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Temporal changes in endogenous estrogens and expression of behaviors associated with estrus during the periovulaory period in Murrah buffaloes (*Bubalus bubalis*)

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Abstract The objective of this study were (1) to establish the duration of behavioral estrus signs and timing of ovulation in Murrah buffaloes (n=10) and (2) to determine relationship between behavioral estrus signs with change in plasma estrogen concentrations. Estrus and its behavioral signs were detected at hourly intervals by visual observations, per recta examination of genitalia and bull parading four times in a day for 30 minutes each. Among the behavioral signs of estrus, swollen vulva (80%) was the best indicator of estrus followed by excitement (70%). Among the duration of behavioral estrus signs the first and longest duration of estrus signs was swollen vulva which was seen upto 19.8±0.8 h after onset of estrus. The mean total duration of estrus symptoms from appearance to disappearance of all the behavioral estrus symptoms was 23.5±1.7 h. All the behavioral estrus symptoms were observed during the period of estrogen surge. Endocrine profile during the periestrus period showed that the mean peak concentrations of total estrogen 23.9±3.9 pg/ml occurred at 8.8±1.7 h after onset of estrus. The average number of estrus symptoms observed per animal during onset of spontaneous estrus was 5.7. Ovulation occurred after 37.4±1.7 h after onset of estrus and 13.4±1.0 h after end of total estrogen surge respectively. In conclusion, our results suggest that all signs of behavioral estrus occurred during the preovulatory rise in estrogens. The first sign of estrus to be observed was a swollen vulva and this symptom persisted the longest.

Keywords Buffalo · Estrus behavior · Estrogen · Ovulation

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Introduction

In the northern states of India, many farmers prefer to keep buffaloes as opposed to native cattle because buffaloes provide meat as well as milk and draught power. Cattle cannot be slaughtered for meat. Buffaloes are very efficient in the utilization of poor quality, native roughages. Good reproductive performance is critical for the efficient production of any livestock. Riverine buffaloes however are sluggish breeders, beset with reproductive problems. A high percentage of buffalo cows (30–40%) experience a prolonged period of anestrus with the Indian farmers incurring an estimated loss of 19–20 million tonnes of milk each year due to this problem.

Subestrus or silent estrous is perhaps the most important factor leading to poor reproductive efficiency in buffaloes (Kanai and Shimizu 1983; Prakash 2002; Madan and Prakash 2007) especially during hot summer months. Proper estrus detection is essential for artificial insemination (AI) practices so as to achieve maximum improvement in reproductive efficiency. Inefficient and inaccurate detection of estrus limits the economic success of many dairy operations in large herds. As the estrus signs in buffaloes are less obvious than in cattle, the accuracy of estrus detection is one of the major problems limiting the use of A.I. in this species. Understanding of the endocrine factors that control estrus is fundamental for the construction of strategies aimed at improving the detection of estrus and fertility improvement. Estrogen plays a key role in the regulation of the endocrine and behavioral events associated with estrous cycle. Estrogen induces the preovulatory surge in LH and estrus behavior (Hafez and Hafez 2000). The occurrence, intensity and duration of behavioral estrus in cattle is quite variable (Roelofs et al. 2005). Although several reports are available on expression of various symptoms of estrus in buffaloes (Gill et al. 1973; Singh et al. 1984; Paul 2003) there are no reports on the timing and duration of estrus symptoms in relation to the preovulatory estrogen surge and ovulation in the Murrah buffalo—the most important milch buffalo breed. Therefore, the present study was designed to record and relate the duration and intensity of estrus to the periovulatory total estrogen release and timing of ovulation in this species.

Materials and methods

Experimental animals

The study was conducted at the National Dairy Research Institute (NDRI) Farm, Karnal, Haryana. Ten cycling Murrah buffaloes (2nd–5th parity) were selected from NDRI herd for experiments. The animals selected for the study were free from any anatomical and reproductive disorders and were not suffering from any health problems. All the animals were kept under loose housing system in the experimental animal paddock and milking paddocks. Each paddock had brick flooring, asbestos roofing and sufficient space for the free movement of the animals. All the animals were fed a ration consisting of concentrate mixture containing maize grain, groundnut cake, mustard cake, wheat bran, mineral mixture, salt and roughages (either berseem and maize or oat fodder as per the availability in the farm). The animals were fed as per the standard feeding practices employed in the NDRI farm.

