Np
DOI: 10.5958/2277-3371.2017.00038.9

Theriogenology Insight: 7(3): 199-203, December 2017

Relationship between Estrus Signs and Subsequent Fertility Rates in Buffaloes Subjected to Estradiol based Synchronization Treatments

H.S. Sandhu[1*], S.S. Dhindsa[2], M. Honparkhe[3], Bilawal Singh[4], S. Singhal[5] and R. Jindal[6]

[1,3,5]Department of Veterinary Gynaecology and Obstetrics, [2]Directorate of Livestock Farms, [4]Department of Veterinary Extension, [6] Department of Veterinary Physiology and Biochemistry, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India 141001

*Corresponding author: sharad20march@yahoo.com

ABSTRACT

The study was carried out on 60 postpartum cyclic buffaloes subjected to Presynch-Heatsynch (n=30) and Heatsynch (n=30) synchronization treatments. In Presynch-Heatsynch group, two injections of $PGF_2\alpha$ analogue were administered at 12 days apart (Day -14 and -2) with last injection two days before GnRH administration (Day 0). A third $PGF_2\alpha$ alpha was given on day 7 followed by estradiol benzoate injection on day 8 and fixed time artificial insemination (FTAI) on day 10. In Heatsynch group, first two PGF2 alpha injections were not given; however, rest of the treatment was same. Frequent urination, vulvar swelling and bellowing were prominent estrus signs observed in all buffaloes of both groups at the time of FTAI. The study revealed significantly pronounced ($p<0.01$) cervico-vaginal mucus (CVM) discharge and high uterine tonicity in buffaloes that became pregnant (100.00 and 70.00 %) than those failed to become pregnant (60.00 and 20.00 %) in group I and II, respectively. Further, it was observed that none of the buffaloes that became pregnant had low uterine tonicity, whereas 40.00 and 55.55% buffaloes which failed to become pregnant showed low uterine tonicity on rectal examination. In conclusion, appearance of CVM at vulva and rectal finding of high uterine tonicity at the time of AI had positive influence on the pregnancy rates in buffaloes subjected to estradiol based synchronization protocols. Thus, successful pregnancy may be predicted if a buffalo shows above two estrus signs at the time of FTAI.

Keywords: Buffalo, Estrus signs, Estrus synchronization, Heatsynch, Presynch-Heatsynch

Buffalo is an important component of livestock production, which contributes more than 50% of the total milk produced in India. For sustainable milk production, buffalo must reproduce efficiently. However, the reproductive efficiency of buffalo is impaired by poor estrus expression, delayed puberty and prolonged calving intervals (Baruselli *et al.,* 2003). The incidence of silent or sub estrus is high in buffalo leading to poor estrus detection, missed estrus and difficulty in predicting the time of ovulation and insemination (Mohan *et al.,* 2009). Due to the above mentioned reasons, use of artificial insemination (AI) in buffalo has been limited. It has been reported that only 21% of buffaloes are inseminated at improper time (Kumaresan *et al.,* 2001). Such limitations substantially reduce the farmer's financial income because of overall reduced pregnancies and milk production (Paul and Prakash, 2005). This consideration indicated a need for estrus synchronization for enhancing reproduction in buffaloes (Presicce *et al.,* 2004 and Ali and Fahmy, 2007). Numerous synchronization protocols have been proposed in buffalo with variable success rate till date. The failure of timed ovulation in synchronized buffaloes has been suggested as an important cause of poor fertility (Hattab *et al.,* 2000). Now days, dairy farmers in India are adopting techniques of estrus synchronization to enhance reproductive and productive efficiency of the buffalo. In recent years, estradiol based

synchronization protocols have been preferred over conventional treatments owing to reduced hormonal costs, easy implementation cum scheduling and intense estrus signs shown by animals following estradiol based treatments. An estrus synchronization treatment is easy to implement as most of the injections are given intramuscularly in the neck region which a farmer can easily execute by his own. However, to assess ovulatory response in order to predict success of the treatment, ultrasonographical examination is mandatory which is not feasible at field level particularly in rural or remote areas of the country. It is hypothesized that success of FTAI program may also be predicted through expression and intensity of certain estrus signs exhibited by buffaloes at the time of FTAI. This study was, therefore, conducted to in investigate the influence of behavioural estrus signs recorded at the time of FTAI on conception rate in buffaloes subjected to estradiol based synchronization protocols.

