Radioimmunoassay of Luteinizing Hormone in the Carabao

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A sensitive radioimmunoassay method for the measurement of luteinizing hormone (LH) in carabao serum was validated. LH surge concentrations in 21 confined female carabaos were determined.

Majority of the animals showed the preovulatory LH peak. Twelve of 21 carabaos (57.1%) had high values (5 ng/mL or greater); others gave medium or low values. Basal, tonic values (0.4 ± 0.2 ng/mL) were observed during the estrous cycle except for the brief LH surge which appeared at either early or late estrus stage. During pregnancy, LH concentrations also remained basal (0.5 ± 0.3 ng/mL), apparently suppressed by the sustained higher levels of progesterone.

The time of LH peak appearance and intensity relative to onset of estrus are useful in selecting carabaos which ovulate normally. Optimum time of insemination can also be determined for a successful breeding program. Carabaos with a late LH surge during estrus require another insemination 96 h after prostaglandin treatment to improve the conception rate. LH profiles can also be used to evaluate reproductive problems related to ovulation.

INTRODUCTION

Interest in the Philippine swamp buffalo (carabao) as a multipurpose animal for draft, meat, and milk has increased in the wake of rising costs of petroleum fuel and the need for self-sufficiency in animal protein to feed our increasing population (1,2,3). Reproductive and genetic constraints, however, are observed as major deterrents to its full utilization.

Endocrine factors are known to influence gonadal function, modulating physiological events such as estrus and ovulation. In the past, hormones were measured by bioassays, but these were insensitive, time-consuming, and entailed the use of many animals (4). Radioimmunoassay techniques can now characterize endocrine events by measuring hormones as low as 5 pg/mL in blood serum or plasma, unlike bioassays or chemical methods which could measure to the µgram level only (5).

Progesterone concentrations during the estrous cycle and other reproductive phases of the carabao were reported earlier (6,7). This study validates a heterologous system for the radioimmunoassay of LH in the carabao, and presents data on LH values during estrus, the estrous cycle, and pregnancy.

LH, a gonadotropin, stimulates estrogen and progesterone secretion, causing ovulation to take place (8). Final maturation of the graafian follicle and the development of the *corpora lutea* are also stimulated. In the female, there is cycling of plasma LH levels, with midcycle or ovulatory peaks many times the basal level. This phenomenon (the LH surge) is therefore of interest as an indi-

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cator of ovulation. A knowledge of LH patterns during the estrus period would serve as a guide in determining the optimum time of insemination. It would also help in the evaluation of reproductive disorders.

MATERIALS AND METHODS

Twenty-one adult female carabaos (3 years old and over) were confined at the National Artificial Breeding Center, Alabang (6). They were monitored periodically for infectious and parasitic diseases.

Bovine LH (LER-1716-2-bLH in 0.5 M phosphate buffer, obtained from the National Pituitary Agency, Bethesda, Md., U.S.A.) was reacted for 60-90 sec with ^{125}I (^{125}I-NaI for protein iodination, in NaOH, pH 7-11, 1 mCi, from Amersham International Ltd., Eng.). Chloramine-T was used as catalyst, and the reaction ended by adding $NaHSO_3$. The mixture was purified through a Biogel P-60 column (100-200 mesh, Bio-Rad Labs., U.S.A.). One mL serial fractions in phosphate buffer (PBS-G) were collected and 10-µL aliquots were measured for radioactivity in a gamma spectrometer, to determine the peak fraction of the radiolabelled hormone (9).

An evaluation of the purity of the ^{125}I-LH after separation through the Biogel P-60 column was performed by electrophoresis. Pure and damaged ^{125}I-LH in the freshly prepared and in the 3-week old purified LH were compared.

Radioimmunoassay of LH in carabao serum/plasma was performed using a heterologous system for the double antibody method described by Niswender *et al.* (10). Bovine LH (NIAMDD-bLH-4) standard was used since a carabao LH standard was not available.

