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Research Article

PLASMA LEUTINIZING HORMONE PROFILING IN NORMAL AND REPEAT BREEDING KANKREJ COW

N. F. CHAUDHARI*, B. N. SUTHAR[1], SANDHYA S. CHAUDHARI[2]
C. F. CHAUDHARI AND L. C. MODI

Department of Veterinary Gynaecology and Obstetrics
College of Veterinary Science and Animal Husbandry
Navsari Agricultural University, Navsari
Gujarat - 396 450, India

The present investigation on plasma leutinizing hormone (LH) profile of 12 Kankrej cow (6 normal and 6 repeat breeding) was carried out on the day of estrus. The mean plasma LH concentration at 2 hours intervals from 8 hours to 36 hours post estrus was significantly ($p<0.05$) higher in control as compared to repeat breeder group at all the period of sampling except during first two collection where non-significant higher level was observed. Further, in control group the level of plasma LH was increased from 8 hrs. after onset of estrus and reached a peak level at 20 hours (4.62 ± 0.52 mIU/mL) and thereafter it declined. Whereas, no such trend was observed in repeat breeder group.

Key words: ELISA, Estrus, Kankrej cow, LH, Repeat breeding

Following breeding, fertilization and maintenance of normal pregnancy and its successful termination are the major reproductive traits of economic concern. If these traits not followed normally, results into increased service period associated mainly with repeat breeding due to certain intrinsic and extrinsic ailments. The animals suffered with repeat breeding possesses normal reproductive tract and express regular oestrous cycle but fail to conceive within 2-3 services to a fertile bull or inseminations. This condition has been studied extensively in cattle (Reddy *et al.*, 2001; Sharma *et al.*, 2006). The synchrony between embryo survival and maternal environment is essential to restore normal fertility in repeat breeding animals. Lack or delayed LH surge will result into the anovulation or delayed ovulation which ultimately makes the animal repeat breeder. It can be maintained by correlation of

*Corresponding Author
[1]Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Sci. & A.H., SDAU, Dantiwada, Gujarat
[2]Department of Veterinary Physiology & Biochemistry, College of Veterinary Sci. & A.H., NAU, Navsari, Gujarat

adequate luteal function either by exogenous supplementation of progesterone or stimulations of steroidogenesis by advocating leutinizing hormone. Hence, in the present study, an attempt was made to ascertain the plasma LH profile of randomly selected repeat breeder cows and to compare it with normal fertile animals.

MATERIALS AND METHODS

The present study was performed as a part of M.V.Sc. research work, which was approved by Director of Research, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat in the year 2008. For the investigation, total 12 cyclic and apparently healthy female Kankrej cows irrespective of age and parity, comprised of 6 normal fertile (LH CON), which conceived at 1st or 2nd breeding and 6 repeat breeder (LH RB) cows maintained at Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar were selected. All the cows were maintained under identical condition of feeding and management at the Livestock Research Station, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The estrus detection was carried out by teaser bull parading two times in a day. The repeat breeder cows were selected on the basis of history of failure to conceive even after 2 or more breeding with fertile semen and having the normal genital tract, healthy discharge and nearly normal estrus cycle length.

The blood was collected aseptically from the jugular vein taking all the aseptic precautions using EDTA vaccutainers after proper restraining of the animals at every 2 hours, starting from 8 hours to 36 hours post estrus in all the cows. The plasma was separated by centrifuging the blood sample at 4500 rpm for 5 minutes and stored at 20°C till analyzed. Plasma LH was estimated using ELISA (Enzyme Linked Immuno-Sorbant Assay) by employing standard kit manufactured by Syntron Bioresearch, Inc. California. Sample, standards and marker were added into the antibody (anti LH) coated wells of 96-well flat bottom microplate. The microplate was incubated at 37°C for 30 minutes. Then the wells of microplate were washed for five times using triple distilled water followed by addition of tetramethyl benzidine base (TMB) substrate to each well. The plate was then incubated at room temperature (15-28°C) for 10 minutes. Stop solution was added in to each well in dark to check the reaction. The absorbance of these wells was read against blank at 450 nm. The values were calculated against a standard curve of 0 to 200 mIU/mL on the graph paper and were expressed as mIU/mL. The sensitivity of assay was 5.5 mIU/mL. The average linearity of assay was 104%. The inter and intra-run precision had a co-efficient of variation of 6.3% and 5.5% respectively.

