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 effectivel y 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 1st to 5th parity raised on semi intensive management conditions at Kundhi Buffalo Farm Rohri and 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 day 6am 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 A1 and B1 were watched closely after 2nd GnRH 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 6am, 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

<table>
<thead>
<tr>
<th>Estrus symptoms</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucous vulvar discharge</td>
<td>3</td>
</tr>
<tr>
<td>Flehmen</td>
<td>3</td>
</tr>
<tr>
<td>Restlessness</td>
<td>5</td>
</tr>
<tr>
<td>Sniffing the vagina of another cow</td>
<td>10</td>
</tr>
<tr>
<td>Chin resting</td>
<td>15</td>
</tr>
<tr>
<td>Mounted but not standing</td>
<td>35</td>
</tr>
<tr>
<td>Mounting (or attempt) other cows</td>
<td>45</td>
</tr>
<tr>
<td>Standing heat</td>
<td>100</td>
</tr>
</tbody>
</table>

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.

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

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.

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.

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

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 (PNE) groups at different 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%),
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 anestrus 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 PGF2α non-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 primiparous and 10.8 hours in multiparous 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 compared 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 (ONE), PGF2α bull-exposed (PBE) and PGF2α 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**

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.
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.

ACKNOWLEDGEMENT

The Authors wish to acknowledge Pakistan Agriculture Research Council, Islamabad for funding of this study.

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