-fig. 2). Marked increases ($P < 0.05$) were noted at 8–9 and 15–19 months of age (0.4 and 3.6 respectively).

LH values in sera of buffalo bull calves were high at birth (2.12 ± 0.47 ng/ml) and showed an

early rise in the first few weeks of life. Serum concentrations of LH decreased to a nadir (1.48 ± 0.45 ng/ml) at 3–4 months of age, followed by gradual increases as age advanced to reach a plateau (3.29 – 3.50 ng/ml) between 8 and 15 months of age. Thereafter, serum LH values decreased with advancing age and averaged 2.14 ± 0.56 ng/ml in 17–19-month-old buffalo bulls.

Discussion

The present serum androstenedione profile in buffalo bull calves conflicts with the findings of Lindner (1969), Singal & Gomes (1978) and Bedair & Thibier (1979) who noted increases at 2–4 months and a decrease to negligible values after 5–6 months of age in bull calves (*Bos taurus*). However, a pattern similar to serum testosterone concentrations in buffalo bull calves has been found at earlier ages in domestic bull calves (Lindner, 1959; MacMillan & Hafs, 1969; Rawlings *et al.*, 1972; Karg *et al.*, 1976). Peripheral testosterone concentrations have also been described as showing an oscillatory pattern after 6.5 months of age (Secchiari *et al.*, 1976) or to increase at a linear rate with age (Karg *et al.*, 1976; Lunstra *et al.*, 1978; McCarthy *et al.*, 1979; Thun, Leuch, Eggenberger & Zerobin, 1980; Fields *et al.*, 1982; Amann & Walker, 1983) in young bull calves. This discrepancy might be due to species and breed differences, seasonal variation, nutritional factors and differences in frequency of sampling and number of animals (Lunstra *et al.*, 1978; Sundby & Tollman, 1978; Lacroix & Pelletier, 1979). Also, season of birth was found to influence testosterone profiles (Amann & Walker, 1983). However, Karg *et al.* (1976) and Secchiari *et al.* (1976) concluded that there is no evidence of seasonal effect, as bulls born at different times showed a similar testosterone pattern during development.

The serum LH profile recorded in the present study disagrees with the conflicting reports on LH concentrations in bull calves; concentrations have been found not to vary with age (Schams & Butz, 1972; Karg *et al.*, 1976), to increase between 1 and 7 months (Rawlings *et al.*, 1972; Mori, Masaki, Wakabayashi, Endo & Hosoda, 1974), to increase between 3 and 4 months only (Amann & Walker, 1983), or to increase from 7 months of age to adulthood (Gombe, Hall, McEntee, Hansel & Pickett, 1973). Some of these discrepancies could be due in part to a pulsatile pattern of release (Gombe *et al.*, 1973; Thibier, 1975; McCarthy *et al.*, 1979; Schanbacher, 1981) and to insufficient blood sampling or the low number of animals studied. Season of birth had no influence on LH profile (Amann & Walker, 1983).

Study of testosterone and LH profiles in 3 buffalo bulls aged 1.5 to 2 years showed number of LH peaks ranging from no peak in one bull to 2 in the other two bulls, whereas the number of testosterone peaks varied from 1 to 10 (Chantaraprateep *et al.*, 1981). No information concerning seasonal influences on gonadotrophins and testicular steroids in buffaloes has been found in the available literature. In *Bos taurus* bulls, there are conflicting reports in the literature with regard to seasonal variation in LH and testosterone secretion (Thibier, 1975, 1976; Karg *et al.*, 1976; Secchiari *et al.*, 1976; Sundby & Tollman, 1978; Amann & Walker, 1983).

The finding that serum testosterone increased steeply in buffalo bulls 13–15 months of age or older, in spite of the rather constant LH concentrations, could be attributed to higher testicular sensitivity to LH as pubertal development proceeds (Catt, 1977) or to the fact that higher levels of testosterone, approaching the physiological concentration of the adult, suppress LH secretion (Lee *et al.*, 1976; D'Occhio, Schanbacher & Kinder, 1982). In *Bos taurus* bulls, a further increase in systemic concentration of testosterone occurs until at least 36 months of age (Hafs & McCarthy, 1979).

For many species, the pubertal period is associated with rapid testicular growth, increased secretion of gonadotrophins, secretion of testosterone in response to discharges of LH and initiation of spermatogenesis. The most dramatic changes occurred from 5 to 9 months of age in *Bos taurus* bulls (Rawlings *et al.*, 1972; 1978; Lunstra *et al.*, 1978; Thun *et al.*, 1980; Curtis & Amann, 1981; Schanbacher, 1981; Amann & Schanbacher, 1983). Results from the present study indicate that, in buffalo bull calves, the pubertal period occurs from about 8 to 15 months of age.

For pubertal buffalo bulls 15–19 months of age, serum androstenedione and LH concentrations were comparable with values reported for pubertal dairy and beef bulls (Thibier, 1975; Lacroix *et al.*, 1977; Bedair & Thibier, 1979; McCarthy *et al.*, 1979). The concentration of peripheral testosterone in buffalo bulls was much lower than that in *Bos taurus* bulls (Katongole, Naftolin & Short, 1971; Karg *et al.*, 1976; Thibier, 1976; Rawlings *et al.*, 1978; Amann & Walker, 1983).

The radioimmunoassays were conducted at the Department of Veterinary Biosciences, College of Veterinary Medicine, Urbana, Illinois, U.S.A., utilizing laboratory facilities under the direction of Dr J. Hixon. We thank Dr P. G. Weston for technical assistance.

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