MATERIALS AND METHODS

A total of 60 postpartum cyclic buffaloes (>45 days in milk) maintained at dairy farm, Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India were included in the study. The selected clinically healthy buffaloes were in 2nd to 6th lactation and had BCS 2.5 to 3.5 (scale of 1-5) with the body weights ranging from 400-650 kg. All the animals were kept under loose housing system. The selected buffaloes were divided into 2 groups on the basis of type of synchronization treatment used. In group I, Presynch-Heatsynch (n=30) and in group II, Heatsynch (n=30) protocols were applied. In Presynch-Heatsynch (Group I), on day -14, a $PGF_2\alpha$ analogue (Cloprostenol, 500mcg, Tradename: Vetmate, 2ml, manufactured by Provimi Vetcare) was administered followed by the same injection on day -2. The GnRH analogue (Buserlin acetate, 10mcg, Tradename: Receptal, 2.5ml, manufactured by MSD) was administered on day 0 of the protocol. On day 7, 3rd injection $PGF_2\alpha$ analogue was administered. On day 8, estradiol analogue (estradiol benzoate, 1 mg dissolved in benzyl alcohol, Tradename: PregHeat, 1ml, manufactured by Virbac) was administered followed by FTAI 48 h later on day 10. In Heatsynch (Group II) first 2 $PGF_2\alpha$ analogue injections at -14 and -2 days were not administered, however, rest of the treatment was similar as explained in Presynch-Heatsynch group. All the injections were given by intramuscular route of administration at 9.00 to 10.00 am. The behavioural estrus signs viz. frequent urination, vulvar swelling, appearance of CVM at vulva, bellowing and uterine tonicity were recorded at FTAI and compared between the both treatments. Uterine tonicity was graded as no, low, moderate and high upon rectal examination as explained in Table 1.

Table 1: Assessment of uterine tonicity at the time of FTAI in buffaloes subjected to Presynch-Heatsynch and Heatsynch treatments

Uterine tone	Rectal finding
No	Flaccid uterine horn
Low	Flaccid uterine horns that got toned after massage
Moderate	Toned horns that were easy to lift and straighten up
High	Toned uterine horns that were difficult to lift and straighten up

In addition, a comparison between pregnant and non pregnant buffaloes of both groups was also carried out. To assess the pregnancy status, ultrasonographical examination (Z 5 diagnostic ultrasound machine, Shenzhen Mindray Biomedical Electronic Co. Ltd) was performed on day 45 post AI. To compare the behavioural estrus signs between animals that became pregnant or failed to become pregnant in both groups, “Chi square test” and “Fisher’s exact test” were applied (IBM SPSS STATISTICS VERSION 24).

RESULTS AND DISCUSSION

The various behavioural estrus signs recorded at the time of FTAI in Presynch-Heatsynch

 Print ISSN: 2249-6610

(Group I) and Heatsynch (Group II) buffaloes and in pregnant vs. non pregnant buffaloes of both groups are presented in Fig. 1 and Table 2, respectively.

Poor estrus expressivity and silent estrus are the major limitations which contribute to the low reproductive efficiency of buffaloes (Baruselli *et al.,* 2003). To maximize the chance of successful AI practices, proper estrus detection is essential. The accuracy of estrus detection is one of the major problems limiting the use of AI in buffaloes. Moreover, the duration of estrus is also variable in buffaloes making the prediction of time of ovulation difficult (Ohashi 1994). The findings of the present study indicate that the problem of poor estrus expression, variable duration of estrus and silent estrus in buffaloes can be eliminated through implementation of estradiol based synchronization programs as appreciable number of animals (73-100%) exhibited major signs of estrus viz. frequent urination, vulvar swelling and CVM at the time of FTAI.

It was observed that frequent urination, vulvar swelling and bellowing were observed in all the buffaloes of both groups. Mucus discharge (CVM) was shown by 86.66 (26/30) and 73.33 (22/30)% buffaloes in group I and II, respectively. Another important estrus sign was uterine tonicity. Low uterine tonicity was shown by 13.33 (4/30) and 33.33 (10/30)% buffaloes of group I and II, respectively. Further, moderate uterine tonicity was observed in 33.33 (10/30)% buffaloes of each group. High uterine tonicity was observed in 53.33 (16/30) and 33.33 (10/30)% buffaloes of group I and II, respectively. Dhindsa *et al.* (2014) and Bilal *et al.* (2017) also observed frequent urination (100 %), swollen vulva (100 %), mucus discharge (82 %) and (70 %) respectively, at the time of AI in buffaloes following synchronization treatments. Another study by Mirmahmoudi and Prakash (2014)

Table 2: Comparison between behavioral estrus signs exhibited by pregnant and non pregnant buffaloes of Presynch-Heatsynch and Heatsynch treatments at the time of FTAI

Parameter		Pregnancy status	Presynch-Heatsynch (Group I)	Heatsynch (Group II)
Frequent urination		Pregnant	100.00 (20/20)	100.00 (12/12)
		Non pregnant	100.00 (10/10)	100.00 (18/18)
Vulvar Swelling		Pregnant	100.00 (20/20)	100.00 (12/12)
		Non pregnant	100.00 (10/10)	100.00 (18/18)
CVM		Pregnant	100.00 (20/20)**	100.00(12/12)**
(Cervico vaginal mucus)		Non pregnant	60.00 (6/10)**	55.55 (10/18)**
Bellowing		Pregnant	100.00 (20/20)	100.00 (12/12)
		Non pregnant	100.00 (10/10)	100.00 (18/18)
Uterine tonicity	**No**	Pregnant	0.00 (0/20)	0.00 (0/12)
		Non pregnant	0.00 (0/10)	0.00 (0/18)
	Low	Pregnant	0.00 (0/20)**	0.00 (0/12)**
		Non pregnant	40.00 (4/10)**	55.55 (10/18)**
	Moderate	Pregnant	30.00 (6/20)	33.33 (4/12)
		Non pregnant	40.00 (4/10)	33.33 (6/18)
	High	Pregnant	70.00 (14/20)**	66.66 (8/12)**
		Non pregnant	20.00 (2/10)**	11.11 (2/18)**

