Serum LH concentrations in cyclic buffalo (*Bubalus bubalis*)

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Summary. Circulating LH levels of 14 Murrah buffaloes were determined by a double-antibody radioimmunoassay using an homologous bovine system. At the onset of oestrus, the mean peak circulating LH level was 20.80 ± 3.43 (s.e.m.) ng/ml in the hotter months (June–August) and 21.24 ± 0.98 ng/ml during the cooler months (October–December), values remaining high for about 4 h. Basal levels throughout the rest of the cycle averaged 1–2 ng/ml. Conception occurred in 7 of the animals showing LH peaks (2/8 in June–August, 5/6 in October–December).

Introduction

Luteinizing hormone (LH) has a vital role in various ovarian events and is one factor that influences reproductive cyclicity in female mammals. There have been several reports on circulating LH levels during the oestrous cycle in cattle (*Bos taurus*) (Henricks, Dickey & Niswender, 1970; Snook, Saatman & Hansel, 1971; Madan & Johnson, 1973; Dobson, 1978) and for the zebu (*Bos indicus*) (Rao, Rao, Rao, Reddy & Rao, 1979). Information on LH concentrations in water buffaloes (*Bubalus bubalis*), however, is scanty (Heranjal, Sheth, Moodbidri, Desai & Rao, 1976; Sheth, Wadadekar, Moodbidri, Janakiraman & Parameswardan, 1978). During the hotter months, many buffalo have poor conception rates and exhibit weak symptoms of oestrus (Johari, 1960; Kodagali, 1968; Roy *et al.*, 1968; Roy, Bhattacharya & Luktuke, 1972) and this reduced fertility is of economic importance because the buffalo contributes greatly towards milk and meat production in many countries. We have therefore examined circulating LH concentrations in buffaloes at various stages of the oestrous cycle during hotter and cooler months.

Materials and Methods

Animals

The 14 animals were Murrah buffaloes (*Bubalus bubalis*). They had a known breeding history and had been cycling regularly (20–22 days) before being taken at random from the University Farm. A young vasectomized buffalo bull was taken to the experimental animals three times a day (05:00, 13:00 and 21:00 h) for detection of oestrus. An animal detected in oestrus was isolated immediately. In addition, the animals were kept under constant surveillance. Eight of these animals (Group 1) were detected in oestrus in the hotter months of June to August (32.5–40.3°C mean maximum ambient temperature); the rest (Group 2) came into oestrus in the cooler months of October to December (23.2–34.5°C). The animals in oestrus were rectally palpated once to confirm follicular growth in the ovaries, and smears of the vaginal mucus were examined for the fern patterns associated with oestrus. At this oestrus the females were mated by a bull of proven fertility.

Blood samples were collected when oestrus was first detected and then every 4 h for 24 h, followed by sampling on every 3rd day for a further period of 26 days. Serum was separated at 5°C and stored frozen until used for assay of LH.

LH assay

Method. The double-antibody radioimmunoassay system of Niswender, Reichert, Midgley & Nalbandov (1969) was used, with slight modifications, to measure LH concentrations. An homologous complete bovine system formed the basis of the assay. Purified bovine LH (LER-1072-2) was radioiodinated with ^{125}I at room temperature (28°C) by a modification of the method of Greenwood, Hunter & Glover (1963): 2.5 µg bLH were reacted for 1 min with 1 mCi sodium ^{125}I in the presence of 50 µg chloramine T. The reaction was stopped by adding 125 µg sodium metabisulphite. This was followed by purification of the labelled bLH by passing it through a Sephadex G-50 column (0.8 × 20 cm). The labelled bLH stock solution was repurified every time before use. The antiserum to bLH (B-225) was raised, characterized and purified for monospecificity by Dr G. D. Niswender, Colorado State University, U.S.A., and its titre was determined before use.

A standard preparation of bLH (NIH-LH-B10) was diluted with 0.1% egg white–0.01 M-phosphate buffer–0.14 M-NaCl, pH 7.0 (EW–PBS) to obtain different standard doses, i.e. 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 ng in 500 µl, for the dose–response curve. Different standards and various amounts of serum (50–300 µl) were placed in the assay tubes (10 × 75 mm), and 0.1% EW–PBS was added to bring the volume to 500 µl in each tube. The non-immune normal rabbit serum (NRS) was diluted 1:400 in 0.05 M-EDTA–PBS at pH 7.0. This diluted serum was used for the dilution of anti-bLH serum (1:80 000). To each of the assay tubes 200 µl diluted anti-bLH serum were added, and the contents were mixed and incubated for 24 h at 4°C; 100 µl labelled bLH (approximately 10 000 c.p.m.) were added before incubation for a further 24 h. Then 200 µl anti-rabbit gammaglobulin, diluted 1:12, were added to each tube which was kept for 48 h to precipitate the bound fraction. All the tubes were counted for total activity using a manual Gamma Spectrometer (ECIL, GRS-23 A). After addition of 2.5 ml cold PBS to each tube, the contents of each tube were centrifuged at 1000 g and 5°C for 30 min. The supernatant was carefully decanted and the precipitate was counted for activity in the bound fraction. Percentage binding was calculated for each tube. The standard dose–response curve was prepared by plotting standard doses and percentage binding on logit-log paper. The slope of the curve was determined by linear regression analysis. The correlation coefficient was also calculated. The concentration of LH in the serum samples was determined from the standard

Text-fig. 1. Logit-log transformation of a representative dose–response curve for the assay of bovine LH in buffalo serum.

curve. The tubes containing the standards and the serum samples were run in duplicate and the average of the two taken for calculation. A set of standard doses was run and a standard curve was prepared for each assay of the serum samples.

