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Original Research Article

Evaluation of estrus behavior and fertility rate following estrus synchronization in Kundhi buffaloes

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Abstract

The present study was designed to compare the efficacy of estrus synchronization protocols with or without Biostimulation in Kundhi buffaloes at and surroundings of Kundhi buffalo farm, Rorhi. Selected forty Buffaloes (n=40) of 1st to 5th parity were divided into group A (Bull exposed; BE) and B (Non-exposed; NE). These both groups were further subdivided into subgroups (A1 and A2, B1 and B2). Subgroups A1 and B1 received Ovsynch GnRH at day 0 followed by PGF2α day 7th and second dose of GnRH at day 9th. Whereas, in subgroups A2 and B2, estrus was synchronized with double PGF2α protocol at interval of 11days. The groups did not show differ (P>0.05) estrus response but found high in A1 (80%) than A2 (70%), B1 (70%) and B2 (60%). Estrus duration differs significantly (P<0.05) among groups. The progesterone level in group A1 and B1 increased from day 0 (1.25±0.095 and 1.08±0.11ng/ml, respectively) to 7th (2.25±0.363 and 2.57±0.281ng/ml, respectively). Similarly in group A2 and B2 it was increase from day 0 (1.11±0.14 and 1.03±0.11ng/ml, respectively) to 11th (1.25±0.17 and 1.39±0.14ng/ml, respectively). While at 18th and 21st day, the progesterone level (ng/ml) in A1 (1.38±0.152) and A2 (1.35±0.16) found high compared to B1 (1.12±0.143) and B2 (1.09±0.166) groups. Pregnancy rate was high in group A1 (60%) and A2 (50%) than B1 (50%) and B2 (40%), but the differences existed non-significant. Present study concludes that biostimulation with ovsynch protocol can effectively be used to induce cyclic activities and increases estrus response and fertility rate in Kundhi buffalo.

Key words: Artificial Insemination, Biostimulation, Estrus synchronization, Kundhi buffalo

INTRODUCTION

Estrus synchronization has been developed as an important tool to induce estrus and increasing the rate of implementation of AI (Parry *et al.,* 2014). It enables a group of females to come in estrus at predetermined time, so that females can be bred with normal fertility during a short, predefined period. Synchronization of estrus also improves the reproductive performance of female animals which were not showing a consistent estrus sign. Several estrus synchronization methods have been developed in bovine to intensify the estrus and increases conception rate (Kumar *et al.,* 2014). In these estrus synchronization protocols gonadotropin releasing hormone (GnRH), progesterone (P4), prostaglandin F2α (or PGF2α analogs), and estrogen alone or in various combinations have been utilized to enhance estrus detection, there by facilitating the use of fixed timed

artificial insemination (FTAI). Use of PGF2α and its synthetic analogues was the earliest drugs to synchronize estrus. It was used as a good management tool in repeat breeder cattle and buffaloes. Prostaglandin (PGF2α) used for estrus synchronization singly or in combination with other protocols and fixed timed insemination was performed 72 and 80 hours after 2nd prostaglandin injection (Archbald *et al*., 1992). Gonadotropin-releasing hormone (GnRH) is considered as a drug of choice in the improving reproductive performance of female animals. GnRH or its analogue causes ovulation of a large follicle. Estrus response and fertility rate enhanced in cyclic and non-cyclic animals by using GnRH and PGF2α in combination (Chaikhun *et al.* 2010). Biostimulation conventionally used for induction of cyclicity in females. It is a stimulatory effect of a male on female to induce estrus and ovulation through olfactory and sensory cues (Chenoweth, 1983). The stimulus can act through olfactory, visual, and auditory signals (Ungerfield, 2007). Berardinelli and Joshi, (2005) reported that excretory products of males and cervical mucus from estrus females enhance ovarian function. However, the response of biostimulation is inconsistent due to variability in parity, season, cow to bull ratio, body condition score of female and postpartum stages at which bulls are exposed. Information about the effect of biostimulation in combination with estrus synchronization protocols in buffalo is scare. Therefore, the present study was designed to compare efficacy of estrus synchronization protocols with or without biostimulation in Kundhi buffalo.

