



## Lame cows have a subdued hypothalamus-pituitary axis as revealed by LH release in response to exogenous endocrine challenges\*

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### ABSTRACT

Lameness is a serious concern in cows due to its multilevel effects and increasing incidences. The ability of anterior pituitary and hypothalamus to release LH, a prime reproductive hormone, is unknown in lame cows. Our study tested this ability by exogenous endocrine challenge using Buserelin acetate (GnRH analogue) and estradiol valerate (EV). The study included moderately lame (L; n=7) either cyclic (LC; n=3) or anestrus (LA; n=4); and normal cyclic, non-lame (NL; n=5) cows. Progesterone blocks LH release, so it was lowered by injecting PG in cyclic cows. After 14 h, all cows were given a walk for 5 min and immediately thereafter injected with 0.008 mg GnRH and 4 h later with 1 mg of EV. Blood plasma progesterone, cortisol and LH were quantified, periodically. There was a significant delay in the onset (105.0 vs 75.0±7.0 vs 54.0±9.0 min) and duration (22.5±5.3 vs 80.0±10.8 vs 117.0±2.7 min) of LH surge in LA vs LC vs NL cows; the surge was absent in two LA cows. The altered LH profile was attributed to a 3 fold (in L) vs 1.6 fold (in NL) rise in cortisol. LH released (ng/ml) in response to EV was also low in LA (31.5±2.4) and LC (69.9±7.2) than NL (79.2±7.0) cows. In conclusion, lame cows subjected to walk exhibited reduced pituitary response to release LH following exogenous GnRH as also a reduced ability of hypothalamus to stimulate LH release in response to exogenous estradiol.

**Key words:** Cows, Endocrine challenge, Exogenous, Lameness, LH production

Lameness impairs fertility, reduces milk output, incurs treatment costs and increases culling rate thereby rendering it to be the third most expensive cattle disease (Wells *et al.* 1998). The incidence of lameness is on rise in exotic (Tadich *et al.* 2010) and Indian cattle (Sood and Nanda 2013). Lameness exerts pain whose severity depends on the extent of lameness (Alawneh *et al.* 2012). Moving around for feeding, milking and other routine locomotory activities are inevitable in cows. A brief locomotion is stressful in lame cows (Sood *et al.* 2012), which could exert a profound multilevel inhibitory effect on the hypothalamo-pituitary-gonadal axis with adrenal gland also coming into play (Goodman *et al.* 1996). Resultantly, this may disrupt the sensitive reproductive mechanisms including estrus expression (Sood and Nanda 2006) and follicular dynamics (Sood *et al.* 2009a, b) in lame cows. Luteinizing hormone (LH) has a pivotal role in regulating different facets of cattle

reproduction (Crowe and Mullen 2013) and may be the common denominator for impaired reproductive processes cited *ut supra*. However, the ability of hypothalamus and anterior pituitary to release LH has not been documented earlier in lame cows, which was investigated in this study using exogenous hormonal challenges.

### MATERIALS AND METHODS

**Animals, lameness scoring and groups:** The work was done on 12 apparently healthy and lactating Holstein Friesian predominated crossbred cows, either non-lame (NL, n=5) or moderately lame (L, n=7) in the post-voluntary waiting period of third to sixth lactation under standard feeding practices. The L cows were either - cyclic (LC, n=3) or true anestrus (LA, n=4). Lameness was assessed and quantified as per Wells *et al.* (1993).

