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Full Length Research Paper

Evidence of multiple preovulatory LH and FSH surges in mithun *(Bos frontalis)*

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The experiment was conducted on 14 postpartum mithun (*Bos frontalis*) cows to establish the preovu-latory LH and FSH surge patterns during estrus in peripheral circulation. Blood samples from jugular vein were collected every 2 h until 72 h and on alternate days till day 22 (day 0 = day of estrus) following the day of estrus onset. Plasma progesterone (P4), LH and FSH levels were estimated. Plasma P4 profile of the estrous cycle indicated that all the animals were ovulated following the onset of estrus. Also plasma LH and FSH concentrations were found to be significantly ($p<0.01$) higher around estrus. Two different preovulatory LH and FSH surge patterns were observed during the study. Pattern-1 indicated multiple and concomitant LH and FSH rises during estrus (43% animals, 6 out of 14), while pattern-2 showed multiple LH rises without concomitant FSH rises during estrus (57% animals, 8 out of 14). The basal concentrations of LH and FSH, the area under LH and FSH curves and the number of LH peak did not vary significantly during estrus between the patterns. However, the amplitude of LH and FSH peaks ($p<0.05$) and the number of FSH peak ($p<0.01$) were found to be significantly higher during estrus in pattern-1. The result indicated that the multiple preovulatory LH surges along with increased FSH activities were responsible for ovulation in mithun.

Key words: LH surge, FSH surge, estrus, mithun.

INTRODUCTION

Mithun (*Bos frontalis*) is a domesticated free-range bovine, which is distributed mainly in the North-Eastern hilly region of India and in many pockets of South-East Asia. This unique bovine species probably originated more than 8000 years ago and is believed to be the domesticated form of wild gaur (Simoons, 1984). Presently mithun and gaur (*Bos gaurus*) are grouped into the same species *B. frontalis* and mithun refers to the domesticated form (Wilson and Reeder, 1993).

The recent molecular genetic studies indicate a close relationship of mithun with zebu cattle (Lan et al., 1993; Ray et al., 1999). This unique bovine species is primarily used for beef production and it has an important role in the economic, social and cultural life of its rearers (Mondal and Pal, 1999). At present farmers rear this animal under free grazing condition in its natural habitat. However, the recent initiatives to popularise mithun farming under the semi- intensive condition and implementation of controlled breeding programme in this species further emphasise the economic importance of this multipurpose animal.

Any profitable animal production system largely depends on the proper estrus detection and subsequent successful breeding. Understanding the temporal relationship between estrus onset and associated endocrine changes is critical for ensuring the successful breeding. In mammalian species, ovulation is associated with the morphological, chemical and physiological changes in the ovulatory follicle. All these changes occur in response to the preovulatory LH and FSH surges.

These changes cause the rupture of ovulatory follicle and release of fertilizable ovum (Guraya and Dhanju, 1992) . The occurrence of a single high-amplitude preovu-latory LH surge is evident in goat, cattle, buffalo and sheep (Mori et al., 1987; Peters and Ball, 1995; Steven-son et al., 1998; Bakker and Baum, 2000; Singh et al., 2001; Kaim et al., 2003; Gonzalez-Bulnes et al., 2004). In

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contrast, the peak plasma FSH concentration following estrus coincides with the LH surge followed by a less well defined peak 4 to 30 h after the LH surge (Peters and Ball, 1995). The hallmark action of FSH during preovulatory follicular development is the induction of LH recaptors on granulosa cells (Zeleznik et al., 1981).

There is paucity of information on preovulatory LH and FSH surge patterns in mithun. The present study was conducted with the objective to establish the preovulatory LH and FSH secretion patterns in peripheral circulation during estrus in mithun.