No treatment was given to induce or synchronize the estrus in these buffaloes. Occurrence of estrus in the animals was monitored by hourly observation of various behavioral estrus signs and by vasectomised bull (teaser) parading at 0600, 1200, 1800, 2400 h for 30 minutes and further confirmed by observing uterine tone on rectal palpation. Timing of ovulation was determined by per rectal ovarian examination of all animals at 2 h intervals from the first observation of behavioral signs of estrus until 2 hours post occurrence of ovulation. The timing of ovulation was confirmed by disappearance of the dominant follicle at palpation following consecutive examinations and change of ovarian surface from turgid to flaccid at ovulation. The changes and duration of behavioral estrus signs were also recorded from the time of estrus onset until ovulation.

Blood sampling

Blood samples were collected in heparinized vacutainer (BD Vacutainer^{TM}, UK) tubes (10 ml) at 2 h intervals from the first observation of behavioural signs of estrus until 6 h after the occurrence of ovulation. All the blood samples were kept in box containing ice (4°C) and carried back to laboratory immediately. Blood samples were centrifuged immediately at 3,000 rpm for 30 min and plasma was stored at—20°C until assayed for total estrogen by a sensitive heterologous enzymeimmunoassay previously developed in the laboratory (Mondal et al. 2006).

Parameters recorded

i) The incidence of behavioural estrus signs (excitement, bellowing, frequent urination, swollen vulva, uterine tone, bull mounting and mucus discharge) after the onset of spontaneous estrus

Table 1 Incidence of various behavioural estrus signs exhibited by buffaloes during spontaneous estrus

Estrus signs	Spontaneous estrus (8)*	
	Number of Responders	Intensity** (Mean)
Excitement	7	++
Bellowing	2	+
Frequent urination	5	++
Bull mounting	5	+
Swollen vulva	8	++
Chasing by bull	4	++
Mucus discharge	4	+
Chin resting on other animal	2	+
Uterine tone	6	++
Tail raising	3	++
No. of symptoms and average intensity of estrus signs observed/animal	5.7	1.71

*indicates the total number of animals observed for estrus signs

**On visual appraisal on a 3 point scale (+ Low ; ++ Medium ; +++ High)

ii) Periestrus and periovulatory plasma total estrogen profile in animals exhibiting spontaneous estrus.
iii) Timing of total estrogen surge after onset of spontaneous estrus.
iv) Timing of ovulation after onset of spontaneous estrus.

Data analysis

The data was subjected to appropriate statistical analysis to draw scientific inferences. Mean and standard errors were calculated by using GraphPad Prism 4.01 software. Number of behavioral signs of estrus exhibited by individual animal were added and the mean score (± SEM) estrus per animal was used for further analysis of data. The relationship of estrus behavior with total estrogen surge was determined by using GraphPad Prism software 4.01.

Results

Estrus behaviour

Out of 10 buffaloes selected, 8 animals exhibited estrus symptoms while ovulations were recorded in 7 buffaloes. The incidence of various behavioral estrus signs exhibited during spontaneous estrus in 8 buffaloes are presented in Table 1. Swollen vulva was the main estrus symptom and was seen in all buffaloes; excitement was the next most exhibited symptom seen in 7 of 8 buffaloes. Other major behavioral estrus signs shown were uterine tone, frequent urination, bull mounting, chasing by bull and mucus discharge. Tail raising, chin resting and bellowing were minor symptoms also observed in buffaloes after onset of spontaneous estrus. The average number of estrus symptoms observed per animal during onset of spontaneous estrus was 5.7.