Aliquots of carabao serum (0.2 mL) were diluted with 0.3 mL PBS-G (0.05 M phosphate buffer saline, with and without gelatin). The first antibody (200 µL No. 15 rabbit antiovine LH, 1:50,000, in normal rabbit serum PBS-EDTA) was added to each assay tube, vortexed gently and the solution incubated at 4°C overnight. A tracer consisting of the labelled LH (5,000 cpm/0.1 mL) was added, and the solution again incubated at 4°C overnight. The second antibody (200 µL , sheep anti-rabbit gamma globulin, 1:40 in PBS-EDTA) was added, and the resulting solution incubated for 3 more days at 4°C. Three mL PBS buffer was then added to each assay tube, except the total counts (TC) tubes, prior to centrifugation at 3,000 rpm for 30 min. The supernate was aspirated and the radioactivity of the precipitate was measured in the automatic gamma spectrometer (Siemens-Germany).

Validation of RIA procedure for LH in carabao serum. Standard solutions of bovine LH (NIAMDD-bLH-4) with concentrations ranging from 0.05 to 0.5 ng/assay tube were prepared for the calibration curve to determine the sensitivity of the test.

To evaluate test specificity, increasing amounts of carabao serum (0.07-0.5 mL/assay tube) were analyzed for LH and compared with increasing concentrations (0.05-0.5 ng/assay tube) of standard solutions of bovine LH (NIAMDD-bLH-4).

Coefficients of variability were used to determine precision. Six replicates of carabao control serum in a single assay (intra-assay test), and replicates in six assays (inter-assay test), were analyzed for LH concentrations.

The accuracy of the RIA procedure was determined by a recovery study which consisted of adding known amounts of LH to carabao serum, and its subsequent radioimmunoassay. This was done for 3 concentrations: 0.1, 0.3, and 0.5 ng/mL.

Analysis of LH in the adult female carabao was done using blood samples collected every 6 h from individual carabaos during natural and induced estrus, for the duration of estrus (overnight to succeeding day). Estrus was induced using prostaglandin analogs (dinoprost tromethamin, Lutalyse, Upjohn Co., 25 mg/mL dose). The samples were allowed to clot and the separated sera were stored in the freezer until assayed. Blood samples which were collected daily during the estrous cycle and weekly during pregnancy, were analyzed for progesterone and LH.

RESULTS AND DISCUSSION

Radioiodination and purification of

LH. The bovine LH (LER-1716-2-bLH) was successfully iodinated with ^{125}I by the chloramine-T method. Passage of the reaction mixture through a Biogel P-60 column gave the ^{125}I-LH peak in fractions 4 to 6 (Fig. 1). A second peak representing the unreacted radioiodine was observed in fractions 11 and 12. Table 1 shows an evaluation of the labelled LH by electrophoresis. When this purified hormone was stored for 3 wk, appreciable degradation took place with the liberation of free ^{125}I (21%); the amount of damaged labelled hormone (43%) was greater than that of the pure ^{125}I-LH (36%). This shows the need to use the labelled LH within 2 wk from radioiodination to ensure the quality of the radiotracer for RIA work.