MATERIALS AND METHODS

Animals and Management

Forty healthy adult Kundhi buffaloes of 1stto 5thparity raised on semi intensive management conditions at Kundhi Buffalo Farm Rohriand its surroundings were used in this study. Only those animals having no any reproductive problem were included in this study. All the animals were confirmed non pregnant by rectal palpation before the treatment. The routine management practices were followed during the study period.

Experimental design

The selected animals were divided into two groups i.e., A (Bull exposed) and B (Non-exposed). Group-A were further divided into two subgroups based on treatments, A1 Ovsynch bull exposed and A2 Double PGF2α bull exposed. Similarly group B were also divided into two sub groups B1 Ovsynch non-exposed and B2 Double PGF2α non bull exposed group.

Subgroup-A1.Ovsynch bull exposed group (OBE) N=10

Animals of this group was treated with Ovsynch (GnRH at day 0 followed by PGF2α day 7 and repeat dose of GnRH on day 9) for estrus synchronization. Treated animals were exposed to an intact, aproned bull for half an hour, twice a day6am and 6 pm from start of synchronization treatment to A.I.

Subgroup-A2. PGF2α bull-exposed (PBE) N=10

Estrus was synchronized with double PGF2α protocol at 11 day interval. Treated animals were exposed to an intact, aproned bull for half an hour, twice a day 6am and 6 pm from start of synchronization treatment to A.I.

Subgroup-B1. Ovsynch non-bull exposed group (ONE) N=10

Animals of this group were synchronized with same protocol as used for animals of group A1 but these animals were not exposed to bull from start of synchronization treatment to A.I.

Subgroup-B2. PGF2α non-exposed group (PNE) N=10

Estrus synchronization was done with same protocol as used for animals of group C but these treated animals were not exposed to bull.

Estrus detection

The buffaloes of group A1and B1 were watched closely after 2ndGnRH injection for behavioral changes to confirm the heat. Buffaloes in Group A2 and B2 were observed closely after 2nd PGF2α injection to confirm the estrus. The animals were inseminated twice with interval 12 and 24 hours after the second GnRH injection in group A1 and B1. In group A2 and B2, animals were inseminated following 72 and 96 hours after last PGF2α injection using frozen thawed semen obtained from Directorate of Animal Breeding, Livestock Department, Government of Sindh. The following parameters were recorded in each group.

Intensity of estrus

Intensity of estrus was recorded four times a day 6 am, 12 pm and 6 pm, 12 am for 30 minutes in each occasion. Estrus behavior was recorded according the scale developed by Van Eerdenburg *et al.,* (1996) with some

Table 1. Scoring scale

Estrus symptomsPoints	
Mucous vulvular discharge	3
Flehmen	3
Restlessness	5
Sniffing the vagina of another cow	10
Chin resting	15
Mounted but not standing	35
Mounting (or attempt) other cows	45
Standing heat	100

Table 2. The effects of estrus synchronization with and without biostimulation on occurrence of estrus, estrus duration and pregnancy rate in Ovsynch and Double PGF2α groups of Kundhi buffaloes.

Parameters			
Groups	**Estrus response (%)**	**Estrus duration (hours)**	**Conception rate (%)**
OBE (A1)	10/8 (80%)	21.62 ± 0.979	10/6 (60%)
ONE (B1)	10/7 (70%)	15.71 ± 1.047	10/5 (50%)
PBE (A2)	10/7 (70%)	18.85 ± 1.047	10/5 (50%)
PNE (B2)	10/6 (60%)	15.00 ± 1.13	10/4 (40%)

modification (Table - 1). Each time a symptom was observed and if the sum of the points exceeded 100 during a whole day, estrus response was considered strong. If the sum of the points were below 100, the estrus response was considered weak.

Collection storage and transportation of milk samples for determination of progesterone concentration

For determination of progesterone concentration, milk samples (10ml) were collected in a plain labeled vaccutainer tube on day 0, 7 and 18 in Subgroup A1 and B1 and on day 0, 11 and 21 in Subgroup A2 and B2. Immediately after the collection, milk samples were brought to the laboratory Department of Physiology and Biochemistry in an ice cooler for analysis of progesterone concentration.