Duration of behavioral estrus signs

The duration of various behavioral estrus signs exhibited during spontaneous estrus in the 8 buffaloes are presented in (Table 2). The first appearance of swollen vulva was observed 0.57±0.37 h (range 0.0 to 2.0 h) after onset of estrus and ended at 20.20± 1.07 h with a range of 16.0 to 24.0 h. Excitement was

Table 2 Duration of estrus signs (Mean ± SEM) in buffaloes (n=8) during spontaneous estrus

Estrus signs	Mean±SEM(h)		Duration(h) (Mean±SEM)	Range (h)
	Onset (h)	End (h)		
Swollen vulva (n=8)	0.57±0.37	20.0±1.07	19.75±0.80	0.0–20.0
Frequent urination (n=5)	2.00±1.16	12.50±1.26	10.80±0.80	0.0–16.0
Excitement (n=7)	1.33±0.99	9.33±1.23	8.00±0.44	0.0–14.0
Bellowing (n=2)	1.00±0.25	10.0±2.00	9.00±1.00	0.0–12.0
Mucus discharge (n=4)	12.0±1.63	23.50±1.71	11.50±2.06	8.0–28.0
Bull mounting (n=5)	13.20±1.49	23.20±1.36	10.00±2.09	8.0–28.0

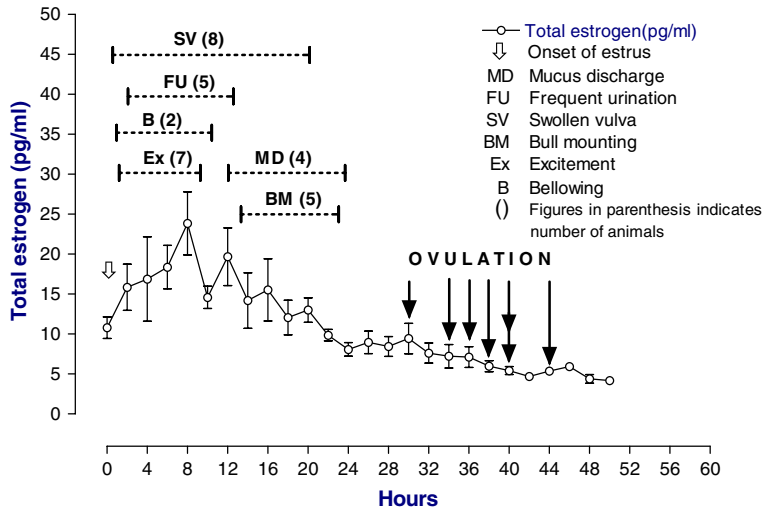

Fig. 1 Changes in plasma total estrogen profile (Mean ± SEM) and mean duration of estrus signs in Murrah buffaloes (n=8) after onset of spontaneous estrus

observed from 1.33±0.99 h (range 0.0 to 6.0 h) upto 9.33±1.23 h (range 6.0 to 14.0 h). Frequent urination was recorded in 5 out of 8 buffaloes from 2.00± 1.16 h (range 0.0 to 4.0 h) upto 12.50±1.26 h (range 10.0 to 16.0 h) post estrus onset. Bellowing was observed from1.00±0.25 h (range 0.0 to 2.0 h) upto 10.00±2.00 h (range 8.0 to 12.0) after onset of estrus in only 2 animals. The appearance of mucus discharge was observed at 12.0±1.63 h (range 8.0 to 16.0 h) upto 23.5±1.71 h (range of 20.0 to 28.0 h). Mounting of buffalo by teaser bull was seen from 13.20±1.49 h (range 8.0 to 16.0 h) upto 23.20±1.36 h with a range of 20.0 to 28.0 h.

Peri-ovulatory plasma total estrogen profile

The post estrus onset and periovulatory changes in plasma total estrogen are presented in (Fig. 1; Table 3). Individual variations in onset of total estrogen surge after initial expression of estrus signs were observed in the buffaloes. Estrogen surge was observed after the onset of estrus in all the 8 buffaloes. In one buffalo (Mu 4952) no ovulation was recorded even though estrus signs were expressed. The mean total estrogen peak concentration recorded was 23.85±3.94 pg/ml; the duration being 24.0±0.98 h (range 20.0 to 28.0 h).