MATERIALS AND METHODS

Animals and management

The experiment was conducted on 14 postpartum mithun cows 4 to 7 years of age, maintained at the Institute mithun farm, N.R.C. Mithun, Medziphema, Nagaland. The animals were allowed free grazing from 0600 to 1600 h daily on mixed pasture (19% dry matter and 12.3% crude protein). 2 kg concentrate mixture (89% dry matter and 15.4% crude protein) fortified with mineral mixture and salt was offered to each animal daily evening. The animals had free access to water throughout the day. The detection of estrus was done from day 30 postpartum onwards with healthy and fertile mithun bulls in early morning and late evening. The onset of estrus in the experimental animals were confirmed by plasma progesterone (P4) concentration of less than 0.5 ng/ml and mounting behaviour exhibited by bull. During the third or fourth estrus following calving, the heat detection in animals was done every 2 h starting from the estrus onset by bull parading. The animals were considered to be out of estrus when bull failed to detect heat and stopped mounting (Dhali et al., 2006).

Blood collection

During the third or fourth estrus following caving, blood samples from jugular vein were collected every 2 h starting from the onset of estrus to until 72 h. Blood samples (2 ml) were collected by means of an indwelling jugular catheter in heparinised polystyrene tubes (20 IU/ ml blood) to determine the plasma LH and FSH concentrations. Further to monitor the variation in plasma P4, LH and FSH concentrations during the estrous cycle, blood samples were collected on alternate days starting from the day of estrus onset (day 0) and continued until day 22. The samples were placed on ice immediately after collection. Plasma was separated within 1 h of collection by centrifugation (1200 ×g) for 20 min at 4ºC and stored at -20ºC until used. The Institute Animal Care and Use Committee regulations were followed during the experiment.

Hormone assay

Progesterone

The plasma P4 concentration was estimated using a commercially available EIA kit (Progesterone Enzyme Immunoassay Kit, Assay Design Inc., USA). The kit was validated for estimating P4 in mithun plasma as described by Dhali et al. (2006). The sensitivity of the assay was 0.25 ng/ ml. The mean intra-assay and inter- assay CVs were 4.8 and 9.1%, respectively.

FSH

The plasma FSH concentration was measured using a validated radioimmunoassay protocol for mithun plasma as described by Dhali et al. (2005). The assay procedures included the use of USDA-bFSH-1-2 (bFSH), rabbit anti-oFSH and NIDDK-oFSH- I. The sensitivity of the assay was 0.25 ng/ ml. The intra-assay and inter-assay CVs were 4.7 and 12.1%, respectively.

LH

The plasma LH concentration was measured using a validated enzyme immunoassay procedure for mithun plasma as described by Dhali et al. (2005) . The assay procedures included the use of bovine LH standards (USDA- Blh-B6) and LH antibody (USDA-309-684P-Beltsville, USA). The sensitivity of the assay was 0.31 ng/ ml. The intra-assay and inter- assay CVs were 4.9 and 11.5%, respectively.

Statistical analysis

All the statistical analyses were performed using the SPSS software package, version 10.0.1 (SPSS Inc., USA). The plasma P4 concentrations of the entire estrous cycle were plotted for individual animal to confirm ovulation and cyclicity.

Further individual patterns of LH and FSH during estrus were plotted to determine the secretion patterns and occurrences of peak concentrations. The basal hormonal concentration was defined as the mean of all concentrations excluding the concentrations that were greater than overall mean + 1 SD. A peak concentration was defined as a concentration increase greater than basal concentration + 2 SD with at least two encompassing concentrations towards the upslope or downslope of the curve. The peak amplitude was calculated as the difference between the peak concentration and basal concentration. To study the variation in LH and FSH secretion patterns during estrus, the experimental animals were categorised into two groups according to the LH and FSH secretion patterns. The variations in basal LH and FSH concentrations, area under LH and FSH curves, number and amplitude of LH and FSH peaks and duration of estrus were analysed by means of general linear model procedure. The model included the group as source of variation. The variation in plasma P4, LH and FSH concentrations of the estrous cycle were analysed by means of repeated measure ANOVA. The model included day of estrous cycle as source of variation. To study the association between the area under LH and FSH curves, the Pearson correlation analysis was performed.