Progesterone assay

Milk progesterone level was determined using Abraxis Progesterone (bovine) ELISA Kit (USA). The Standards were 0, 0.23, 0.47, 0.94, 3.75 and 20 ng/ml.

Pregnancy rate

The Pregnancy was confirmed on day 45 post A.I by rectal palpation.

Statistical Analysis

The data were analyzed by ANOVA and chi square test using Graph pad instate 3.05 versions of statistical package. Difference was considered significant at $P<0.05$.

RESULTS

The present study was undertaken to evaluate the efficacy of estrus synchronization protocols (ovsynch and double PGF2α) with or without biostimulation in Kundhi buffalo. The results are presented in different tables. The effects of estrus synchronization with and without biostimulation on occurrence of estrus, estrus duration and pregnancy rate in Ovsynch and Double PGF2α groups of Kundhi buffalo are presented in Table 2. The estrus response did not different significantly ($P>0.05$) among all the four treatment groups. Furthermore, higher estrus response was observed in Ovsynch bull-exposed (OBE) group (80%) as compared to PGF2α bull-exposed (PBE) (70%). Similarly, Ovsynch non-exposed (ONE) treatment induces estrus in (70%) buffaloes than the PGF2α non bull exposed (PNE) group (60%) respectively.

Estrus duration differ significantly among the groups ($P<0.05$). Estrus duration was longer in the Ovsynch bull-exposed group (21.62 ± 0.979 hours) as compared to PGF2α bull-exposed (18.85 ± 1.047 hours), Ovsynch non- exposed (16 ± 1.047 hours) and PGF2αnon-bull-exposed (15.00 ± 1.13 hours) group.

Pregnancy rate did not differ significantly among the four treatment groups ($P>0.05$). While pregnancy rate

Table 3. Signs of estrus (%) displayed by different groups of Kundhi buffaloes.

Estrus signs	OBE(A1)	ONE (B1)	PBE (A2)	PNE (B2)
Mucous vulvar Discharge	87.5	42.85	71.14	66.66
Flehmen	87.5	57.14	85.71	50.00
Restlessness	62.5	42.85	57.14	33.33
Sniffing the vagina of another cow	75.0	42.85	71.14	66.66
Chin resting	75.0	42.85	57.14	33.33
Mounted but no standing	50.00	42.85	71.42	50.00
Mounting (or attempt) other cows	50.00	0.00	42.85	0.00
Standing heat	75.00	57.14	71.42	66.66

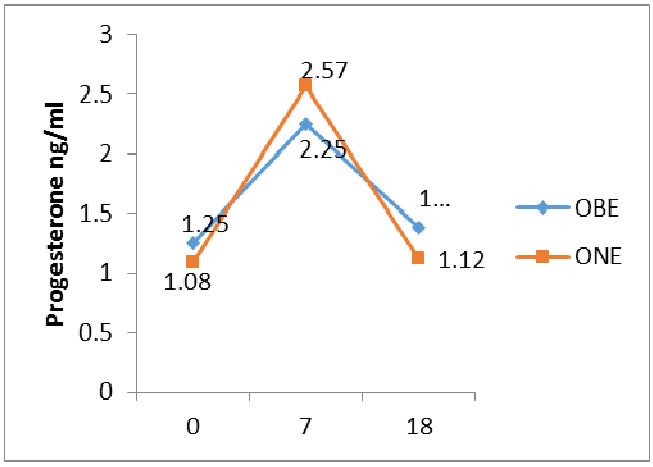

Figure 1. Milk progesterone concentrations in Ovsynch bull-exposed (OBE) and Ovsynch non-exposed (ONE) group at different days of treatment.