**: Significantly different ($p<0.01$) within the column, (Pregnant vs. Non pregnant)

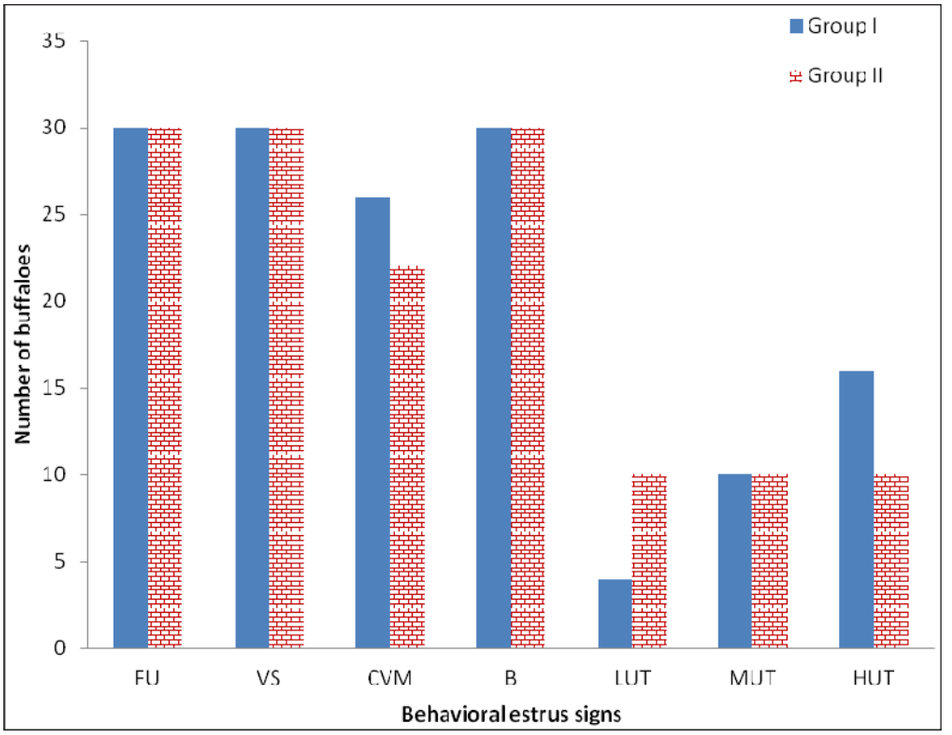

Fig 1: Behavioral estrus signs exhibited by buffaloes in Presynch (Group I) and Heatsynch (Group II) at the time of FTAI

Where, FU, VS, MD, B, LUT, MUT and HUT represent frequent urination, vulvar swelling, cervico vaginal mucus, bellowing, low uterine tone, moderate uterine tone and high uterine tone, respectively.

revealed frequent urination (100 %), swollen vulva (90 %) and mucus discharge (55 %) estrus signs following Heatsynch protocol in Murrah buffaloes. In the present study, various estrus signs observed viz. frequent urination, vulvar swelling, cervico vaginal mucus, bellowing and uterine tone did not differ significantly (P>0.05) between two groups.

A comparison of various behavioural estrus signs exhibited by pregnant and non-pregnant buffaloes of both groups (Table 2) revealed that estrus signs of frequent urination, vulvar swelling and bellowing were exhibited by 100% buffaloes irrespective of their pregnancy status. Furthermore, CVM at vulva was observed in 100% pregnant buffaloes of group I and II which was significantly higher (p<0.01) than their counterparts (60.00 and 55.55 %, respectively). In I and II respective groups, 40.00 and 55.55% of non pregnant buffaloes had low uterine tonicity, however none of the pregnant buffaloes of both groups exhibited this sign. High uterine tonicity was observed significantly higher (p<0.01) in pregnant buffaloes of both groups compared to those that failed to became pregnant (70.00 and 66.66 % vs. 20.00 and 11.11 %, respectively).

To summarize, inadequate estrus detection is considered as a major limit to buffalo reproductive performance. Introduction of estradiol based synchronization programs in buffalo reproduction management may eliminate the problem of estrus detection by application of FTAI. The success of synchronization program may be predicted through intensity of various

 Print ISSN: 2249-6610

estrus exhibited by buffaloes at the time of FTAI. Higher conception rates can be predicted in buffaloes exhibiting mucus discharge (CVM) at vulva and rectal finding of high uterine tonicity at the time of FTAI following estradiol based synchronization protocols.

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