The data generated on various parameters was subjected to statistical analysis using FCRD (Factorial Completely Randomized Design) (Snedecor and Cochran, 1994).

RESULTS

Plasma LH concentration in group of normal fertile cows was non-significantly increase from 1.8 ± 0.20 mIU/mL at 8 hours to 1.87 ± 0.22 mIU/mL at 10 hours after the onset of estrus. However, from 12 hours onwards it was fluctuated non-significantly up to 18 hours and reached at peak level (4.62 ± 0.52 mIU/mL) at 20 hours after the onset of estrus. The significantly ($p < 0.05$) elevated LH level in control group was maintained up to the 24 hours post estrus. The concentration measured at this time was greater than that measured at any other time during estrus indicated that LH reach definite peak at 20 to 22 hours post estrus (Table 1 and Fig. 1).

However, in case of LH RB group no such fluctuations in the LH concentration was recorded and the highest (2.13 ± 0.29 mIU/mL) level observed in this group was only at 16 hours post estrus. While during all other periods LH levels remained within the range of 1.22 ± 0.26 to 1.92 ± 0.34 mIU/mL. Further, the concentration of LH was at par between LH CON and LH RB groups during 8 and 10 hours post estrus. However, 12 hrs. post estrus onwards the LH concentration was significantly ($p < 0.05$) low in LH RB group as compared to LH CON group till 36 hours post estrus (Table 1 and Fig. 1).

Table 1. Plasma luteinizing hormone profiles in Kankrej cow during estrus

Time of collection (hrs.) after onset of estrus	Groups	
	LH CON (n = 6) (Mean ± SE) (mIU/mL)	LH RB (n = 6) (Mean ± SE) (mIU/mL)
8	$1.80 \pm 0.20_{x}^{a}$	$1.67 \pm 0.35_{x}^{ab}$
10	$1.87 \pm 0.22_{x}^{a}$	$1.82 \pm 0.30_{x}^{ab}$
12	$2.80 \pm 0.49_{x}^{bc}$	$1.73 \pm 0.29_{y}^{ab}$
14	$2.50 \pm 0.42_{x}^{ab}$	$1.82 \pm 0.24_{y}^{ab}$
16	$2.40 \pm 0.45_{x}^{ab}$	$2.13 \pm 0.29_{y}^{b}$
18	$2.78 \pm 0.56_{x}^{bc}$	$1.92 \pm 0.34_{y}^{ab}$
20	$4.62 \pm 0.52_{x}^{g}$	$1.30 \pm 0.19_{y}^{a}$
22	$4.43 \pm 0.73_{x}^{fg}$	$1.27 \pm 0.39_{y}^{a}$
24	$4.02 \pm 0.24_{x}^{efg}$	$1.70 \pm 0.35_{y}^{ab}$
26	$3.75 \pm 0.27_{x}^{def}$	$1.37 \pm 0.30_{y}^{a}$
28	$3.50 \pm 0.19_{x}^{cde}$	$1.40 \pm 0.12_{y}^{a}$
30	$3.67 \pm 0.34_{x}^{de}$	$1.68 \pm 0.15_{y}^{ab}$
32	$2.76 \pm 0.32_{x}^{b}$	$1.43 \pm 0.14_{y}^{ab}$
34	$3.11 \pm 0.70_{x}^{bcd}$	$1.34 \pm 0.08_{y}^{a}$
36	$2.76 \pm 0.44_{x}^{b}$	$1.22 \pm 0.26_{y}^{a}$

Means bearing different superscripts within a column (within the groups, viz. a, b...) and means bearing different subscripts within a row (between the groups, viz x, y...) differ significantly ($p<0.05$)