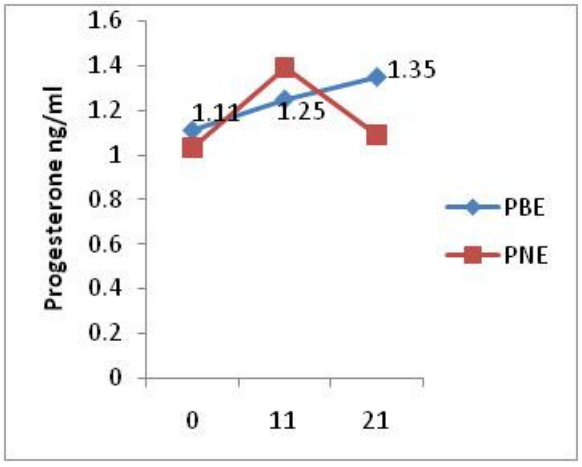

Figure 2. Milk progesterone concentrations in Prostaglandin bull exposed (PBE) and Prostaglandin non exposed buffaloes at different treatment days.

was higher in the Ovsynch bull-exposed group (60%) as compare to PGF2α bull-exposed (50%), Ovsynch non-exposed (50%), and PGF2α non- bull-exposed group (40%) respectively.

Signs of estrus displayed by different groups of Kundhi buffalo are presented in Table 2. Estrus signs were observed in all groups but Bull exposed (BE) groups showed intense estrus signs as compare to non-bull exposed (NE) groups. The most frequent estrus signs which were observed mucus discharge (87.5%),

(42.85%), (71.14%), (66.66%), flehmen (87.5%), (57.14%), (85.71%), (50 %), sniffing the vagina of another animal (75%), (42.85%),(71.14%), (66.66%) and standing heat (75%), (57.14%), (71.42%), (66.66%) in ovsynch bull-exposed group (OBE), ovsynch non-exposed (OBE), PGF2α bull-exposed (PBE) and in PGF2α non-bull-exposed (PNE) groups respectively. Animals showing milk progesterone concentrations in Ovsynch bull-exposed (OBE), Ovsynch non-exposed (ONE), $PGF_{2\alpha}$ bull-exposed (PBE) and $PGF_{2\alpha}$ non-exposed (PNE) buffaloes at different days of treatment are shown in Figure 1 and 2. The Progesterone level increased from day 0 to 7 in the ovsynch bull exposed and ovsynch non exposed group but it was lower in ovsynch bull exposed (1.25 ± 0.095), (2.25 ± 0.363) group, as compare to ovsynch non exposed (1.08 ± 0.11), (2.57 ± 0.281) group. Similarly in prostaglandin group it was increase from 0 and 11 in prostaglandin bull exposed and prostaglandin non bull exposed, but it was lower in prostaglandin bull exposed (1.11 ± 0.14), (1.25 ± 0.17) group, as compare to prostaglandin non bull exposed (1.03 ± 0.11),(139 ± 0.14) group, and on 18 and 21ovsynch bull exposed (1.38 ± 0.152) and prostaglandin bull exposed (1.35 ± 0.16) group showed higher progesterone level as compare to ovsynch non exposed (1.12 ± 0.143) and prostaglandin non bull exposed (1.09 ± 0.166) group. Some animals showed milk progesterone > 1ng/ml at day 8 post AI these were considered pregnant.

DISCUSSION

Silent estrus and seasonality of breeding are two important factors limiting reproductive efficiency of buffalo. Estrus synchronization may be used to overcome these problems. Gonadotropin releasing hormone (GnRH), prostaglandin F2α (PGF2α) and/or their different synthetic analogues have been used for estrus synchronization (Khumran *et al*., 2012). The ovsynch protocol is a sequence of GnRH-PGF2α-GnRH treatments that became popular for estrus synchronization in cattle over the last decade, resulting in an acceptable fertility to timed AI (TAI) (Jabeen *et al.,* 2012). Ovsynch treatment has been successfully used in buffaloes with acceptable fertility rates (Chaikhun *et al.,* 2010). It reduced the incidence of anestrous from 45% before treatment to 18% after treatment (Roy and Prakash, 2009).Similarly in the present study, the estrus response was higher in the Ovsynch bull-exposed group compared to Ovsynch non-exposed (80%), PGF2α bull-exposed (70%) and PGF2αnon- bull-exposed group (60%) but estrus response did not differ significantly (P>0.05) among the groups. These findings are in line with the results reported by Berardinelli *et al.* (2001). They reported that the estrus response was greater in postpartum cows exposed to males during estrus synchronization with GnRH and PGF2α, than non-exposed group. Similarly they also noted non-significant difference (P>0.05). Ahmed *et al.* (2011) found 90% estrus response in Bull-exposed cows as compared to 65% non-bull exposed cows with GnRH + PGF2α protocol. A lower estrus response was reported by Alberio *et al.* (1987) they found 67.9% estrus response in bull exposed and 32.7% in non-bull exposed animals without ES protocol. The result of current study lies in the range of above studies. Estrus duration was longer in the both Bull exposed (22 ± 0.663 hours), (20 ± 2.05 hours) and non-Bull exposed group (16 ± 0.954 hours), (14 ± 0.841 hours) with ovsynch and prostaglandin protocol. These findings are in agreement with Khanh *et al.,* (2012) they reported 23.64 ± 4.15 hours estrus in bull exposed and 13.40 ± 1.50 in non-bull exposed group. Our results are higher than those reported by Roelofs *et al.* (2005) who found 13.6 hours estrus duration in primiparious and 10.8 hours in multiparious cows. The difference in results may be due to methods of estrus detection, type of housing, frequency of handling, environmental condition etc.

