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IMPACT OF ESTRADIOL BASED MODIFIED SYNCHRONIZATION PROTOCOL ON EXHIBITION OF BEHAVIOURAL ESTRUS IN BUFFALO (*Bubalus bubalis*)

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ABSTRACT

The present study was aimed to compare the behavioral estrus signs and uterine tone exhibited by buffaloes subjected to traditional heat synch and a modified heat synch (G6G-Heatsynch) protocol in which a gonadotropin releasing hormone (GnRH) injection was administered two days after prostaglandin ($PGF_{2\alpha}$) and six days prior to the Heat synch protocol. A total of 50 postpartum clinically healthy and cyclic buffaloes were randomly divided into two groups. Buffaloes of Group 1 (n=25) were treated with G6G-Heatsynch protocol whereas Group 2 (n=25) buffaloes received the traditional Heat synch protocol. Various behavioral estrus signs *viz*. frequent urination, vulvar swelling, cervical-vaginal mucus (CVM) discharge, bellowing and uterine tonicity were recorded on the day of fixed time artificial insemination (FTAI) and a comparison was carried out between both groups based on display of these estrus signs. Frequent urination, vulvar swelling and bellowing were present in all the animals of both groups whereas CVM was observed in 80 and 68% buffaloes of Group 1 and 2, respectively. High uterine tone was exhibited by 44% buffaloes of Group 1 compared to 32% buffaloes of Group 2. Furthermore, frequent urination, vulvar swelling and bellowing were observed in all the animals of both groups irrespective of their subsequent pregnancy status. On the other hand, higher proportion of pregnant buffaloes showed CVM discharge compared to non-pregnant ones in Group 1 ($P<0.01$) and 2 ($P<0.05$). Pregnant buffaloes also had higher uterine tonicity at the time of FTAI than non-pregnant buffaloes of Group 1 ($P<0.05$) and 2 ($P<0.01$). In conclusion, estradiol-based synchronization methods were successful in eliminating estrus detection problem. Further, CVM discharge and high uterine tonicity at the time of AI were prominent signs to predict successful pregnancy in buffaloes subjected to estradiol-based synchronization protocols.

Keywords: *Bubalus bubalis*, buffaloes, behavioral estrus, heat synch, G6G-Heatsynch, artificial insemination, uterine tone

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INTRODUCTION

To enhance the chances of conception through artificial insemination (AI) practices, proper and timely detection of estrus becomes an obligation in buffalo. However, use of AI becomes limited because of the lesser degree of accuracy in estrus detection practices (Singh, 2017). Poor estrus detection, a variable duration of estrus and the difficulty encountered in predicting the time of ovulation are the main reasons responsible for the failure of AI (De Rensis and Lopez-Gatius, 2007). So inadequate heat detection has acted as a limiting factor for reproductive management of buffalo herds over the years. If the estrus is not detected at proper time, there is a loss of 21 days as far as productive performance is concerned because the farmer must wait for the next estrus to inseminate the animal (Verma *et al*., 2014). As per the earlier studies, it has been reported that almost 50% of heats often go unnoticed whereas nearly 21% animals are inseminated at an improper time even in the organized dairy farms (Kumaresan *et al*., 2001). Furthermore, the problem of poor estrus expressivity and a greater incidence of silent heat act as the major contributing factors responsible for the low reproductive efficacy of buffaloes (Baruselli *et al*., 2003). The main behavioral signs of estrus shown by buffaloes are frequent urination, vulvar swelling, cervical-vaginal mucus discharge and bellowing but these signs shown by buffalo are less overt in comparison to the cattle (Vale *et al*., 1990) as most of the buffaloes exhibit estrus during late night and early morning which often goes undetected. Although with the introduction of FTAI protocols, the problem of visual estrus detection has been largely overcome, but still the success of any synchronization program largely depends upon the intensity of various estrus signs exhibited by buffaloes on the day of artificial insemination (Sandhu *et al*., 2017). Heat synch protocol developed by Pancarci *et al.* (2002) which makes use of GnRH, $PGF_{2\alpha}$ and estradiol benzoate (EB: in place of second GnRH of Ovsynch protocol) is thought to be advantageous over Ovsynch protocol in buffaloes because of reduced hormone costs, increased estrus intensity and improved uterine tone following EB injection which further enhances the success of the protocol (Mohan *et al*., 2014; Singh, 2017). In addition, incorporating a resynchronization strategy before starting any protocol increases pregnancy rates by 10% to 12% in bovines (Moreira *et al*., 2001; Navanukraw *et al*., 2004). Since presynchronization is reported to enhance the conception rate, it may also increase the intensity of behavioral estrus signs and uterine tone on the day of AI (Singh, 2017). Therefore, the present study was planned to compare the behavioral estrus signs and uterine tone at the time of insemination and their impact on subsequent pregnancies in buffaloes subjected to traditional heat synch and a modified heat synch (G6G-Heatsynch) protocol.