RESULTS

Plasma P4 concentration varied significantly ($p<0.01$) during the entire estrous cycle. The plasma P4 profiles confirmed that all the experimental animals were normally cyclic and ovulated following the onset of estrus (Figure 1). In the present study, two different preovulatory LH and FSH surge patterns were observed during estrus. These were pattern-1: multiple and concomitant LH and FSH rises during estrus and pattern-2: multiple LH rises without concomitant FSH rises during estrus. The representative LH and FSH secretion patterns during estrus are depicted in Figure 2 and 3 respectively. The pattern-1 was found in 43% (6 out of 14) experimental animals and the pattern-2 was found in 57% (8 out of 14) experimental animals. In all the experimental animals, the LH and FSH

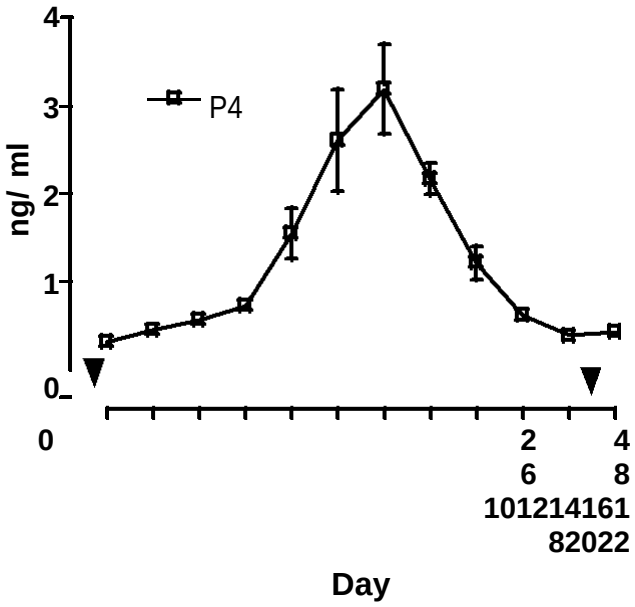
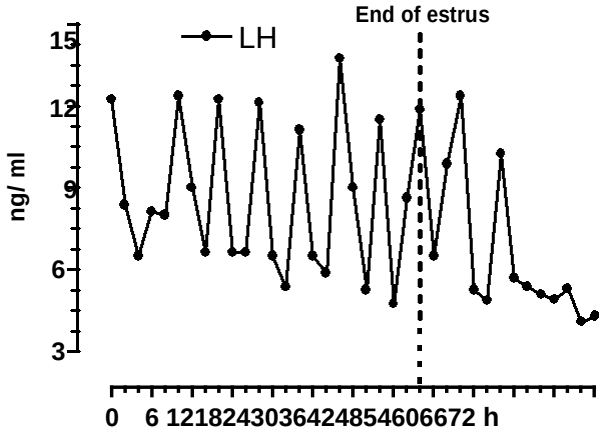


Figure 1. Plasma progesterone (P4) profile (Mean ± SE) during the estrous cycle in mithun (n = 14); the arrows indicate the day of estrus onset.



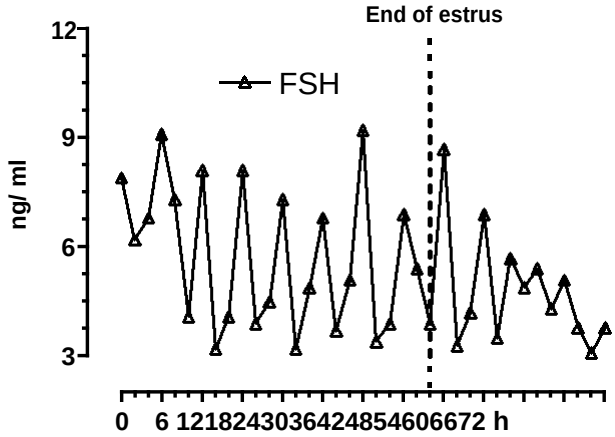
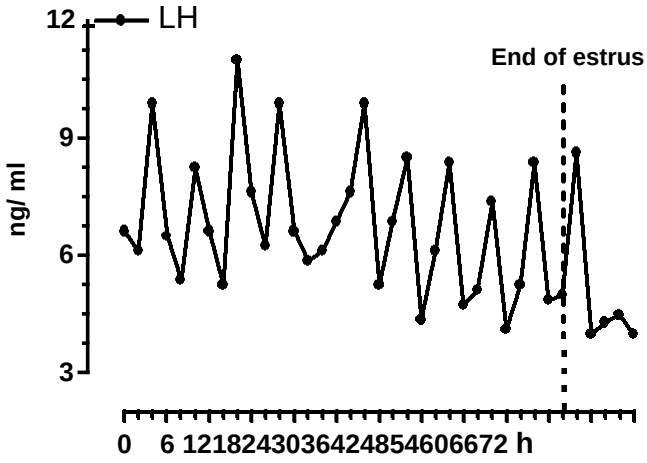