**Catheterization, application of walk, endocrine challenges and blood sampling:** An indwelling jugular vein catheter was fitted in each cow a day before the experiment. The patency of the catheter was maintained with heparin in normal saline solution (50 IU/ml) during the course of experiment. Each cow was injected with PG (5 ml, i.m, Iliren, each ml containing 0.196 mg tiaprost trometamol equivalent to 0.15 mg tiaprost, Intervet-Germany) at 8.55 PM on the day of catheterization. Fourteen hours later, the cows were made to walk for 5 min. This was achieved by

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following the cow from its rear side so that it maintained gentle strides for the entire duration. Walking was immediately followed by a single GnRH injection (2 ml, i.v, Receptal, each ml containing 0.004 mg Buserelin acetate, Hoechst, India). Four hours after GnRH injection (18 h after PG) each cow was injected with estradiol valerate (EV - 0.1 ml in 1.9 ml groundnut oil, i.m, Progynon, each ml containing 10 mg estradiol valerate, German Remedies Limited, Mumbai, India). The GnRH challenge was given to test the response of anterior pituitary, while EV challenge tested the ability of hypothalamus to stimulate anterior pituitary to release LH. The exogenous hormonal challenges were based on earlier studies (Dobson and Alam 1987, Dobson *et al.* 1988).

Heparinised blood samples were collected at 15 min interval for 2 h (-120, -105, -90, -75, -60, -45, -30, and -15 min) before GnRH administration and every 15 min for a 3 h period (15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 165, 180 min) after GnRH. All these samples were utilized for LH estimation. One hour after the last sample, EV was injected and a blood sample was collected. On the next day, nine blood samples were collected every 2 h for a period of 16–32 h after EV injection for estimation of LH. Cortisol was estimated in the blood samples collected at -120, -60 -15 min (timings listed *ut supra*), 5 min after walk, at 30, 75 min (timings listed *ut supra*) as well as 180 and 240 min after walk. Progesterone was estimated in the blood samples collected at the time of jugular catheterization and immediately before GnRH and EV injections. The blood samples were transported to lab on ice and centrifuged to harvest plasma that was stored at -20°C pending analysis of total cortisol (Prakash and Madan 1984), LH (Prakash *et al.* 2002) and progesterone (Kamboj and Prakash 1993). The assay sensitivity for cortisol estimation was 0.2 ng/ml with intra- and inter-assay coefficients of variation being 9.2 and 10.1%, respectively. The assay sensitivity for LH was 2.0 ng/ml with intra- and inter-assay coefficients of variation being 8.3 and 10.5%, respectively. The assay sensitivity for progesterone was 0.1 ng/ml with intra- and inter-assay coefficients of variation being 9.5 and 10.0%, respectively. The behaviour and reactions of the animals were noted during the course of bleeding.

*Assessment of LH secretory pattern:* LH pulse, basal LH, pulse amplitude, time to respond to treatment, LH surge and duration of LH were evaluated (Mc Neilly and Baird 1983, Dodson *et al.* 1988, Nanda 1989, Sood *et al.* 2015). AUC (area under curve) was calculated as per Simpson's 1/3<sup>rd</sup> rule (Jain *et al.* 1993).

*Statistical analysis:* Difference in LH characteristics between the three groups was compared using Kruskal – Wallis H Test. A difference of  $P < 0.05$  (atleast) was considered to be significant, whereas  $P = 0.10$  was considered as a tendency to differ between the groups.

## RESULTS AND DISCUSSION

*Progesterone, cortisol and GnRH induced LH response:* At the start of experiment, plasma progesterone

concentration was similar in NL ( $2.4 \pm 0.1$  ng/ml) and LC ( $2.2 \pm 0.1$  ng/ml) indicating a luteal phase. Progesterone values reduced, 12h after PG, to  $0.9 \pm 0.1$  ng/ml in NL cows and 0.4 to 1.5 ng/ml in LC, respectively. Progesterone concentration in LA remained between 0.4 to 0.7 ng/ml during the course of experiment.

Table 1 shows LH values in all groups after GnRH administration succeeding walk. All groups exhibited one LH pulse during a 2 h period. The NL cows showed higher basal LH concentrations compared to LC and LA cows. Studies have reported similar basal LH concentration (1.5–3.5 ng/ml) in cycling cows, 12 h after PG administration (Dobson and Alam 1987).

