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RESEARCH ARTICLE

# Effect of Biostimulation on Estrus Expression, Resumption of Ovarian Activity and Conception Rate in Postpartum Anestrus Nili-Ravi Buffaloes during Low Breeding Season

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**ABSTRACT**

This study evaluated the effect of biostimulation by bull exposure on estrus expression, resumption of ovarian activity, and fertility in anestrus, pluriparous buffaloes (*Bubalus bubalis)* during the low breeding season (May to July, 2012). Non pregnant, lactating Nili-Ravi buffaloes that depicted no corpus luteum (CL) on ovaries as palpated per rectum twice at 11 days interval, were divided in to bull exposed (24 h exposure per day, N=20, BE), bull partial exposed (1 h per day, N=20, BP) and bull non-exposed (no bull exposure, N=20, BN) groups. Estrus expression and interval to service from start of experiment were recorded over 60 days (June and July). Pregnancy was assessed at day 60 post service. The number of animals that showed behavioural estrus was significantly higher ($P<0.05$) in BE (60%) and BP (40%) than BN (5%) group while BE and BP groups did not differ significantly. Progesterone profile in 5 animals per group (once a week blood sampling) indicated ovarian activity matching with visually observed estrus expression. Interval to service from start of experiment was less ($P<0.05$) in BE than BP and BN groups (26.3±4.9, 37.0±3.7, 40.0±0.4 days, respectively); the difference being non-significant between BP and BN groups. Pregnancy rate was significantly higher in BE (40%) and BP (20%) groups than BN (0%) group ($P<0.05$); the difference being non-significant between BE and BP groups. It was concluded that biostimulation for 24 h per day revived ovarian cyclicity in 60% anestrus buffaloes during low breeding season.



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## INTRODUCTION

Buffalo is an indispensable livestock resource for thousands of poor farmers in Pakistan. A majority of buffalo grows under low input and low output system and although they can adapt to harsh environment and live on poor quality forage, reproductive and productive efficiency is often compromised under such conditions (Paul *et al*., 2002). Buffalo is a polyestrus animal and can breed round the year, however, reproductive seasonality in buffalo has been reported from all buffalo rearing countries with winter being the peak breeding season (Barile, 2005; Das and Khan, 2010; Perera, 2011). Information from Pakistan, India and Egypt indicated that 34-49% of buffaloes showed estrus during the first 90 days after calving and 31-42% remained anestrus for more than 150 days (El-Wishy, 2007). A study on postpartum ovarian activity over one year by taking twice weekly samples, measuring blood serum P4 and E2 levels indicated that 30% Nili Ravi buffaloes did not resume postpartum ovarian activity and showed anestrus for one year, whereas, 70% buffaloes resumed postpartum ovarian activity but then underwent anestrus during summer months (April to August) (Ullah *et al*., 2010). These buffaloes had calved in September to November. Under-nutrition, high environmental temperatures, poor management practices (Anwar *et al*., 2003) and photoperiod (Zicarelli, 1995) have been found to influence the frequency of postpartum and summer anestrus period in buffaloes.

Strategies like hormonal therapy, nutritional management and environmental modification have been tried to improve the reproductive performance in buffaloes (Jabeen *et al.,* 2015; Phogat *et al.,* 2016). Improved management practices are one of the options in this respect. Biostimulation by bull exposure represents

one of the possible management tools to improve reproductive efficiency in this species. This may be an effective strategy to reduce the postpartum anestrus and summer anestrus in buffaloes. Bull exposure to postpartum beef cows has been used to reduce the interval from calving to the resumption of luteal activity (Burns and Spitzer, 1992). The mechanism of this biostimulatory effect involves pheromones produced and excreted by bulls (Berardinelli and Joshi, 2005). Pheromones in the urine, faeces, or from cutaneous glands can be perceived through the olfactory system (Archunan *et al.*, 2014) and affect hypothalamic pituitary-gonadal activity in female rodents (Mora and Sanchez-Criado, 2004) and cattle (Tauck *et al*., 2007). Information regarding effect of biostimulation on anestrus and the reproductive performance in buffaloes is meagre. So, the present study was designed to evaluate the effect of bull exposure on estrus expression, resumption of ovarian activity, and fertility in anestrus buffaloes during low breeding season.