Figure 2. Representative multiple and concomitant LH and FSH rises during estrus; 0 h indicates the estrus onset.



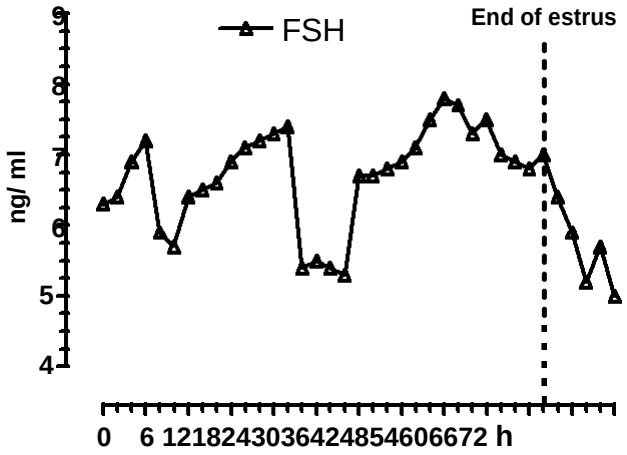


Figure 3. Representative multiple LH rises without concomitant FSH rises during estrus; 0 h indicates the estrus onset.

concentrations decreased to the basal level after the end of estrus.

The basal LH and FSH concentrations, area under LH and FSH curves and number of LH peaks during estrus did not differ significantly between the patterns (Table 1).

In contrast, the number of FSH peaks during estrus was found to be significantly ($p<0.01$) higher in the experimental animals under pattern-1. The amplitudes of LH and FSH peaks during estrus were also found to be significantly ($p<0.05$) higher in the experimental animals under pattern-1 (Table 1) . The duration of estrus did not differ significantly in the experimental animals under different LH and FSH secretion patterns (Table 1) . A significant ($p<0.01$) positive correlation ($r = 0.89$) was observed between the area under LH and FSH curves during estrus. The concentrations of plasma LH and FSH varied significantly ($p<0.01$) during the entire estrous cycle (Figure 4).

Table 1. Basal concentrations of LH and FSH, area under LH and FSH curves, number and amplitude of LH and FSH peaks during estrus and, duration of estrus in mithun under different preovulatory LH and FSH surge patterns

Particular	Pattern-1 Mean ± S.E. N=6	Pattern-2 Mean ± S.E. N=8
Basal LH concentration, ng/ ml	5.79± 0.38	5.77± 0.35
Basal FSH concentration, ng/ ml	5.04± 0.11	5.12± 0.60
Area under LH curve, arbitrary unit	351 ± 48	447 ± 86
Area under FSH curve, arbitrary unit	278 ± 48	366 ± 62
Number of LH peak during estrus	6.7± 0.2	6.7± 0.7
Number of FSH peak during estrus	6.4 ± 0.2**	2.8 ± 0.1**
Amplitude of LH peak, ng/ ml	4.54 ± 0.27*	3.78 ± 0.19*
Amplitude of FSH peak, ng/ ml	4.32 ± 0.29*	2.97 ± 0.31*
Duration of estrus, h	48.5 ± 5.0	57.2 ± 6.1

Pattern-1: Animals showed multiple and concomitant LH and FSH rises during estrus; Pattern-2: Animals showed multiple LH rises without concomitant FSH rises during estrus; * indicates the values within row differ significantly at p<0.05 and ** indicates the values within row differ significantly at p<0.01.