Table 1. Basal and surge characteristics (Mean $\pm$ SEM) of LH 2 h before and 3 h after administration of GnRH in non-lame (NL; n=5) and lame – cyclic (LC; n=3) or anestrus (LA; n=4) cows

Parameter	Non-lame	Lame		Level of significance (Kruskal - Wallis H Test)
		Cyclic	Anestrus*	
Basal LH (ng/ml)	2.7 $\pm$ 0.9	2.0 $\pm$ 1.0	1.7 $\pm$ 0.4	$P > 0.99$
Basal LH pulse amplitude (ng/ml)	5.3 $\pm$ 1.4	3.0 $\pm$ 1.2	2.9 $\pm$ 0.6	$P = 0.9$
LH pulse frequency/2 h	1.0	1.0	1.0	$P = 1.0$
<i>LH surge characteristics</i>				
Time of response (min)	30.0 $\pm$ 8.4	30.0 $\pm$ 7.0	33.8 $\pm$ 9.7	$P = 0.95$
Onset (min)	54.0 $\pm$ 9.0	75.0 $\pm$ 7.0	105.0	$P < 0.10$
Duration (min)	117.0 $\pm$ 2.7	80.0 $\pm$ 10.8	22.5 $\pm$ 5.3	$P > 0.025$
Peak levels (ng/ml)	25.0 $\pm$ 1.6	23.5 $\pm$ 2.8	15.2 $\pm$ 1.2	$P < 0.10$
AUC (mm <sup>2</sup> )	47.6 $\pm$ 3.0	32.3 $\pm$ 2.2	20.0 $\pm$ 3.5	$P < 0.10$

\*Two cows did not elicit an LH surge.

The average cortisol concentration was higher in NL cows (15.1 $\pm$ 2.9 ng/ml) compared to LC (6.8 $\pm$ 2.0 ng/ml), during 2 h period after GnRH injection. Relatively higher cortisol concentration in NL cows could be due to nervous and aggressive behaviour of 2 of the NL cows during experiment. Excluding the cortisol values of the aggressive NL cows, the average cortisol concentrations (8.8 $\pm$ 1.1 ng/ml) became comparable to L cows and a previous study (Shutt and Fell 1985). Normal cortisol concentration in L cows could be justified due to an inherent adjustment to prevent them from over stimulation of hypothalamo-pituitary-adrenal axis, which otherwise causes prolonged glucose metabolism, altered vascular dynamics and deranged immune response (Ganong 1991). Walking was stressful and manifested relatively early only in L, as cortisol increased by 3 times (19.0 $\pm$ 4.6 ng/ml) after 22.6 $\pm$ 4.1 min of walk, while in NL cows cortisol increased only 1.6 times

(25.1±6.7 ng/ml) after 61.0±22.0 min of walk.

The pituitary responded to GnRH challenge in all except two LA cows. The time of response was similar in all groups and ranged from 30.0±8.4 to 33.8±9.7 min. Contrarily, Kanchev *et al.* (1984) reported response time of five minutes after GnRH injection in a dose dependant manner; this suggests a delayed pituitary response in present study. The gradual increase in plasma LH lead to LH surge like concentrations. Lame cows showed delayed onset, shorter

duration and lower peak values for LH surge. This could be due to rapid rise in cortisol, after walk. Elevated glucocorticoids, exogenous or endogenous, have anti-gonadal effects (Chrousos *et al.* 1998) and suppress mean LH concentration, LH release in response to GnRH and LH surge, the latter may even be delayed or blocked (Daley *et al.* 1999).

