The Journal of Animal & Plant Sciences, 29(3): 2019, Page: 645-649
ISSN: 1018-7081

ESTRUS DURATION AND EXPRESSION IN NATURAL AND INDUCED ESTRUS IN INDIGENOUS *(Bos indicus)* CATTLE

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ABSTRACT

For the rapid genetic improvement of non-descript indigenous *(Bos indicus)* cattle of Azad Jammu and Kashmir through artificial insemination (AI) both in natural and induced AI program heat detection is a key. The study was undertaken to describe the duration and intensity of natural and induced estrus in indigenous heifers and cows. A total of 12 heifers and 18 cows were synchronized using the Ovsynch protocol (day 0 GnRH-1, day 7 PG, day-9 GnRH-2). In natural estrus group 8 heifers and 6 cows were utilized in this study. They were observed for total duration of estrus and standing estrus. The primary and secondary estrus signs were observed. The total duration of heat in heifers was 18.63±1.03 and 18.00±1.58 h in induced and natural group respectively. The duration of standing estrus in induced and natural groups was 10.63±0.60 and 8.25±0.73 h, respectively. In cows, the total duration of estrus in induced and natural groups was similar, whereas, the duration standing estrus was longer in induced group (9.69±0.62 h) compared to natural group (6.66±0.80 h) ($P<0.05$). The estrus intensity score was not affected significantly in both heifers and cows between induced and natural estrus. The duration of standing estrus in indigenous heifers and cows has been shortened in natural compared to induced estrus.

Key words: *Bos indicus*, duration of estrus, natural estrus, induced heat.

INTRODUCTION

Dairying has become an important subsidiary source of income for thousands of rural families with an important role in generating earning opportunity in Azad Jammu and Kashmir (AJ&K). The indigenous cattle in AJ&K are of *Bos indicus*, small in size and require small amount of feed to meet nutrient requirement for maintenance. These animals are well adapted to graze on hilly and mountainous areas, and resistant to diseases especially ecto-parasites. However, the low productivity of indigenous cattle of AJ&K may be because of poor genetic makeup (Kuthu *et al*., 2007; Khan *et al.,* 2014). In *Bos taurus* cows a reasonable genetic improvement has been made by applying artificial insemination (AI) and it has the potential to do likewise in *Bos indicus* cows (Galina and Arthur, 1990). Scattered and small-scale farms, and difficult movement due to hilly terrain are some of the most important obstacles in large scale use of the AI technique in AJ&K. The study of estrus behavior in *Bos indicus* cattle can be made easier by estrus induction as a large number of cows in estrus can be observe within a short period. One major problem of dairy industry is lack of accurate estrus detection (Senthilkumar and Chandrahasan, 2015). Therefore, the animals in estrus are diagnosed based on mounting or allowing to be mounted, mucus discharge and restless (van Eerdenburg *et al.,* 1996). However, the duration of estrus and intensity (Orihuela *et al.,* 1983; Pinheiro *et al.,* 1998) can be altered through the utilization of pharmacological agents in estrus synchronization protocol. For an effective breeding program estrus detection is the key and this can be achieved by close observation, timed AI and sound record keeping (Roelofs *et al.,* 2010). The application of estrus synchronization program has the potential to choose the calving season, shorten the postpartum interval, increase calf uniformity and facilities of AI (Udin *et al.,* 2017)**.** It is a reproductive management tool that can increase production efficiency and economic returns in the relative shorter time (Malik *et al*., 2018).

Knowledge of estrus behavior is essential for estimating the best time to artificially inseminate, as the understanding of the factors involved in the expression of estrus and occurrence of ovulation is an obstacle limiting the success of artificial insemination (Galina *et al.*, 1996). In *Bos taurus* breeds estrus behavior lasts approximately 18 h and ovulation occurs 28 to 31 h after the onset of spontaneous or synchronized estrus, while in *Bos indicus*, naturally occurring estrus is shorter, approximately 11 h, and ovulation occurs around 25 h after the start of estrus (Pinheiro *et al.*, 1998). The effects of synchronization treatments on expression of estrus are very important aspects that need to be considered when designing

strategies for controlled breeding programs (Quezada-Casasola *et al.,* 2015), keeping in view that both hormonally-induced, and naturally-occurring estrus may differ among different types and breeds of cattle (Zárate-Martínez *et al.*, 2006). There is no information available on the reproductive behavior of indigenous cattle of AJ&K and the AI results have often not been very encouraging. It is very important to know the estrus behavior of the indigenous cattle for the rapid genetic improvement of indigenous cattle through AI.