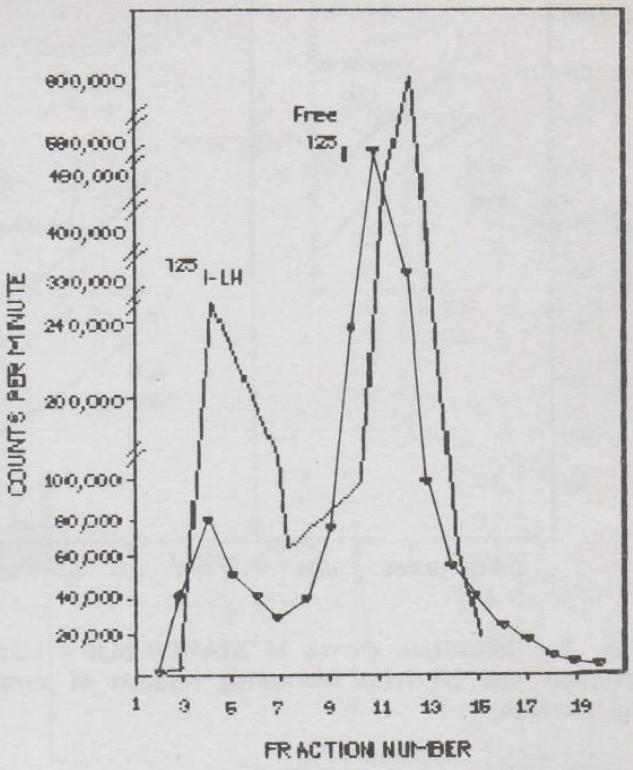

Fig. 1. Elution patterns of ^{125}I-LH purified through Biogel P-60 column.

Validation of RIA procedure for carabao serum LH. The sensitivity of the assay is 50 pg. Optimum dilutions of 1:50,000 and 1:60 were found for the first antibody and second antibody, respectively. Good specificity was shown by parallelism between the endogenous LH in buffalo serum (0.07-0.5 mL/assay tube) and bLH (NIAMDD-bLH-4) solutions at concentrations ranging from 0.05 to 0.5 ng/assay tube (Fig. 2). The intra-assay coefficient of variability ranged from 12.2 to 14.1% while the inter-assay CV was 18.1%. Mean recovery was 99% for all 3 levels of LH (0.1, 0.3, and 0.5 ng/mL) added to carabao control serum (Fig.3). The results show that with the use of a heterologous system for the double antibody method of radioimmunoassay, it is possible to quantify the LH content in carabao serum or plasma.

Circulating LH in the carabao Majority of the animals were observed to

Table 1. Test for purity of ^{125}I-LH after column chromatography through Biogel P-60 by electrophoresis.

	ONE DAY AFTER IODINATION			THREE WEEKS AFTER IODINATION		
FRACTION NO.	Fr 4	Fr 5	Fr 6	Fr 4+5	Fr 11	Position
^{125}I-LH, intact	51.0%	42.4%	38.2%	36%	0	origin
^{125}I-LH, damaged	3.7%	-	-	43%	14%	segm 5,6
^{125}I, free	4.3%	5.5%	6.7%	21%	86%	segm 12
% Purity ^{125}I-LH	87.9%	88.5%	85.1%			

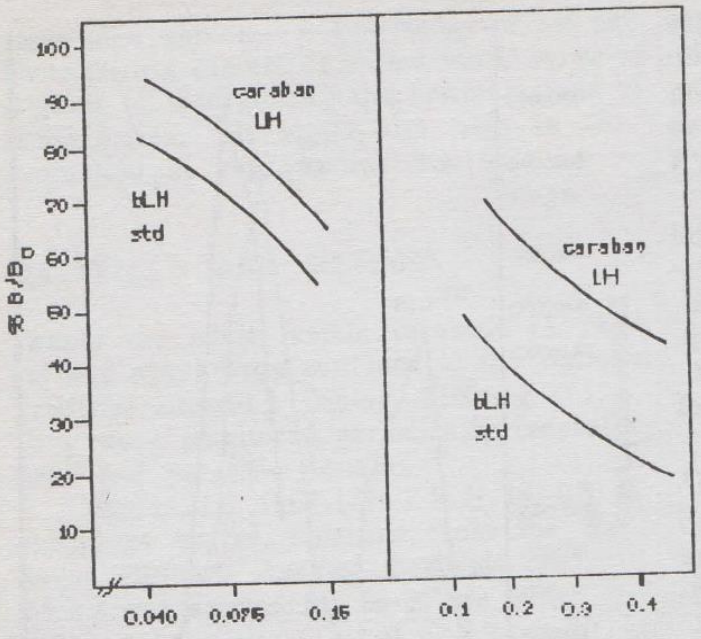

Fig. 2. Inhibition curves of NIAMDD-bLH-4 standard solutions and LH from increasing volumes of serum of the carabao.