*EV induced LH response:* The average progesterone concentrations were less than 1ng/ml in all groups at the

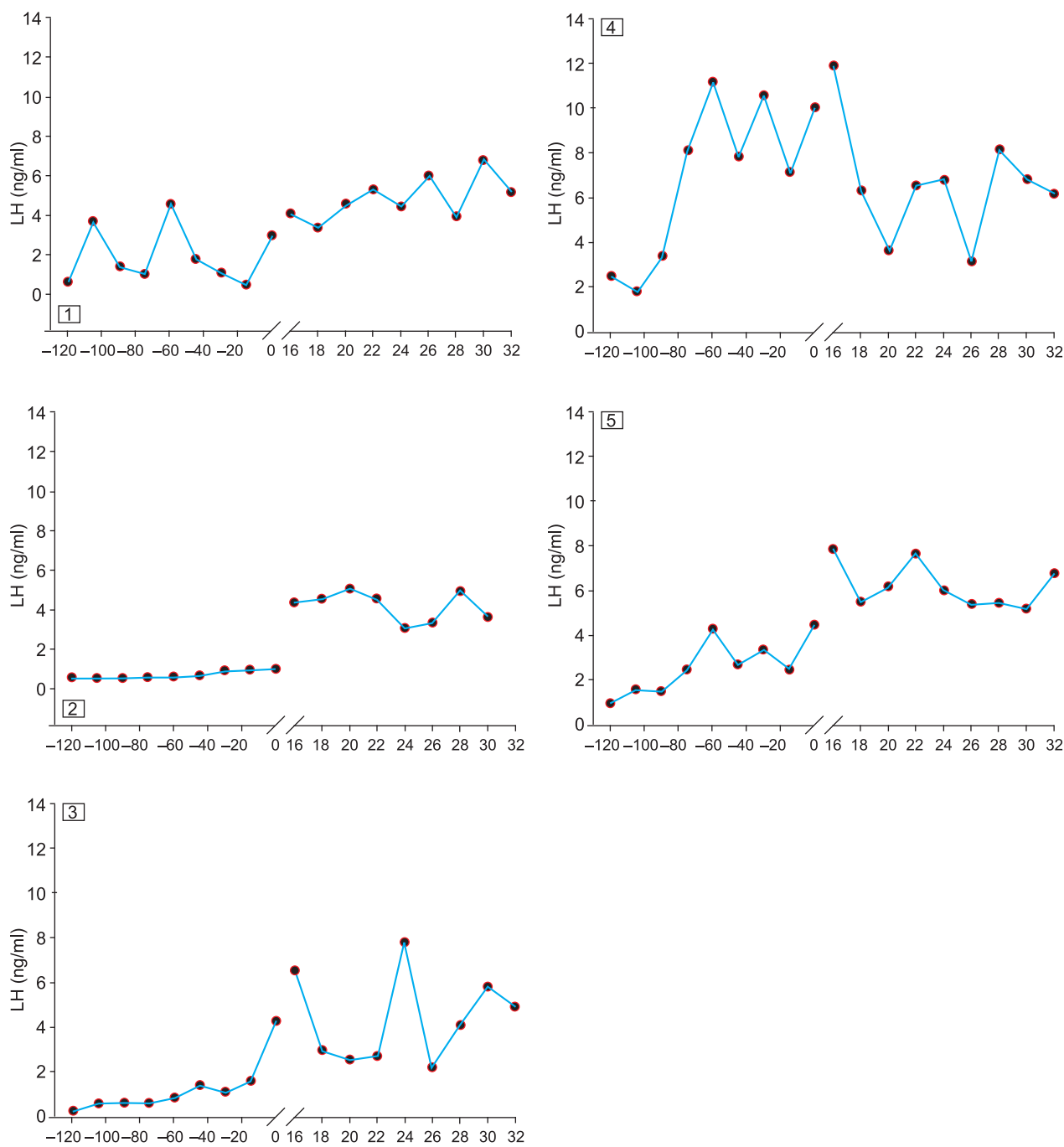


Fig. 1. LH concentration (ng/ml) between 12–14 h after PG administration (basal concentration; shown in min before break in X-axis) and between 16–32 h after estradiol administration (shown in h after break in X-axis) in individual non-lame cyclic cows (NL; n=5).