Therefore, the hypothesis for the present study was that, in non-descript cattle; mechanisms involved with natural and synchronized estrus differ. Therefore, the objective was to evaluate the behavioral estrus intensity and duration after synchronization with Ovsynch treatment or naturally-occurring estrus in indigenous cattle of AJ&K.

MATERIALS AND METHODS

A total of 44 indigenous cattle at Livestock Development Research Center Muzaffarabad were used in this experiment. The age of heifers and cows was ranges between 3 to 4 years and 6 to 10 years, respectively. Whereas the body condition score of all the animals was 3.0 - 3.5 (scale -1 to 5) recorded at the beginning of the experiment (Wildman *et al.,* 1982). Animals with inactive ovaries which were small, flat and smooth or rounded with follicle or with a cyst were excluded from the study after examining by rectal palpation. All the cows and heifers were kept in tail to tail system with double row in a semi closed sheds provided with asbestos sheet roof, iron bar and concrete floor. All the cattle were stall fed on Total Mixed Ration (TMR) with adequate supply of fresh, clean and soft drinking water under the same management and environmental conditions. Animals were maintained on TMR manufactured by Big Feed Pvt. Ltd Pakistan at the rate of 2% body weight. Common salt in the form of lump was placed in feeding pan and cows were free to lick.

The cows and heifers were synchronized using Ovsynch treatment (Pursley *et al.,* 1995) in which injection of 100 mg of GnRH analogue (Dermarelin; lecirelin acetate 25 mcg/ml, FATRO S.p.A.-pharmaceutical veterinary Industry, Italy) was administered on day 0, 25 mg of PGF2α (Lutalyse^{TM}, Dinoprost tromethamine 5 mg/ml, Pfizer manufacturing Belgium NV- Puurs- Belgium) on day 7 and 2nd injection of GnRH on day 9 (Figure 1). The signs of estrus were starting to be observed 12 h after PGF2α administration and recorded by well trained personnel at 3 h intervals (8 times daily). The time of start of estrus was considered the average time between the first observation of the behavioral signs of estrus and the previous check without any such signs. Similarly, the end of the estrus was considered as the midpoint between first finding of an animal to be off estrus and the previous check in estrus. Estrus duration (h) was calculated from the onset and cessation of estrus. Estrus expression ranking (maximum score being 100) was done based on cardinal signs (standing heat, mucous discharge, vulvar swelling, micturition, restlessness, bellowing sound, mounting, redness of vaginal mucosa, sniffing, chin resting, aggression). These signs were assigned with numbers according to their importance based on some modification in the format described by Layek *et al.* (2011) as shown in Table 2. During the period from January to May 2017, the duration and intensity of estrus was recorded for 8 heifers and 6 cows, in natural estrus.

Statistical Analysis: Data analysis was done by unpaired t-test (non-parametric) between the two groups using Graph Pad Prism version 6.0. Statistical results were considered to be significant at $P<0.05$.

RESULTS

Estrus Length: The results of present study obtained on mean duration of induced and natural estrus (h) of indigenous heifers are given in Table 1. The duration of induced estrus was similar ($P>0.05$, $t_{(18)}=0.346$) to natural estrus. Whereas, the duration of standing estrus, was a significantly longer in induced group compared to natural group. Time from PGF2α to the initial onset of estrus and standing estrus was 47.00±0.51 h (46-49 h) and 53.33±0.98 (50-56 h). In cows, the duration of estrus in induced group was similar to natural group. The duration of standing estrus was significantly longer in induced compared to natural groups. Time from PGF2α to the initial onset of estrus and standing estrus was 46.56±0.24 h (46-48 h) and 52.67±0.55 h (51-55 h). The onset of estrus and standing estrus was similar in heifers and cows in both induced and natural groups.