Timing of ovulation in relation to onset of estrus and plasma estrogen surge

'The timing of ovulation after onset of estrus and post total plasma estrogen surge is depicted in Table 3 and Fig. 1. Ovulation occurred 37.43±1.73 h in 7 of 8 buffaloes (range 30.0 to 44.0 h) after the onset of spontaneous estrus and at 13.43±0.98 h (range of 9.43 to 17.43 h) post onset of total estrogen surge (Table 3).

Table 3 Plasma total estrogen peak characteristics and timing of ovulation in buffaloes (n=8) exhibiting spontaneous estrus

Parameters	Animals	Mean±SEM	Range
Mean total estrogen peak concentration (pg/ml)	8	23.85±3.94	(9.04–44.52)
Duration of total estrogen surge (h)	8	24.00±0.98	(20–28)
Time from:			
a) onset of estrus to mean total estrogen peak (h)	8	8.75±1.73	(2–16)
b) onset of estrus to ovulation (h)	7*	37.43±1.74	(30–44)
c) after end of total estrogen surge to ovulation (h)	7*	13.43±0.98	(9.43–17.43)

*Out of 8 buffaloes, ovulatory response was recorded in 7 animals

Discussion

To the best of our knowledge, this is the first report in Murrah buffaloes on duration of estrus behavior in relation to onset of estrus, timing of ovulation and estrogen surge. No reports are available in either cattle or buffaloes with which comparisons can be made.

The various behavioral estrus symptoms expressed during onset of spontaneous estrus in cycling Murrah buffaloes were swollen vulva, excitement, presence of uterine tone, frequent urination, bull mounting, mucus discharge bellowing, chasing by bull and tail raising (Table 1). The three most important symptoms were swollen vulva, excitement and presence of uterine tone. Other major estrus signs were frequent urination, bull mounting, and chasing by bull or teaser. Behavioral estrus signs like bellowing, frequent urination, restlessness, swollen vulva and mucus discharge were also observed by Singh et al. (1984) during estrus in buffaloes. Gill and Gangwar (1972) and Gunasekaran (1998) suggested that uterine tone and turgid or coiled cornuae on rectal palpation could be used for confirmation of estrus in Murrah buffaloes. Other reports suggest that discharge of cervical mucus was one of the most important estrus signs in buffaloes (Gill et al. 1973; Rao and Kodagali 1983; Singh et al. 1984; Danell et al. 1984). Mucus discharge however, was observed only in 50 percent of the buffaloes in the present study. The variations observed in the present study could be attributed to different feeding, management, environmental conditions and breed of buffaloes. Janakiraman (1978) considered frequent urination at the onset of estrus as a sure constant sign of estrus in Surti buffalo heifers. Although not seen in all Murrah buffaloes in the present study, frequent urination was a major symptom.

The overall average number of estrus symptoms exhibited per buffalo during spontaneous estrus (5.7) was similar to those recorded earlier (6.0) in Murrah buffaloes (Paul 2003).

Among the estrus symptoms, swollen vulva appeared to be the most notable estrus symptom the duration of which was also the longest (Fig. 1). Our observations also indicate that all the estrus symptoms were recorded during the course of the estrogen surge and basal estrogen levels were recorded at the time of ovulation (Fig. 1). In our opinion the magnitude and duration of the estrogen surge could therefore play a major role in overt estrus behavior influencing not only the incidence of various estrus symptoms but also their duration at estrus. In an earlier study from this laboratory a distinct improvement of estrus symptoms was recorded in winter months in buffaloes when the estrogen surge was greater (Roy and Prakash 2007) in comparison to that recorded in summer months. From a practical viewpoint our results suggest that the optimum time of inseminating the Murrah buffalo is 24–36 h post estrus onset as ovulations occur 30 to 44 h post estrus onset.

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