

RELIABILITY OF ESTRUS BEHAVIORAL SIGNS FOR ESTRUS DETECTION IN HORMONE TREATED POST PARTUM SUMMER ANESTRUS BUFFALOES

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ABSTRACT

Fifty anestrus buffaloes (over 3 months post-partum) maintained by individual farmers at village Jharli (Jhajjar, Haryana) were treated with various combinations of MGA (Melengesterol acetate) or Hydroxyprogesterone caproate (HPC) with eCG (equine chorionic gonadotrophin) and PGF2 α (ProstaglandinF2 alpha) during summer months. The anestrus status of the animals was also determined by per rectal palpation of ovaries, twice at 10 days interval. All the treated and control animals were observed for estrus signs twice daily for 14 days from the day of last treatment, both visually as well as by presentation to a fertile bull for mating. The visual signs of estrus observed, included vulvar swelling, hyperemia of vaginal mucosa, vaginal mucus discharge, bellowing, restlessness and response to vulvar massage. In addition, behavioral responses of bull towards female particularly sniffing of vulva, following the female, resting chin on female's rump and mounting and mating were also considered. Vulvar hypremia, bellowing, restlessness and response to vulvar massage were the most reliable signs of estrus whereas, standing to be mounted by the bull was a sure sign of estrus. Highest frequency of accurate signs was observed in MGA-PGF2 α treated buffaloes. Study of behavioral signs suggests that vaginal hyperaemia, bellowing, restlessness and response to massage of vulvar lips were more reliable signs whereas, standing to be mounted by bull was a sure sign of estrus. Further, it was observed that estrus can be better detected by careful observation of number of estrus signs and highest frequency of accurate estrus signs in MGA-PGF2 α treated females warrant more emphasis on this protocol.

Key words: Estrus signs, hormone, summer anestrus, buffaloes

In buffaloes, signs of estrus are less intense than in cattle (Aboul-Ela, 1988) and lower estrogenic levels at the time of estrus have been suggested to be the main reason for this lower intensity of estrus behavioral signs (Zicarelli *et al.*, 1993). Moreover, during low breeding season, estrus behavioral signs of estrus in buffaloes were even weaker (Raizada and Pandey, 1981). On the other hand, estrus detection in buffaloes, particularly under field conditions, is difficult and needs careful observation (Jainudeen, 1982). Present study was designed to find out the reliability of estrus signs for estrus detection in hormone treated summer anestrus buffaloes in rural area.

MATERIALS AND METHODS

The study was conducted during summer

months (May-July) on a total of 50 anestrus buffaloes (over 3 months post-partum) maintained by individual farmers of village Jharli, district Jhajjar, Haryana. The buffaloes were genetically heterogeneous with majority of Murrah breed and a few nondescript buffaloes. All animals were in good health and with apparently normal genitalia. The animals had a history of anestrus with no observation of vaginal discharge and mating since calving. Since no proper records were being kept by the farmers therefore, post-partum interval was stated in months. Acyclicity was confirmed by per rectal ovarian palpation carried out twice at 10 days interval, which revealed smooth ovaries on both the occasions. Animals were kept fastened in covered sheds during day time and in open yards during the night. All these animals were suckled and milked twice daily and were fed according to their individual requirement by the respective owner. Experimental animals were randomly

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Table 1
Hormone therapy schedule of buffaloes under study

Group (n)	Treatment	Lactation (Range)	Months post-partum (Range)
I (10)	0.4 mg MGA*/head/day in feed for 14 days plus eCG** 500 IU, i/m on last day of MGA feeding	2 -6	4.0 -10
II (10)	0.4 mg MGA plus 500 IU, eCG as in group I plus 25 mg PGF2 α ***, 17 days after the last MGA feeding.	1 -6	3.5 -10
III (10)	500 mg Hydroxyprogesterone caproate**** (HPC) s/c plus 1000 IU, eCG, i/m given 7days after HPC injection	1 -4	4.5 -10
IV (10)	500 mg HPC s/c plus 1500 IU eCG i/m, given 7 days after HPC injection	1 -4	3.5 -11
V (10)	Untreated control	1 -5	3.5 -11

*MGA: Melengesterol acetate

**eCG: Equine chorionic gonadotrophin

*** PGF2 α : Prostaglandin F2 alpha

****HPC: Hydroxyprogesterone caproate

distributed in different groups and administered hormone therapy as described in Table 1.