have an LH surge lasting for 6 h during the estrus period with values of 5 ng LH/mL or greater. LH concentrations were basal and tonic during the rest of the estrous cycle, varying from 0.1 to 0.7 ng/mL, with a mean of 0.4 ± 0.2 ng/mL. Table 2 presents LH values in 5 carabaos with significant LH surge. During pregnancy, LH concentrations remained basal (0.5 ± 0.3 ng/mL), apparently suppressed by the sustained higher progesterone level of at least 1 ng/mL (6). The data agree with those of Kamonpatana for the Thai swamp buffalo on estrus and pregnancy LH values (11,12).

The intensity of the LH surge varied among the carabaos studied, 12 of 21 (57.1%) showing high LH values (5 ng/mL or greater), and 6 of 21 (28.3%) showing relatively low to insignificant values. In the former case, ovulation is expected to occur normally. In the latter, the weak or negligible peak indicates insufficient LH secretion from the anterior pituitary, which may not be enough to cause ovulation. Attempts at breeding the animal may therefore not be successful.

The time at which the LH peak appeared relative to the onset of observed estrus varied (Fig.4), with 38.7% of the animals showing an early LH surge (C-1, C-3) and 61.3% giving a late surge (C-7, C-9, and C-14). Where the LH surge is delayed, and where a fixed-time insemination regime (e.g., 72 and 96 h from prostaglandin treatment) may fail to catch the late ovulation, a third insemination will improve the chances of conception. Hafs *et al.* reported higher fertility rates in cows and heifers inseminated 3 to 5 days after treatment with prostaglandin (13).

The presence of double LH peaks during estrus was observed in 3 carabaos (C-9, C-22, and C-29). This could indicate double ovulation, and warrants further investigation.

The data on carabao serum LH obtained by the validated radioimmunoassay procedure, in conjunction with clinical and behavioral observations, have provided valuable insights into the ovulatory processes of

Table 2. LH concentrations during estrus in individual carabaos with significant LH peak.

Carabao Number	LH surge ng/mL	(μIU/mL)
C-2	6.50	(15.6)
C-7	8.50	(20.4)
C-29	6.75	(16.2)
C-15	11.75	(28.2)
C-16	10.00	(24.0)

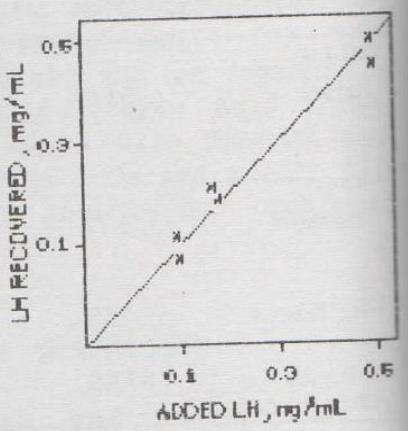

Fig. 3. Recovery of NIAMDD-bLH-4 added to carabao serum.