MATERIALS AND METHODS

Experimental animals

The present study was performed on 50 postpartum clinically healthy and cyclic buffaloes maintained at the Directorate of Livestock Farms, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. Buffaloes having a body condition score of 3 to 4 on a scale of 1 to 5 basis were included in the study (Alapati *et al*., 2010; Anitha *et al*., 2011). All selected buffaloes were multiparous (in their 2^{nd} to 6^{th} lactation) having a body weight greater than 400 kg and were kept

under semi-intensive housing system.

Design of the experiment

All buffaloes (n = 50) included in the study were divided into two equal groups. A modified heat synch protocol (G6G-Heatsynch) was applied to animals in the Treatment group (Group 1, n = 25). An equal number of buffaloes were subjected to Heat synch protocol, which served as a control group (Group 2, n = 25). The detailed experimental design for both groups has been depicted in Figure 1.

Observation of the behavioral signs of estrus

All the animals subjected to the synchronization protocol in both groups were observed for behavioral signs of estrus *viz*. frequent urination, vulvar swelling, cervico-vaginal mucus and bellowing three times a day (Summer: 5.00 to 7.00 A.M., 12.00 to 2.00 P.M., 6.00 to 8.00 P.M.; Winter: 6.00 to 8.00 A.M., 1.00 to 3.00 P.M., 5.00 to 7.00 P.M.) following EB administration with special reference to the estrus signs observed on the day of FTAI. Uterine tonicity was also assessed by per-rectal examination at the time of FTAI and graded as nil, low, moderate and high (Table 1).

Pregnancy diagnosis

Pregnancy was diagnosed in all the animals of both groups on day 45 post-insemination using B-mode transrectal ultrasonography (M-Turbo, SonoSite Inc., Bothell, USA, and BestScan S6 Touch Digital Ultrasound Diagnostic System, BMV Technology Co., Ltd., Shenzhen, China) with a 5.0 to 7.5 MHz linear transducer. The pregnancy was diagnosed as positive or negative based on the respective presence or absence of fetal heartbeat and amniotic vesicle containing fluid.

Statistical analysis

To compare the behavioural estrus signs between the animals of both groups as well as the pregnant and non-pregnant animals of both groups, “Chi-square test” and “Fisher’s exact test” were applied using IBM SPSS STATISTICAL VERSION 20.

RESULTS AND DISCUSSIONS

The findings of the present study indicate that the problem of visual estrus detection due to poor estrus expression, variable duration of estrus and silent estrus in buffaloes can be eliminated by implementation of estradiol-based synchronization regimens as a significant proportion of animals exhibited these major signs of estrus. The incidence of various behavioural estrus signs expressed by buffaloes of Group 1 and 2 on the day of AI has been presented in Table 2.

Behavioral estrus signs such as frequent urination, vulvar swelling and bellowing were found to be the most prominent as these were observed in all the buffaloes subjected to synchronization regimens in both the Groups (1 and 2) at the time of FTAI. As far as the CVM discharge is concerned, 80% (20/25) buffaloes in Group 1 and 68% (17/25) buffaloes in Group 2 exhibited mucus discharge at the time of AI ($P>0.05$). Another important estrus sign observed through per rectal examination was uterine tonicity. Interestingly, none of the buffaloes in both groups showed nil uterine tonicity. Low uterine tonicity was shown by 20 and 28% buffaloes of Group 1 and 2, respectively ($P>0.05$) whereas moderate uterine tonicity was observed in 36% (9/25) and 40% (10/25) buffaloes in Group 1 and Group 2 respectively ($P>0.05$). In addition, high uterine tonicity was reported

in 44% (11/25) of Group 1 and 32% (8/25) of Group 2 buffaloes (P>0.05). Although there was no significant difference, but the extent of CVM discharge and uterine tonicity was found higher in G6G-Heatsynch compared to Heat synch group which clearly indicates the beneficial effect of incorporating presynchronization strategies prior to synchronization in inducing better behavioral estrus signs in buffaloes.

Furthermore, out of 25 buffaloes in each group, 14 in Group 1 (G6G-Heatsynch) and 9 in Group 2 (Heat synch) were found pregnant upon ultrasonographical examination with the corresponding conception rate of 56 and 36%, respectively. Thus, a comparison of various behavioral estrus signs exhibited by pregnant and non-pregnant buffaloes of both Group 1 and 2 was also done (Table 3).