During therapy and for 15 days after therapy, all experimental animals were observed visually for sexual behavioral signs and elicitation of estrus twice daily at 6 AM and 6 PM. After each observation, buffaloes were also presented to a fertile bull to recognize female in estrus and for mating the ones in estrus. In addition, each individual farmer was also advised to look for these signs and symptoms. The sexual behavioral signs and symptoms observed were vulvar swelling, hyperemia of vulvar mucus membrane, vaginal mucus discharge, bellowing, drop in milk yield, restlessness and response to massage of vulvar lips. In addition, when the female was presented to bull, the interest of latter in the female was judged by sniffing of vulva, following the female, resting its chin on female's rump, mounting and mating. On any given day of observation, the number of animals showing a particular sign was considered its frequency. In addition, number of estrous behavioural signs expressed by each individual female on any particular day was also recorded.

Frequency of symptoms expressed by buffaloes mated (standing estrus) and not mated but later detected with a CL (silent estrus), was counted on the day of mating and expression of maximum number of symptoms. For silent estrus

buffaloes, the day of estrus thus calculated also coincided with return to estrus, detected 21 \pm 1 days after the calculated day of estrus in a majority of silent estrus buffaloes. The signs expressed by buffaloes on one day before and one day after mating or expression of maximum number of signs were considered to be associated with standing or silent estrus, respectively. Any sign or symptom expressed by these animals on all other days or by any of the remaining animals were considered estrus signs not associated with ovulation i.e. false estrus.

Estrus was considered standing when the buffalo was mounted by a bull apart from exhibiting other behavioral signs, which were followed by the presence of a palpable CL on ovaries on per rectal examination made 15 and 22 days after treatment. Estrus was considered ovulatory but silent / sub-estrus when more than 5 estrus signs were exhibited by buffaloes but without standing to be mounted or mated by a bull. On per rectal palpation, 15 and 22 days after treatment, a CL was detected relative to the day when respective female had expressed more than five estrus signs / symptoms. The remaining animals which did not have a palpable CL and were not mated after treatment were considered as non-respondents and the signs expressed by these females were considered as false symptoms.

Table 2
Frequency of sexual behavior signs and symptoms in relation to estrus in postpartum anestrus buffaloes with or without hormone treatment

Signs	Frequency per cent (no.)			
	Total frequency	Standing estrus*	Silent estrus*	False signs Ovulatory** or nonovulatory
Vulvar swelling	113	37.17 (42)	9.73 (11)	53.09 (60)
Vulvar hyperemia	15	80 (12)	20 (3)	0
Vaginal mucus discharge	153	29.41 (45)	7.84 (12)	62.67 (96)
Bellowing	8	100 (8)	0	0
Drop in milk yield	184	20.65 (38)	8.15 (15)	71.19 (131)
Restlessness	6	100 (6)	0	0
Response to vulvar massage	11	72.72 (8)	27.27 (3)	0
Sniffing of vulva by bull	183	24.04 (44)	8.18 (15)	67.75 (124)
Following of female by the bull	60	60.66 (37)	18.33 (11)	20 (12)
Resting of chin on female rump by bull	20	80 (16)	15 (3)	5 (1)
Mounting and mating by bull	16	100 (16)	0	0

* Including ± 1 day of standing / silent estrus

** Signs exhibited beyond ± 1 day of standing / silent estrus and without estrus

RESULTS AND DISCUSSION

Buffaloes exhibiting estrual signs were grouped in three categories viz. standing estrus, silent estrus and false estrus, depending upon whether the animal was mated, not mated with subsequent presence of CL and without mating and without CL, respectively. Table 2 reflects the frequency of association of individual behavioral sign with standing, silent estrus or false estrus, following therapy. Bellowing, restlessness and response to vulvar massage were exhibited by about half of the treated animals in standing estrus, while vulvar swelling, vaginal mucus discharge, drop in milk yield, sniffing of vulva by bull were recorded in females showing standing or silent estrus. The data for standing or silent estrus also include observations recorded on a day before and a day after estrus.