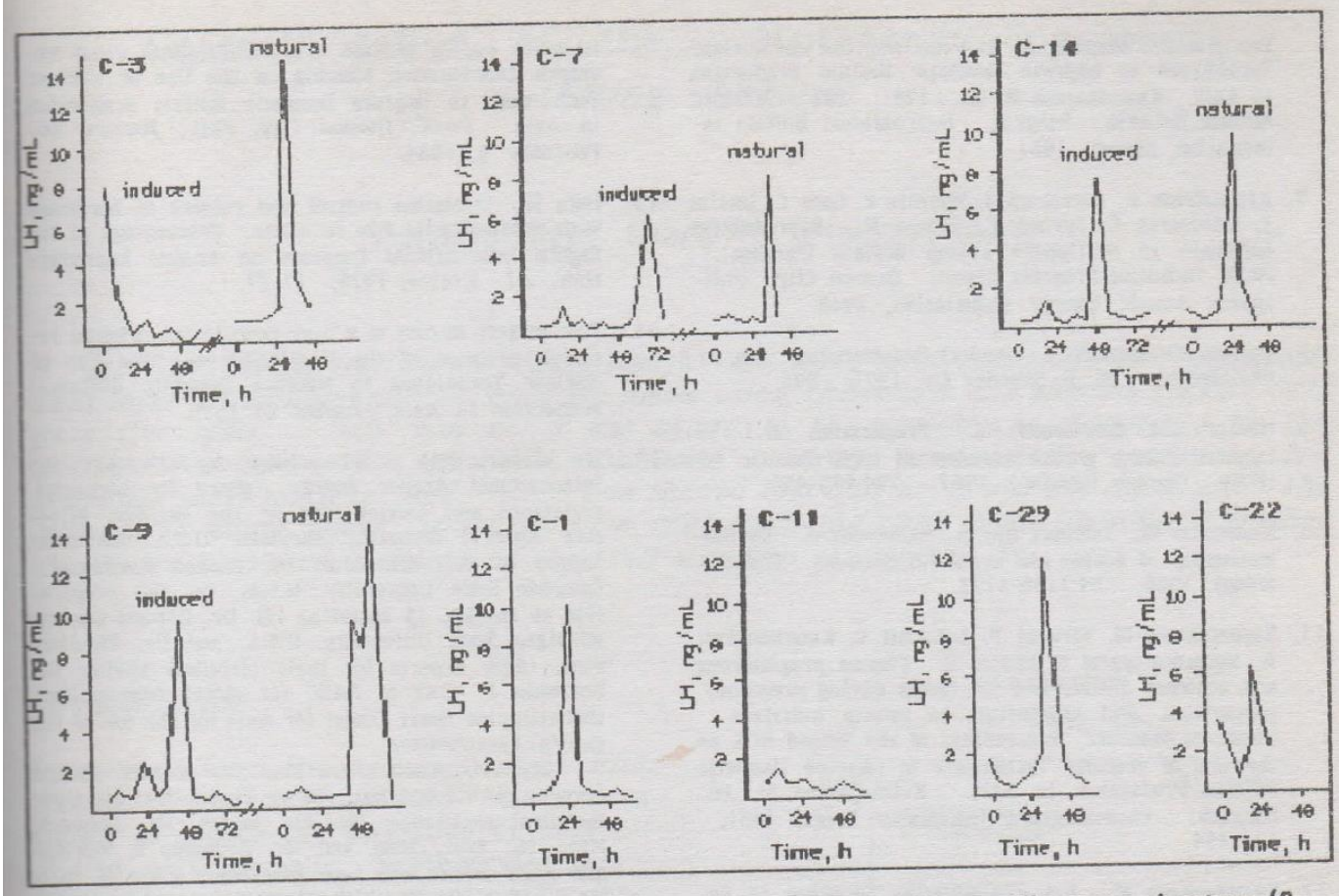

Fig. 4. LH Profiles in individual carabaos during induced estrus and succeeding natural estrus (C-3, C-7, C-14, C-9); induced estrus (C-1, C-11, C-29); and natural estrus (C-22).

the carabao. A knowledge of the capability of the animal to produce the LH preovulatory surge is helpful in the proper identification and selection of a good breeding base. A clue on the determination of the optimum time for insemination, which is an important factor in evolving a more efficient breeding management scheme, was gathered. Further studies on characterizing the LH patterns during estrus are therefore recommended. This has special applications on the Bureau of Animal Industry program on upgrading the carabao for milk production using prostaglandin or its analogs to synchronize estrus. LH profiles can also be used in evaluating problems on reproduction. All these are expected to result in increased carabao production and productivity.

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