Some of the behavioral estrus signs such as frequent urination, vulvar swelling and bellowing were observed in all the animals of both groups irrespective of their pregnancy status. On the other hand, 100% pregnant buffaloes of both groups exhibited CVM compared to only 54.54% and 50% non-pregnant ones of Group 1 (P<0.01) and 2 (P<0.05). Subsequently, none of the pregnant animals of both groups showed low uterine tonicity in comparison to 43.75% and 45.45% non-pregnant buffaloes of Group 1 (P<0.05) and 2 (P<0.01). Moderate uterine tonicity did not differ (P>0.05) significantly between pregnant and non-pregnant buffaloes of both groups but high uterine tonicity varied significantly (P<0.01) between pregnant and non-pregnant buffaloes of Group 1 with their corresponding values being 66.67% (6/9) pregnant animals and 12.50% (2/16), respectively. In the

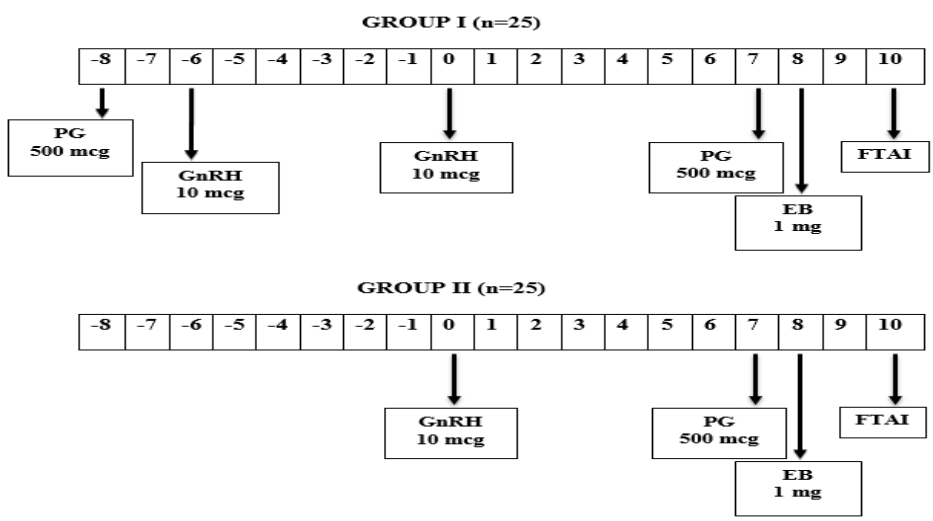

Figure 1. Estradiol based estrus synchronization protocols for fixed time artificial insemination in buffaloes. (US: Ultrasonography, BS: Blood Sampling, PG: Prostaglandin, GnRH: Gonadotropin-Releasing Hormone, EB: Estradiol Benzoate, FTAI: Fixed Time Artificial Insemination, PD: Pregnancy Diagnosis).

Table 1. Assessment of uterine tonicity at AI.

Uterine tone	Rectal finding
Nil	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

Table 2. Behavioral estrus signs exhibited by buffaloes of G6G-Heatsynch and Heat synch treatments on the day of AI.

Parameters		Percent of buffaloes (Number of buffaloes exhibiting estrus sign/ Total number of buffaloes)	
		G6G-Heatsynch (Group 1)	Heat synch (Group 2)
Frequent urination		100.00 (25/25)	100.00 (25/25)
Vulvar swelling		100.00 (25/25)	100.00 (25/25)
CVM (Cervico-vaginal mucus)		80.00 (20/25)	68.00 (17/25)
Bellowing		100.00 (25/25)	100.00 (25/25)
Uterine tonicity	Nil	0.00 (0/25)	0.00 (0/25)
	Low	20.00 (5/25)	28.00 (7/25)
	Moderate	36.00 (9/25)	40.00 (10/25)
	High	44.00 (11/25)	32.00 (8/25)

Table 3. Comparison between behavioral estrus signs exhibited by pregnant and non-pregnant buffaloes of G6G-heat synch and heat synch treatments on the day of AI.