Validation. The anti-bLH serum used bound approximately 30% of ^{125}I -labelled bLH at a dilution of 1:80 000 in the absence of bLH standard or unknown serum. This initial binding was considered to represent 100% for calculation of percentage binding in the tubes containing the standards and serum samples. A representative dose-response curve is shown in Text-fig. 1. The slope of the standard curve was found to be -2.32 and the correlation coefficient was 0.99 . Non-specific binding ranged between 2.5 and 5% in different assays. The quantity of the serum did not affect the LH estimate when tested in volumes varying from 100 to $300\ \mu\text{l}$ for each assay tube. The sensitivity of the assay was up to a minimum hormone level of $0.125\ \text{ng}$. The intra-assay variation was within 5% and inter-assay variation was 8.62% for 7 assays.

Results

Group 1

Rectal palpation of the oestrous animals revealed the presence of a small Graafian follicle and the vaginal mucus smear exhibited a typical fern pattern.

Individual LH values during the first 24 h after the detection of oestrus are given in Table 1 and those on different days after oestrus are shown in Table 2. Buffaloes 1, 2 and 3 had the highest concentrations at the time of detection of oestrus. Buffaloes 4 and 5 showed peak levels at 20 and 24 h. Buffaloes 6 and 7 did not exhibit elevated LH levels within the first 24 h after

Table 1. Serum LH levels (ng/ml) at different times after detection of oestrus in Murrah buffaloes during the hotter months of June–August

Time after detection of oestrus (h)	Buffalo no.							
	1	2	3	4	5	6	7	8
0	22.00	32.00	12.25	1.60	4.15	2.42	3.80	2.84
4	13.00	2.45	4.70	1.90	3.40	2.10	2.75	5.75
8	2.65	1.40	1.38	1.60	2.25	1.60	1.25	21.90
12	2.43	0.85	1.47	5.44	2.03	1.48	1.70	3.79
16	2.86	1.27	1.13	—	2.56	1.78	0.96	4.16
20	1.63	1.83	1.13	15.15	2.13	1.86	1.33	1.69
24	2.30	0.97	0.98	6.99	22.64	2.06	2.17	1.63

Table 2. Serum LH levels (ng/ml) on different days of the oestrous cycle of Murrah buffaloes during the hotter months of June–August

Time after detection of oestrus (days)	Buffalo no.					Mean $\pm$ s.e.m. for Buffaloes 1–5	Buffalo no.		
	1	2	3	4	5		6	7	8
1	22.00	32.00	12.25	15.15	22.64	20.80 ± 3.43	2.06	3.80	21.90
2	1.10	1.30	1.08	1.38	1.26	1.21 ± 0.23	0.90	1.33	1.66
5	0.93	0.64	1.03	1.20	1.96	1.15 ± 0.22	1.08	0.92	—
8	0.85	0.64	0.75	1.20	1.38	0.96 ± 0.14	3.23	1.33	2.19
11	3.50	0.92	0.73	0.78	1.36	1.46 ± 0.52	0.85	3.16	12.39
14	0.70	0.61	0.98	1.96	1.66	1.18 ± 0.27	0.85	0.82	2.88
17	2.63	1.63	1.33	1.80	0.98	1.67 ± 0.28	0.70	2.70	1.91
20	1.71	2.31	0.83	0.70	1.38	1.39 ± 0.29	0.92	—	1.86
23	0.88	4.90	1.03	0.78	1.16	1.75 ± 0.79	1.08	—	1.75
26	0.95	—	—	0.60	0.62	0.72 ± 0.11	0.90	—	—

detection of oestrus or on subsequent days. Buffalo 8 showed an LH peak at 8 h and another peak on Day 11, but values on other days were basal. Non-peak values in the 8 animals ranged from 0.60 to 13.00 ng/ml, although values at 4 h after the peak value were generally higher than those on other days. Buffaloes 3 and 4 conceived but none of the others did so.

Group 2

Graafian follicles were detected by rectal palpation but they were generally larger than those in Group 1 animals. The vaginal mucus dried in a fern pattern as in the summer months.

The LH concentrations in Buffaloes 9–14 are given in Tables 3 and 4. Buffaloes 9–12 showed peak LH levels at 0 or 4 h after detection of oestrus, but there was no detectable peak in Buffaloes 13 and 14 in the first 24 h. However, both animals had their highest concentrations on Day 2, although only that for Buffalo 13 was within the range shown by Buffaloes 9–12. Concentrations were again higher at 4 h after the peak value, while values on other days ranged from 0.37 to 4.99 ng/ml. Buffaloes 9–13 conceived but Buffalo 14 did not.