Estrus signs were observed in all groups but Bull exposed (BE) groups showed more intense estrus signs as compare to non-bull exposed (NE) groups. The most frequent estrus signs which were observed mucus discharge 87.5%), (42.85%), (71.14%), (66.66%), flehmen (87.5%), (57.14%), (85.71%), (50%), sniffing the vagina of another animal (75%), (42.85%), (71.14%), (66.66%) and standing heat (75%), (57.14 %),(71.42 %), (66.66%) in ovsynch bull-exposed group (OBE), ovsynch non- exposed (OBE), PGF2α bull-exposed (PBE) and in PGF2α non-exposed (PNE) groups respectively. The present findings are in agreement with the Mondal *et al.,* (2006) and Khanh *et al.,* (2012) they observed that Bull exposed animals were showed more frequent estrus signs as compare to non-bull exposed animals.

Pregnancy rate was higher in the both treatments Ovsynch bull-exposed group (60%) and PGF2α bull-exposed (50%) group as compare to Ovsynch non-exposed (50%), and PGF2αnon-exposed group (40%) but the difference was non-significant among groups (P>0.05). Findings of the present study are higher than the Tauck and Berardinelli, (2007) who reported (59%) pregnancy rate in bull exposed group and (37%) in non-bull exposed cows. In contrast to this lower pregnancy rate was reported by Purabi *et al.* (2011) and Ahmed *et al.* (2011) who observed (81.82%), (66.7%) and (40.0%), (33.7%) in BE group and NE group respectively. Variability in the results may be due to the fact that exposure to males or excretory products of males during an estrus synchronization protocol based on GnRH and PGF2α varies due to different environmental condition, breed and estrus synchronization treatment etc.

The mean progesterone level (ng/ml) increased from days 0 to7 in the ovsynch bull exposed and ovsynch non exposed group but it was lower in ovsynch bull exposed

(1.25 ± 0.095), (2.25 ± 0.363) group, as compare to ovsynch non exposed (1.08 ± 0.11), (2.57 ± 0.281) group. Similarly in prostaglandin group it was increase from 0 and 11 in prostaglandin bull exposed and prostaglandin non bull exposed, but it was lower in prostaglandin bull exposed (1.11 ± 0.14), (1.25 ± 0.17) group, as compare to prostaglandin non bull exposed (1.03 ± 0.11), (139 ± 0.14) group, and on 18 and 21ovsynch bull exposed (1.38 ± 0.152) and prostaglandin bull exposed (1.35 ± 0.16) group showed higher progesterone level as compare to ovsynch non exposed (1.12 ± 0.143) and prostaglandin in non exposed (1.09 ± 0.166) group. The level of progesterone in the present study is in agreement with the levels recorded by Ali and Fahmy (2007) in skim milk of buffaloes. This pattern of progesterone production during estrous cycle follows the changes of CL function in the ovary (Mondal *et al.,* 2010).

CONCLUSION

It was concluded from the present study that the biostimulation with ovsynch protocol can effectively be used to induce cyclic activities and increases estrus response and fertility rate in Kundhi buffalo.

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