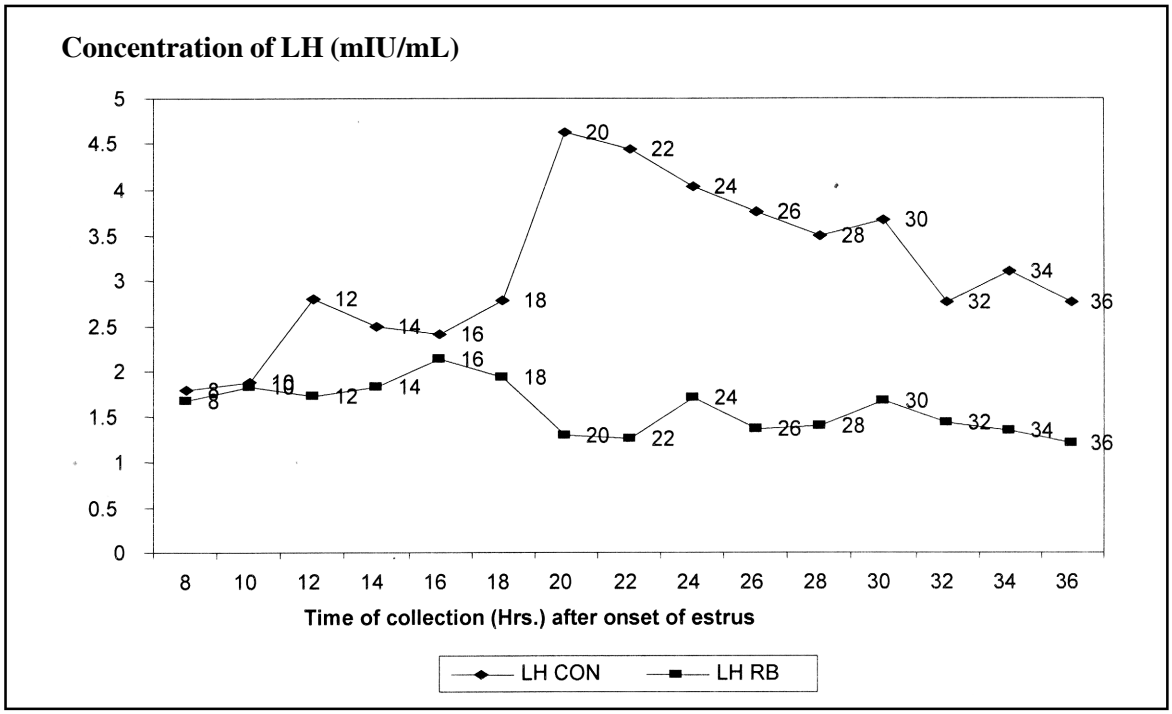

Fig. 1. Average value of leutinizing hormone (mIU/mL) in Kankrej cow during estrus

DISCUSSION

In the present study, the undeniably pre-ovulatory LH surge was observed in normal Kankrej cows which persist about 6 hours whereas, it was only transitory in case of repeat breeding Kankrej cows as the LH concentrations observed in repeat breeding Kankrej cows was remain remarkably stable during the estrus.

Similar findings were observed by Hackett and Hafs (1969) who observed the LH surge varied from 8 hours before to 8 hours after the end of the standing heat. However, Swanson and Hafs (1971) observed the peak of LH 3 hours before the standing heat. Further, Henricks *et al.* (1970) observed peak LH levels at 3 to 6 hours after the onset of estrus in non-lactating cows. The peak of LH at the onset of estrus was lasted between 6 and 16 hrs. have been reported by many authors (Christensen *et al.*, 1974; Kiddy and Odell, 1969; Schams and Karg, 1969; Snook *et al.*, 1971; Sprague *et al.*, 1971; Varian *et al.*, 1968).

Starbuck *et al.* (2006) observed the time of elevation in plasma LH as 54.06 ± 4.0 hours following luteolysis. Roy and Prakash (2008) recorded the LH surge from 6 to 10 hours of induced estrus in Murrah buffalo heifers. Discrepancy in levels of LH reported by various authors may be due to

the frequency and time of sampling. Schams and Karg (1969) opined that LH concentration above a baseline level last less than 6 hrs.

Scanning through the available literature revealed no such studies. However, from the above findings it can be postulated that the low levels of LH in repeat breeder Kankrej cows may be the cause for repeat breeding condition of the animals. Reeves (1987) opined that preovulatory surge of LH lasting for 6 to 12 hours are responsible for ovulation which is in response to the increase in circulatory concentrations of estrogen. Further, Niswender and Nett (1988) reported that principal hormone which stimulates progesterone production by the CL is LH. A lot of literature is available supporting the luteotrophic nature of LH (Wiltbank *et al.*, 1961; Roberts, 1986; Hafez, 1987; Arthur *et al.*, 1989; Ayad *et al.*, 2007).

The surge level of LH in normal breeder last for 6 hours, whereas no such surge level was recorded in repeat breeder Kankrej cows and which might be a reason for repeat breeding condition in animals.

Conflict of interest: Authors declare that there is no conflict of interest regarding the present research work.

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