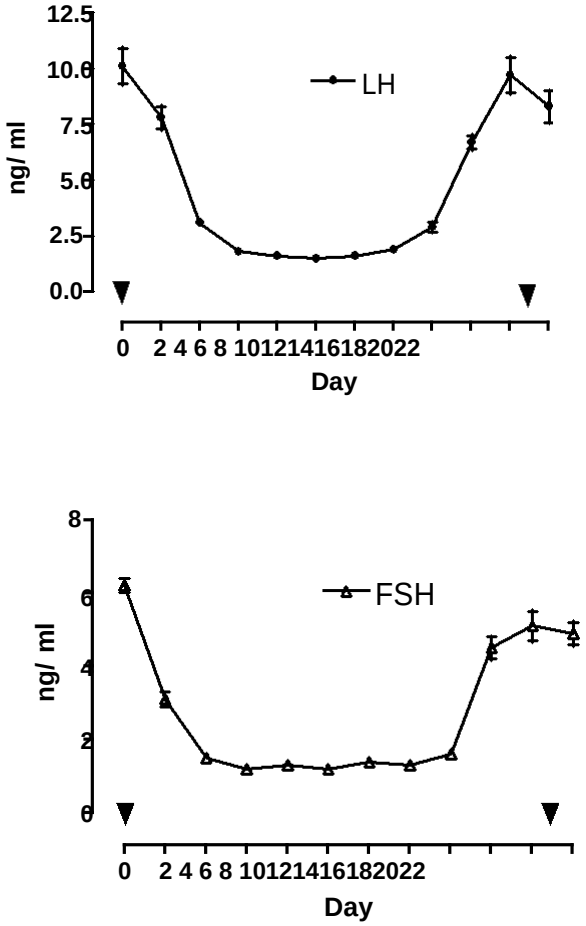


Figure 4. Variation in plasma LH and FSH concentrations (Mean ± SE) during the estrous cycle in mithun (n = 14); the arrows indicate the day of estrus onset.

The LH and FSH concentrations were found to be considerably higher around estrus and decreased to the basal level on day 4 of the estrous cycle.

DISCUSSION

We conducted the present investigation to establish the Preovulatory LH and FSH surge patterns during estrus in mithun. The study revealed a unique preovulatory LH and FSH surge patterns in this animal. The results suggest that the multiple low-amplitude LH surges and associated increased FSH activities during estrus are responsible for ovulation in this species.

Ovulation marks the culmination of follicular growth and development and involves a complex cascade of events stimulated by an estradiol induced surge of gonadotropins (Bridges et al., 2006). In cattle, during follicular phase, estradiol exerts a positive feedback effect on hypothalamic GnRH secretion that in turn increases LH and FSH releases (Peters and Ball, 1995).

Evidences are there that estradiol induces the preovulatory LH surge in cattle by increasing pituitary sensitivity to LHRH and then increasing LHRH release (Kesner et al., 1981). In mammals, the morphological, chemical, physiological and endocrinological changes associated with ovulation occur in the ovulatory follicle in response to the preovulatory LH surge (Thibault and Levasseur, 1988; Espey and Lipner, 1994; Tsafriri and Dekel, 1994; Guraya, 1997). The synthetic mechanisms that start in the ovulatory follicle in response to the LH surge play a crucial role in ovulation (Tsafriri and Dekel, 1994). Indeed, the preovulatory surges of LH and FSH cause a series of orderly sequenced changes in the matured ovulatory follicle, which include resumption of oocyte maturation, germinal vesicle breakdown, initiation of luteinisation process of granulosa cells and restructuring of the follicle wall with resultant rupture of follicle and release of fertilizable ovum (Goetz et al., 1991; Guraya and Dhanju, 1992; Espey and Lipner, 1994). The action of FSH during the preovulatory follicular development is the induction of LH receptors on granulosa cells (Zeleznik et al., 1981; Zeleznik and Hillier, 1984). The granulosa cells of early antral follicle possess FSH receptors and stimulation of