Parameter		Pregnancy status	G6G-Heatsynch (Group 1)	Heatsynch (Group 2)
Frequent urination (Non pregnant)		Pregnant	100.00 (14/14)	100.00 (9/9)
		100.00 (11/11)	100.00 (16/16)	
Vulvar swelling (Non pregnant)		Pregnant	100.00 (14/14)	100.00 (9/9)
		100.00 (11/11)	100.00 (16/16)	
CVM (Cervico-vaginal mucus)		Pregnant	100.00 (14/14)**	100.00 (9/9)*
Non pregnant		54.54 (6/11)**	50.00 (8/16)*	
Bellowing (Non pregnant)		Pregnant	100.00 (14/14)	100.00 (9/9)
		100.00 (11/11)	100.00 (16/16)	
Uterine tonicity	Nil	Pregnant	0.00 (0/9)	0.00 (0/14)
		Non pregnant	0.00 (0/16)	0.00 (0/11)
	Low	Pregnant	0.00 (0/9)*	0.00 (0/14)**
		Non pregnant	43.75 (7/16)*	45.45 (5/11)**
	Moderate	Pregnant	33.33 (3/9)	35.71 (5/14)
		Non pregnant	43.75 (7/16)	36.36 (4/11)
	High	Pregnant	66.67 (6/9)**	64.28 (9/14)*
		Non pregnant	12.50 (2/16)**	18.18 (2/11)*

Superscripts * and ** indicate significant difference within the column between pregnant and non-pregnant buffaloes ($P<0.05$) and ($P<0.01$), respectively for a particular parameter. Figures in parenthesis indicate number of animals.

same way, 64.28% (9/14) are pregnant and 18.18% (2/11) non-pregnant animals of Group 2 displayed high uterine tonicity on the day of FTAI ($P<0.05$).

The findings of the current study support the earlier evidence which state that the problem of decreased display and intensity of estrus can be overcome by using EB injection to induce ovulation (Singh, 2017). Further, the issue of variable duration of estrus and silent estrus can also be resolved to a greater extent if estradiol based FTAI protocols are used. Frequent urination, vulvar swelling and bellowing are the prominent estrus signs observed in buffaloes during estrus (Sandhu *et al*., 2017) and the same trend has also been noticed in this study as all the buffaloes of both groups showed these three estrus signs on the day of FTAI irrespective of their subsequent pregnancy status. Singh (2017) reported that 68 percent of buffaloes exhibit CVM discharge following heat synch protocol whereas in the present study, cervico-vaginal mucus discharge was shown by 80% and 68% buffaloes of Group 1 and 2, respectively. This further indicates that the incorporation of presynchronization prior to heat synch improves the display of CVM discharge on the day of FTAI. Moreover, all the pregnant animals of both groups showed CVM discharge on the day of FTAI which clearly implies that this is an important behavioral estrus sign and its presence or absence can be used to predict the subsequent conception. In some earlier studies, frequent urination (100%), swollen vulva (100%) and mucus discharge (82%), respectively at the time of AI following synchronization treatments have been reported in buffaloes (Dhindsa *et al*., 2016; Bilal *et al*., 2017). Another study revealed frequent urination (100%), swollen vulva (90%) and mucus discharge (55%) as the prominent estrus signs exhibited by buffaloes subjected to heat synch protocol (Mirmahmoudi and Prakash, 2014). High uterine tonicity also appears to be another important parameter which can be used to predict higher conception rates as suggested by the findings of the present study in which a significantly greater proportion of pregnant buffaloes in both the groups showed high uterine tone at the time of FTAI compared to their respective non-pregnant counterparts. In addition, to make any synchronization treatment more successful, it is a must to access the ovulatory response which requires ultrasonographic examination. As the facility of ultrasonography may not be available all the time at the field level in developing countries, it becomes a mandate that the buffaloes display overt behavioral signs of estrus for their timely insemination. Since the estradiol-based synchronization protocols lead to an increased intensity of estrus display as indicated by the present and previous studies, these protocols find a great deal of practical application in buffaloes especially in the summer season when the incidence of silent heat is high. In addition, estradiol-based protocols are more cost effective than GnRH based protocols and follow easier scheduling and implementation (Mohan *et al*., 2014).

In conclusion, inadequate or poor estrus detection practices act as the major contributing factors that decrease the reproductive efficacy of buffaloes. However, with the incorporation of estradiol based FTAI synchronization methods, the problem of estrus detection may be eliminated. Since behavioral estrus signs exhibited by buffaloes help to predict the success of a synchronization program, it is advantageous to use estradiol-based protocols because of the increased intensity of heat signs on the day of FTAI following EB administration. While frequent urination, vulvar swelling and bellowing are the most prominent estrus signs, higher conception rates can be

predicted by the exhibition of CVM discharge and per-rectal finding of high uterine tonicity at the time of FTAI after subjecting the buffaloes to estradiol-based synchronization methods.

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