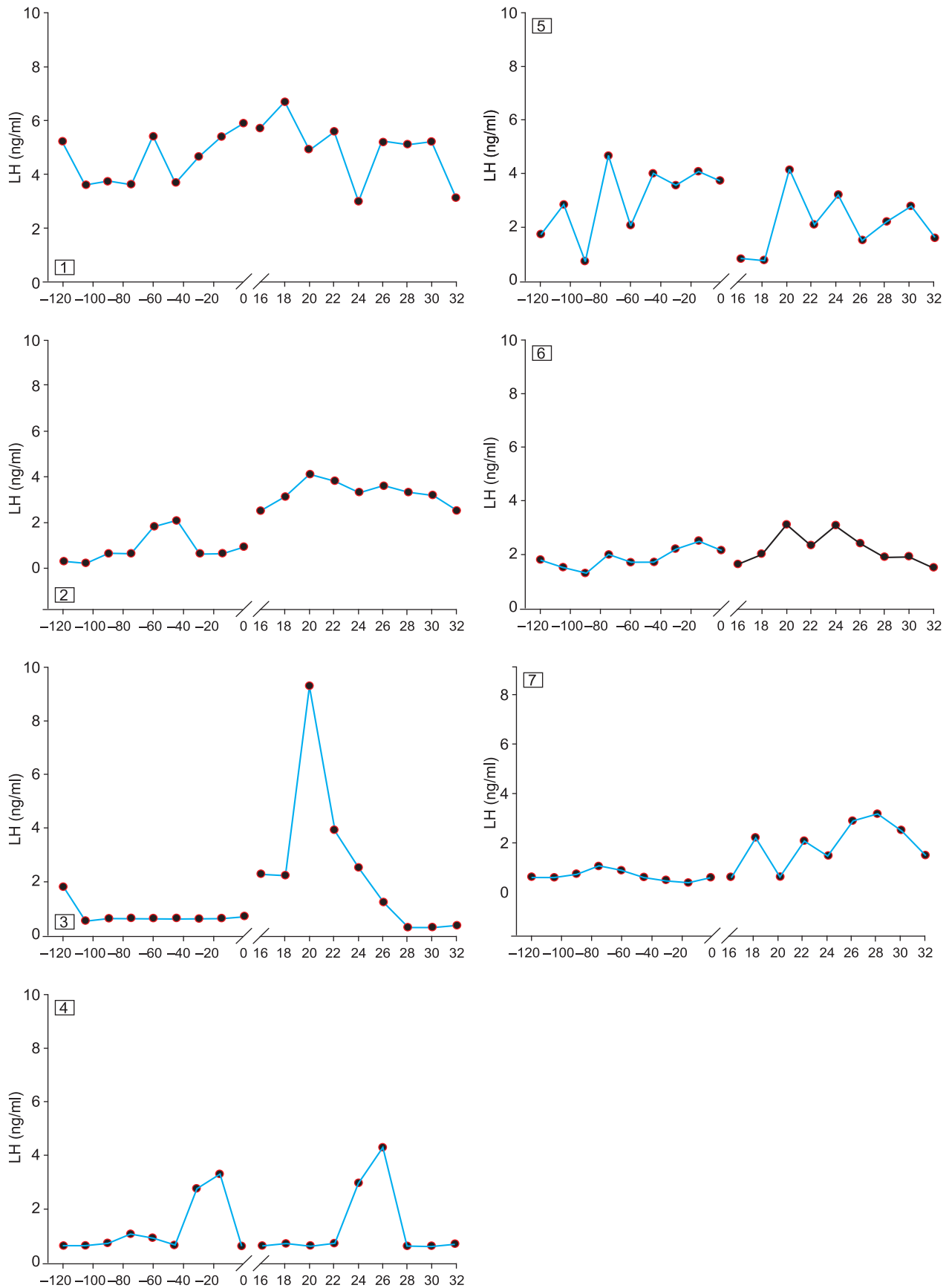


Fig. 2. LH concentration (ng/ml) between 12–14 h after PG administration (basal concentration; shown in min before break in X-axis) and between 16–32 h after estradiol administration (shown in h after break in X-axis) in individual lame cyclic (LC; Panel 1 to 3) and lame anestrous (LA; Panel 4 to 7) cows.