A perusal of data reveals that a high frequency of signs such as vulvar swelling, vaginal mucus discharge, drop in milk yield and sniffing of vulva by the bull, were not associated with ovulatory estrus (false signs). These signs, therefore, can not be relied upon for estrus detection, unless these are associated with other signs of estrus. Practically, to detect vulvar swelling, close observations are needed and more

so because of large variations in shape and size of vulva in any buffalo population depending upon genetic make up, parity, health status and clinical history. Similarly, detection of vaginal discharge, particularly within the vulvar labia, is tedious and needs closer monitoring. Therefore, these signs may not be so important in estrus detection, but may be helpful in estrus confirmation (Barkawi *et al.* 1993). Vale (1994) also considered mucus discharge as an unreliable sign of estrus because of its presence during proestrus as well as after insemination. In addition, there is a tendency of mucus accumulation at the floor of vagina, which may be thrown out as a lump at one time (Janakiraman, 1988). Decrease in milk yield can be associated with asexual parameters such as availability of nutrition, change of diet, climatic changes, change of place, clinical problem etc. Therefore, decrease in milk yield can not be relied upon as a criterion to confirm estrus. Similarly, sniffing of vulva by male proved to be a false sign, thereby suggesting its poor reliability, in contrast to observations of Barkawi *et al.* (1993). The later authors found response of females towards the male as the most reliable signs of estrus, but sniffing of vulva in the present study was also recorded in a large proportion of females prior to or after estrus.

On the other hand, a high frequency of vulvar hyperemia, response to slight vulvar massage, following the female and resting chin on female rump by the bull were associated with ovulatory estrus and can be considered as reliable signs. However, for recording vaginal hyperaemia, close observation is needed and the farmer has to open the vulvar lips manually to detect this sign. But it can be easily observed at farms practicing AI. Similarly, Vale, (1994) suggested vaginal hyperaemia as the main symptom associated with estrus if teaser is not available. Similar to the findings of present study, Singh *et al.* (1984) and Barkawi *et al.* (1993) considered vulvar massage as a reliable sign of estrus detection. Slightly higher frequency (80%) of following the female by the bull was associated with ovulatory estrus but it was also seen in females (20%) showing false symptoms. The sniffing of vulva and following the female by bull may probably be due to secretion of pheromones in the vaginal discharge as well as in the perineal glands (Blazquez *et al.* 1988, Chantarapruteep, 1995). Resting chin on female rump by the male was more frequently (95%) associated with ovulatory estrus and in the females showing standing estrus and it preceded mounting by the bull.

Bellowing, restlessness and mounting and mating by the bull were always associated with standing ovulatory estrus. These results suggest that these signs should be considered most reliable indicators for estrus particularly under field conditions, where no teaser bull is available. Bellowing was reported to be most reliable sign of estrus detection (Singh *et al.* 1984, Aboul-Ela, 1988, Barkawi *et al.*, 1993) and restlessness was reported to be an indicator of most intense heat in Murrah buffalo heifers (Singh *et al.*, 1984). All the females showing ovulatory standing estrus were mounted and mated by fertile bull, a sign which can be considered to be a sure indicator of estrus (Gill *et al.* 1973, Vale *et al.* 1984, Barkawi *et al.*, 1993).

Apart from treated females which failed to exhibit ovulatory estrus, some estrual females also exhibited a combination of behavioral signs (2 to 5) not associated with estrus, all of these were considered as false signs. One of the main

causes of the low fertility in buffaloes with AI has been the frequent occurrence of false estrus (Vale, 1994) and this phenomenon reportedly coincided with second follicular wave (Janakiraman, 1978). Mid-cycle estrual symptoms are not uncommon (Danell, 1987, Vale *et al.*, 1991) and are associated with the presence of large follicle along side a mature corpus luteum. False signs of estrus are also frequently exhibited by anestrus buffaloes (McCool *et al.*, 1989).

Buffaloes exhibiting standing estrus also showed a higher frequency of estrus signs compared with that of silent estrus buffaloes. Similarly, Singh *et al.* (1984) observed only the mucus discharge in silent estrus buffalo heifers, whereas, females in standing estrus showed more number of signs. Barkawi *et al.* (1997) reported 43% of Egyptian buffaloes showing weak and non standing estrus during hot season, while in the present study, only 25% of ovulating females were not in standing estrus, though the period of present study was also during summer months. This also indicates that hot season probably exerts inhibitory effect on estrus activity and this may be attributed to the low level of circulating estrogens during the hot months either during estrus cycle or at estrus (Shafie *et al.*, 1982, El-Wardani, 1995).

Study of behavioral signs suggests that vaginal hyperaemia, bellowing, restlessness and response to vulvar massage of lips are more reliable signs, whereas, standing to be mounted by a bull was a sure sign of estrus.

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