Table 3. Serum LH levels (ng/ml) at different times after detection of oestrus in Murrah buffaloes during the cooler months of October–December

Time after detection of oestrus (h)	Buffalo no.					
	9	10	11	12	13	14
0	21.25	22.22	23.00	4.77	1.00	1.45
4	3.75	9.00	9.90	18.50	0.98	1.51
8	0.97	2.60	1.73	4.90	2.13	1.88
12	0.85	1.83	1.16	1.76	2.06	0.97
16	0.90	0.88	1.21	1.56	3.03	1.20
20	0.61	0.57	1.16	2.23	2.36	0.90
24	0.59	3.75	0.53	1.35	1.23	0.81

Table 4. Serum LH levels (ng/ml) on different days of the oestrous cycle in Murrah buffaloes during the cooler months of October–December

Time after detection of oestrus (days)	Buffalo no.				Mean $\pm$ s.e.m. for Buffaloes 9–12	Buffalo no.	
	9	10	11	12		13	14
1	21.25	22.22	23.00	18.50	21.24 $\pm$ 0.98	3.03	1.88
2	0.96	0.71	0.68	1.18	0.88 $\pm$ 0.12	21.00	3.87
5	1.29	1.83	0.66	0.58	1.09 $\pm$ 0.29	0.73	0.81
8	0.62	0.93	1.11	0.96	0.90 $\pm$ 0.10	2.25	0.79
11	0.80	1.26	0.42	1.05	0.88 $\pm$ 0.18	0.65	0.57
14	0.90	0.80	0.57	1.70	0.99 $\pm$ 0.24	0.96	0.62
17	0.95	0.85	0.48	1.40	0.92 $\pm$ 0.19	—	0.61
20	1.25	0.53	0.62	4.99	1.85 $\pm$ 1.06	0.65	0.59
23	1.12	0.68	0.37	—	0.72 $\pm$ 0.22	1.63	0.45
26	0.69	0.85	0.67	—	0.74 $\pm$ 0.06	1.51	1.01

Discussion

The higher LH concentrations in most of the buffaloes at the time they were detected in oestrus are consistent with observations on cattle (Henricks *et al.*, 1970; Madan & Johnson, 1973; Schams, Schallenberger, Hoffman & Karg, 1977; Dobson, 1978). However, large variations in the peak LH levels of cattle have been reported by different workers. The mean peak LH levels

in buffaloes obtained in our study (about 21 ng/ml in both groups) are comparable to the reports of Webb, Lamming, Haynes, Hafs & Manns (1977), Schams *et al.* (1977, 1978) and Dobson (1978) but are lower than those reported by Niswender *et al.* (1969), Henricks *et al.* (1970), Madan & Johnson (1973), Kaltenbach *et al.* (1974) and Lemon, Pelletier, Saumande & Signoret (1975). Our values are also lower than those obtained by Rao *et al.* (1979) for 2 zebu cows (57.5 ng/ml). In our study, the mean basal LH levels was about 1.28 ng/ml in Buffaloes 1–5 in the hotter months and about 1 ng/ml in Buffaloes 9–12 during the cooler months. These values are comparable to the basal values reported for cattle. Heranjal *et al.* (1976) measured circulating LH levels of 7 Murrah buffaloes during the oestrous cycle and reported peak levels of 14.03–38.5 ng/ml at oestrus and basal levels between 5.4 and 9.8 ng/ml at other times during the cycle. These values are higher than those found in the present study, but we cannot explain them unless it be due to the different assay systems employed. The report of Sheth *et al.* (1978) shows mean circulating LH levels of 19.8 ng/ml at oestrus and 14.8 ng/ml on Day 15 of the cycle in buffaloes of the Surti breed during winter. This high Day 15 value is hard to explain by breed or assay differences.

Conception rate was better in Group 2 (5/6) than in Group 1 (2/8) animals. Of the animals that did not conceive 4 (Nos 1, 2, 5 and 8) had what appeared to be good (in value and timing) LH peaks, and a failure of ovulation seems unlikely. An LH peak was not observed in Buffaloes 6, 7 and 13; it is possible that this was missed during the sampling schedule but none of the 3 animals conceived and they may not therefore have ovulated.

Only one animal (No. 8) showed a small mid-cycle LH peak followed by a fall to basal LH levels. The appearance of a mid-cycle LH peak in buffaloes was reported by Heranjal *et al.* (1976) and has also been found in cattle (Snook *et al.*, 1971): it is thought to be correlated with growth and atresia of anovulatory follicles.

Although there was a difference in conception rate for the buffaloes coming into oestrus in the hotter and cooler months, there was no appreciable difference in the mean peak or basal levels of LH. However, these observations are based on only small numbers of animals and generalizations are unwarranted.

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