Scoring of Estrus Signs: The effect of induced estrus on different cardinal signs in indigenous cattle is presented in Table 2. In heifers that were synchronized with Ovsynch protocol to induce estrus and for the heifers that shows estrus naturally, scoring of estrus sign were carried out during the study period. Numerically the induced group had lower total estrus intensity score (48.83±5.06) compared to natural group (59.67±5.87). However, this difference did not differ significantly between the two groups of heifers. Along with heifer's estrus scoring was also done in eighteen cows that were treated with Ovsynch protocol and in six cows that had natural estrus. More pronounced in some primary and secondary signs of estrus were observed in induced estrus with total estrus intensity score of 58.39±2.99 compared to natural group (54.83±4.45). The total estrus intensity score did not differ significantly between the two groups.

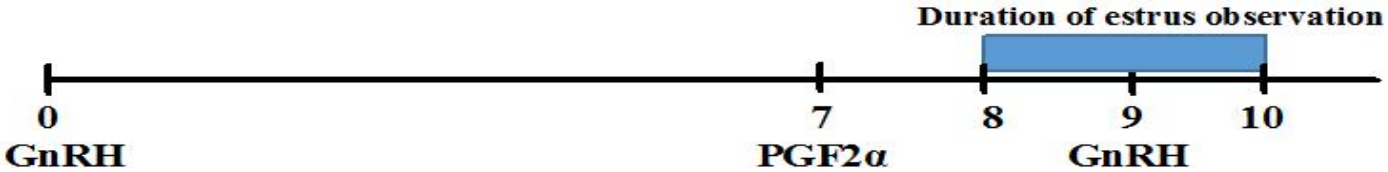

Figure1. Protocol of used for estrus induction and observation of duration of estrus in non-descript (*Bos indicus*) heifers and cows at Azad Jammu and Kashmir.

Table 1. Mean (± SEM) induced and natural estrus duration (h) of non-descript (*Bos indicus*) heifers and cows at Azad Jammu and Kashmir

	Estrus phase	Duration of Estrus (H)				
		Induced (n=12	(Range)	Natural (n=8)	(Range)	P
Heifers	Total Estrus Duration	18.63 ± 1.03	(13-25)	18.00 ± 1.58	(13-24)	0.73
	Standing Estrus	10.63 ± 0.60[a]	(7-13)	8.25 ± 0.73[b]	(6-12)	0.02
Cows	Total Estrus Duration	19.47 ± 1.21	(9-29)	18.83 ± 2.25	(14-29)	0.79
	Standing Estrus	9.69 ± 0.62[a]	(4-15)	6.66 ± 0.80[b]	(5-10)	0.01

The values with different superscripts are significantly different within the same row, (P<0.05)

Table 2. Different estrus signs and total estrus intensity (Mean ± SEM) score observed during induced and natural heat in indigenous heifers of AJ&K.

Estrus observation/ Signs with numbers	Standing heat (25)	Mucus discharge (20)	Vulvar swelling (9)	Micturition (9)	Restlessness (9)	Bellowing (6)	Mounting (6)	Redness of vaginal mucosa (6)	Sniffing (4)	Chin resting (3)	Aggression (3)	Total intensity score (100)
						Heifers						
Induced	15.83±1.8	16.17±1.4	5.50±0.7	2.17±0.7	2.0±0.7	1.41±0.4	1.75±0.6	3.5±0.6	0.16±0.1	0.0±0.0	0.41±0.2	48.83±5.0
Natural	19.22±1.5	16.11±0.5	6.55±0.8	2.33±1.2	4.11±1.2	1.88±0.6	2.55±0.8	4.0±0.6	1.22±04	0.66±0.3	1.0±0.5	59.67±5.8
						Cows						
Induced	18.33±0.7	18.06±0.5	3.78±0.6	2.44±0.6	2.55±0.5	4.00±0.4	3.05±0.6	2.83±0.4	1.22±0.3	0.66±0.3	1.44±0.3	58.39±2.9
Natural	16.83±1.0	13.33±0.6	4.33±1.2	2.33±1.4	2.50±0.9	4.33±0.6	2.83±1.2	3.83±0.4	1.66±0.6	1.0±0.6	1.83±0.5	54.83±4.4