time of EV injection (NL=0.7±0.02 ng/ml; LC=0.5±0.1 ng/ml; LA=0.4±0.1 ng/ml). Such progesterone concentration is essential for estradiol to generate LH surge (Dobson *et al.* 1988). Table 2 presents average LH characteristics of all groups, between 16–32 h, after EV injection, while Figs 1 and 2 shows comparisons between NL and L cows.

The pre-EV / basal LH concentration increased from 9.5 to 51.9% after EV in the three groups. LH concentrations and peak, before and after EV injection, differed markedly;

Table 2. Characteristics of LH release (mean±SEM) 2 h before GnRH administration (basal) and between 16–32 h after administration of 1 mg estradiol valerate in non-lame (NL; n=5) and lame – cyclic (LC; n=3) or anestrus (LA; n=4) cows

Parameter	Non-lame	Lame		Level of significance (Kruskal - Wallis H Test)
		Cyclic	Anestrus	
Basal LH (ng/ml)	2.7±0.9	2.0±1.0	1.7±0.4	P>0.99
16–32 h after EV Concentration (ng/ml)	5.2±0.4	4.2±0.4	1.8±0.1	P<0.01
% increase from basal	51.9	49.0	9.5	P<0.10
Peak (ng/ml)	6.9±0.9	7.0±1.5	3.7±0.3	P<0.05
AUC (mm <sup>2</sup> )	79.2±7.0	69.9±7.2	31.5±2.4	P<0.10

but the LH increase remained comparable for NL and LC cows. LH peak and AUC were much higher in the NL and LC than the LA cows with a significant difference between the three groups.

Three out of five NL (Fig. 1) and one out of three LC cows displayed a distinct LH release after EV, which was missing in all the LA (Fig. 2) cows. Timing and peak LH concentration may vary after estradiol. Studies have reported a peak of 24.6±9.8 and 22–100 ng/ml at 16±1 and 24–36 h, after administration of 1.0 mg estradiol benzoate (Zaied *et al.* 1981 and Nanda 1989). A weak LH response in lame anoestrous cows in present study is similar to no or weak LH surge in anoestrous cows in a previous study in response to estradiol benzoate challenge (Dobson and Alam 1987). Endogenous progesterone concentration over 0.3–0.5 ng/ml blocked oestrogen induced LH surge in cows (Hobson and Hansel 1972). Likewise, progesterone concentration, ranging from 0.4 to 0.8 ng/ml, might have blocked estradiol - induced LH surge after EV application, in present study. All cyclic cows showed a sudden drop in progesterone after PG injection and later exhibited oestrus - suggesting effectiveness of PG action. Relatively high progesterone concentrations are still obscure and could be due to slower or delayed luteolysis or be of adrenal origin; progesterone released from adrenal cortex is also sufficient to block LH surge (da Rosa and Wagner 1981); prolonged isolation and restraining of cows during sampling (for more than five hours); stress of lameness in LC and LA cows, that may or may not be associated with walking stress of 5

min; difference in the kinetics of EV in present study, compared to estradiol benzoate in earlier study (Oriowo *et al.* 1980); breed variation; or a combination of all these. LH surge always occurs during period of 16–32 h, after EV injection. LH surge differed by nearly 10 h, after PG injection, between normal and repeat breeder cows (Sood *et al.* 2015).

Our study infers that walking deters LH profile following GnRH administration in the lame cows. The LH production after estradiol challenge was also weak in lame cyclic and still weaker in lame anestrus cows. Reduced LH production due to attenuated hypothalamic – pituitary axis therefore justifies reduced reproductive capacity in lame cows. In addition, a subnormal LH response needs to be considered in the experiments / treatments targeting endocrine manipulations in lame cows.

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