Plasma concentrations of LH, progesterone and oestradiol during the oestrous cycle in swamp buffaloes (*Bubalus bubalis*)

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Summary. Plasma concentrations of LH, progesterone and oestradiol were measured in 5 cyclic swamp buffaloes. Progesterone levels began to increase after Day 5 (Day 0 = the day of oestrus) and reached a plateau after Day 10. A rapid decrease in progesterone levels occurred during the 5 days before oestrus, followed by a sustained increase in oestradiol concentrations. Basal LH levels decreased towards the mid-luteal phase and then progressively increased during the follicular phase.

Introduction

Although serum LH levels have been measured (Kaker, Razdan & Galhotra, 1980) during the oestrous cycle in the Asiatic buffalo (*Bubalus bubalis*), the changes in circulating LH concentrations concomitant with those of ovarian steroids have not been reported. Furthermore, no comparable data are available in temperate areas. The present study was therefore undertaken to analyse such changes of plasma LH, progesterone and oestradiol levels during the oestrous cycle in buffaloes under temperate climatic conditions.

Materials and Methods

Daily blood samples (30 ml) were collected by jugular venepuncture in 5 cyclic swamp buffaloes, 6–10 years old, starting on the day of oestrus (Day 0). The animals were kept in a loose-housing system and checked for manifestation of oestrus twice daily by using a vasectomized bull (Kanai & Shimizu, 1983). Plasma was obtained by centrifugation and stored at -15°C . LH was measured by a heterologous radioimmunoassay according to the method of Niswender, Reichert, Midgley & Nalbandov (1969). Antiserum for ovine LH (GDN No. 15), which was supplied by Dr G. D. Niswender, was used with reference standard NIH-LH-B7. Purified bovine LH (LER-1716-2) was used as the ^{125}I -labelled preparation. There was a good parallelism in the inhibition curves between standard LH and buffalo plasma (parallel line test, $k = 3$, $P > 0.20$; Text-fig. 1). The sensitivity of the method was 0.3 ng/ml with intra- and inter-assay coefficients of variation (CV) of 9.6 and 13.5%, respectively. Progesterone was measured by a competitive protein-binding assay as described by Neil, Johansson, Datta & Knobil (1967) with a slight modification. Pregnant guinea-pig plasma was used as a binding reactor at 1:2000 dilution. The values were corrected for the efficiency of extraction with petroleum ether (mean $\pm$ s.e.m.: $91.8 \pm 0.9\%$, $n = 10$). The recovery rate of known amounts (0.4–4.0 ng) of progesterone was 98% ($n = 20$). The sensitivity was 0.05 ng with intra- and inter-assay CVs of 12.7 and 12.9%, respectively. In the oestradiol assay (Orczyk, Caldwell & Behrman, 1974) an antiserum against oestradiol-6-oxime-bovine serum albumin was

raised in a rabbit. This antiserum cross-reacts 1.3% with oestrone, 0.1% with oestradiol-17 α and 2.5% with oestriol. Duplicate samples (3–5 ml) of plasma were extracted with 5 volumes of diethyl ether. The extracts were evaporated under N₂, redissolved in 200 μ l benzene : methanol (85 : 15 v/v) and applied to a Sephadex LH-20 micro-column (2 ml). The procedure loss was determined by adding [³H]oestradiol-17 β to separate plasma samples ($81.5 \pm 0.6\%$, $n = 10$). Assay recovery of 10–200 pg oestradiol added to 5 ml plasma samples averaged 96% ($n = 25$). The intra- and inter-assay CVs were 10.3 and 14.5%, respectively, and the sensitivity was 5 pg.

Text-fig. 1. The standard curve for the LH radioimmunoassay. Antiserum for ovine LH (GDN No. 15) was used with purified bovine LH (LER-1716-2) as the ¹²⁵I-labelled preparation. Buffalo plasma, obtained on the day of oestrus, was diluted with 1% BSA-PBS.

Results

Plasma concentrations of LH, progesterone and oestradiol in 5 cyclic buffaloes are shown in Text-fig. 2. Progesterone levels gradually increased after oestrus with the first significant rise on Day 6 (t test, $P < 0.05$) and reached a plateau after Day 10 with a peak value (2.7 ng/ml) on Day -6. A precipitous decrease in progesterone levels occurred during the 5 days before oestrus, followed by a sustained increase in oestradiol levels. The highest concentration of plasma oestradiol was 9.5 pg/ml on Day 0. The basal LH levels decreased towards the mid-luteal phase. The values between Days 6 and -6 inclusive (0.8–1.2 ng/ml) were significantly lower than those for the early and the late 5-day periods ($P < 0.001$). There was a progressive, 3-fold increase ($P < 0.01$) in the basal LH levels during the 5-day period preceding the sharp rise which occurred on Day 0 (16.7 ng/ml). The basal LH levels between Days 1 and -6 were negatively correlated with progesterone levels in all the animals (r : -0.62 to -0.80).

Discussion

The pattern of plasma progesterone and oestradiol concentrations during the oestrous cycle in buffaloes was similar to that reported for cattle (Wettemann, Hafs, Edgerton & Swanson, 1972), although the progesterone levels during the luteal phase were relatively low in buffaloes. This low level of progesterone (peak values: 2–3 ng/ml) supports the findings of Kamonpatana, Kunawongkrit, Bodhipaksha & Luvira (1979) and Rao & Pandey (1982). Oestradiol levels in this study were 3–4 times lower than those reported for river buffaloes (Bachlaus, Arora, Prasad &

Text-fig. 2. Mean $\pm$ s.e.m. plasma concentrations of LH (●), progesterone (▲) and oestradiol (○) for 5 swamp buffaloes during the oestrous cycle. The duration of oestrous cycles, which ranged from 19 to 23 days, was standardized to 20 days.

Pandey, 1979). The discrepancy may be due to differences in the radioimmunoassay used. Plasma LH concentrations in this study were in close agreement with those reported by Kaker *et al.* (1980) in spite of differences in breed, assay system and environmental conditions. Kaker *et al.* (1980), however, did not demonstrate any temporal changes in the basal LH levels during the oestrous cycle, perhaps because blood samples were taken only at 3-day intervals. The decrease in basal LH concentrations towards the mid-luteal phase and subsequent increase during the follicular phase have been clearly observed in sheep (Hauger, Karsch & Foster, 1977) but less convincingly in cattle (Chenault, Thatcher, Kalra, Abrams & Wilcox, 1975). Although single daily samples may not be fully adequate to describe correlations amongst hormones, the data presented here provide circumstantial evidence that negative feedback influence of progesterone is presumably involved in the control of tonic LH secretion in cyclic buffaloes.

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