DISCUSSION

In cattle the duration of estrus lasts from 6 to 30 h, with an average of 20 h (Yizengaw, 2017). In present study the mean duration of total estrus in non-descript *Bos indicus* is shorter that of *Bos taurus* and no difference was observed between the induced and natural estrus length of heifers and cows. Similar finding was observed by Valle *et al.* (1994) in Nelore cows and Alves *et al.* (2009) in Guzera (*Bos taurus indicus*) breed cows. The duration of induced estrus in non-descript heifers and cows in our study was similar to that observed in Brahman cows (Landaeta-Hernandez *et al.,* 2002) and longer than that in German Brown (16.2±0.7 h), Holstein (15±0.8 h) and White Fulani heifers (14.6±0.8 h) (Adeyemo *et al.,* 1979). The estrus duration recorded in the present study is in consonance with Layek *et al.* (2011) who reported the duration of estrus in Sahiwal cows ranged from 6 to 31 h with an average of 14.53±0.80 h. Although, it was suggested that the estrus length is shorter in *Bos taurus indicus* than in *Bos taurus taurus* (Randel, 1994), the average estrus length in non-descript indigenous cows, in this study, was similar to that observed in Holstein cows by Walker *et al.* (1996), 9.6 h.

The duration of standing estrus significantly ($P<0.05$) increased in induced group of heifers and cows as compared to non-induced group of heifers and cows. Similar finding was observed by Yoshida *et al.* (2009) who found the duration of standing estrus in Holstein Friesian dairy heifers on the average (±SD) was 9.7±5.3 h in induced heat. The longer duration of standing estrus in synchronized heifers and cows in present study may be due to administration of exogenous GnRH, due to which increased follicular growth results in secretion of large amount of estrogens that, in turn, cause plasma concentrations of the primary estrogen, estradiol, to reach peak and causes the behavioral signs of longer estrus.

The reduction in estrus behavior involves reduced mounting activity and shorter periods of estrus which limits the use of AI (Hansen and Fuquay, 2011) the increased estrus duration by estrus induction in present study may be beneficial in application of AI in indigenous cattle for a rapid genetic improvement. The duration of estrus in heifers ranged from 12.2 to 16.0 h (Diskin and Sreenan, 2000; Aoyagi *et al*., 2003). In the present study, duration of estrus in indigenous heifers of AJ&K was shorter than the data reported earlier (Diskin and Sreenan, 2000; Aoyagi *et al*., 2003), but relatively longer than previous report by Yoshida and Nakao (2005) that duration of standing estrus in heifers was 6.2±3.9 h.

Heat detection in cattle means fewer wasted insemination and saving time as it improves the submission rate and more accurate timing of A.I., which ultimately increases the profit.

In present study induced group had numerically higher total intensity score in indigenous cows (50.80±2.82 and 51.94±2.37 respectively) compared natural groups. These results of total estrus intensity matched with Layek *et al.* (2011) who reported estrus intensity of 34% to 52% in Sahiwal cattle. The above authors also recorded redness of vaginal mucosa, mucus discharge and uterine tone as evident heat signs as observed in the present study. However, chin resting and sniffing were also prominent in Sahiwal cows (Layek *et al.,* 2011) but were less prominent in indigenous heifers and cows as noted in the present study. A slightly better response with Ovsynch protocol in cows may be due to the reason that GnRH injection at day 0 might have resulted in synchronous follicular emergence and timely follicular development at the start of treatment. Thus, a more finely delineated endocrine regulation might have induced a wave of follicular development. Low intensity of estrus is a limiting factor in the use of AI and other reproductive management techniques depends upon visualization of estrus. Therefore, the higher estrus intensity score achieved by estrus induction may be helpful in application of AI.

Conclusion: Total duration and intensity of estrus were not affected by estrus induction whereas duration of standing estrus was increased by estrus induction in indigenous heifers and cows.

Acknowledgement: This research was supported by a grant from Agricultural Linkages Program, Pakistan Agricultural Research Council Islamabad.

Novelty Statement: *Bos indicus* cattle usually exhibit a shorter duration of standing heat which is a major problem of accurate heat detection. These results show using estrus synchronization protocol (Ovsynch) to induce heat in indigenous heifers and cows has resulted in a longer length of standing heat.

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