these cells by FSH results in the activation of adenylyl cyclase and the production of cAMP. In response to FSH stimulation, the granulosa cells acquire LH receptors and, like that of FSH receptor, occupancy of the LH receptor by LH also results in the activation of adenylyl cyclase and production of cAMP (Goff and Armstrong, 1977).

Therefore, the preovulatory follicle responds similarly to both FSH and LH due to the involvement of common intracellular cAMP pathway. Moreover, at non-saturating levels of LH and FSH, the responses are additive (Kolena and Channing, 1972). The occurrence of a single high amplitude preovulatory LH surge is evident in goat, cattle, buffalo and sheep (Mori et al., 1987; Peters and Ball, 1995; Stevenson et al., 1998; Bakker and Baum, 2000; Singh et al., 2001; Kaim et al., 2003; Gonzalez-Bulnes et al., 2004). Whereas, peak plasma FSH concentration following estrus coincides with the LH surge followed by a less well defined peak at 4 to 30 h after the LH surge (Peters and Ball, 1995).

The current study demonstrates the evidence contrary to this well established phenomenon. The results indicated that instead of a single LH surge, the multiple rises in LH concentration above the basal level during estrus were responsible for ovulation in mithun. It may be suggested that these multiple LH rises were probably the multiple low-amplitude preovulatory LH surges. In present investigation, it was observed that the concentration of LH and FSH were higher during the first three days of the estrous cycle. The LH and FSH concentrations decreased to the basal level on day 4 and again increased on day 18 of the estrous cycle. In mithun, during estrus, the multiple low-amplitude LH surges along with the increased FSH activity either in the form of multiple surges like LH or in the form of gradual surge like increase for longer duration were probably crucial for the final maturation of the ovulatory follicle and subsequent ovulation. A significant association between the areas under LH and FSH curves indicated that the total LH and FSH activities were probably related phenomenon. It was also observed that the amplitude of LH peak was significantly higher in the animals that showed multiple FSH surges during estrus. The result indicated a probable facilitatory action of FSH on LH activity during the final maturation stages of ovulatory follicle.

It is reported that there is great divergence in the patterns of LH and FSH secretions though they are produced in the same cell gonadotrophs under the control of a single releasing hormone GnRH (Schallenberger et al., 1985; Moyle and Campbell, 1996; Roche, 1996).

After synthesis, both the gonadotropins are stored in secretory granules within the cytoplasm and are secreted differentially by exocytosis. It is suggested that the LH release is a regulated pathway mediated by GnRH action, whereas FSH release is a more constitutive pathway, in which synthesis is followed by release rather than by storage (Roche, 1996). The GnRH independent release of FSH is probably due to the selective stimulation of FSH synthesis by activin in association with sequential changes in the activin-binding protein.

Thus the paracrine mechanisms within the pituitary that selectively affect FSH are partially responsible for the differential release of FSH and LH from gonadotrophs (Roche, 1996). Interestingly in the current study, the different FSH secretion patterns were observed during estrus despite a similar LH secretion pattern in all the experimental animals. The result suggests the existence of a probable differential regulatory mechanism of LH and FSH release during estrus in mithun.

In conclusion, the multiple low-amplitude LH surges and associated increased FSH activity during estrus were probably responsible for ovulation in mithun. The existence of a probable differential regulatory mechanism of LH and FSH release during estrus was evident.

However, the further studies on the expression LH and FSH receptors in ovulatory follicle and the associated functions of LH and FSH during the final developmental stages of ovulatory follicle will be helpful to understand this species specific preovulatory LH and FSH milieu.

Besides, the investigation on the effect of different follicular factors on FSH secretion is required to define the differential regulatory mechanism of LH and FSH secretion in this species.

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