## MATERIALS AND METHODS

**Location and breeding practices:** The experiment was conducted at Military Dairy Farms, Okara, Pakistan (latitude 30.8° N, longitude 73.4° E and 180 meters above sea level). Buffaloes at the farm were served naturally. In summer, more than 100 buffaloes were grouped in open yard with one bull at night. In winter, buffaloes were exposed to bull for 2 h in morning and evening. Calves were allowed limited suckling at the time of milking for milk let-down.

**Experimental Animals:** Non pregnant, lactating Nili-Ravi buffaloes (N=60) with a normal calving history in their 2$^{nd}$ to 4$^{th}$ lactation were used for the study. The animals were part of a herd comprising of 1500 animals. The buffaloes used in the study had calved from September to November, 233.1±7.2 days before the start of the experiment. These buffalo had not shown estrus since calving. Ovaries of these animals were examined per rectum twice at 11 days interval before starting the trial and anestrus confirmed through absence of CL. The experimental animals were separated from the main herd during the present study conducted from 28$^{th}$ May 2012 to 31$^{st}$ July. 2012 Animals were allowed to acclimatize for three days after separation from the main herd. The animals were divided into three treatment groups that were balanced for postpartum interval (240±4 (BE), 233.3±6 (BP) and 227.4±5 (BN) days).

**Bull exposed (BE) group (N=20):** Buffaloes were kept with an intact buffalo bull 24 h per day.

**Bull partial exposed (BP) group (N=20):** Buffaloes were exposed to an intact buffalo bull twice daily at 7 am and 7 pm, for half an h each time.

**Bull non exposed (BN) group (N=20):** Buffaloes were kept without a buffalo bull. Heat detection was done by visual observation twice daily at 7 am and 7 pm for half an h each time.

All the three groups were kept at a distance of 0.5 km from each other. In this way the BN group was in complete isolation from the bull so that no excretory product or bull pheromone reached this group. Following observation were made on the three treatment groups.

**Estrus expression:** Estrus was detected by an experienced animal attendant in the three groups for half an h in the morning and evening.

**Ovarian activity:** Ovarian activity was monitored in five randomly selected animals per group by plasma progesterone concentration (once a week sampling). Progesterone was measured in duplicate using solid phase $^{125}$I RIA (Immunotech, Beckman Coulter Company, France) at Nuclear Medicine, Oncology and Radiotherapy Institute, Islamabad. The analytical sensitivity was 0.05ng/ml. The intra and inter assay coefficient of variation (CV) were 10.1and 9.3% respectively. The precision (intra-assay CV, 10.1%) was calculated by comparing the results from repeated assays (6 times) of two samples differing in progesterone concentration. The reproducibility (inter-assay CV, 9.3%) was assessed by repeating three samples in every assay. An increase in baseline plasma progesterone concentration in at least two consecutive samples exceeding 1ng/mL was considered as criterion for resumption of ovarian activity (Barman *et al*., 2011).

**Conception rate:** Animals showing heat signs in BE and BP groups were allowed to be served by bull. In BN group, the animals showing heat signs were separated from the group and brought to a bull for natural service 12 h after observing first heat signs. Pregnancy was checked by palpation per rectum 60 days after breeding. Conception rates were determined through dividing number of animals conceived by total animals and multiplying by 100.

**Statistical analysis:** Estrus expression and conception rates were compared by Chi-square statistics. Data for "Interval (days) to service from the start of the experiment" were analyzed by analysis of variance and means were compared with Tukey's test. The $P<0.05$ was used to define statistical significance. Analysis was performed using the Minitab (version 16) statistical package.

## RESULTS

Estrus expression by buffaloes in three treatment groups, "interval to first service from start of experiment", and number of animals pregnant are shown in Table 1. Number of buffaloes that expressed estrus and were served over 60 days was significantly higher in BE and BP groups than that in BN group ($P<0.05$) while BE and BP groups did not differ significantly. Interval (days) to service from the start of the experiment was significantly less ($P<0.05$) in BE compared to BP and BN animals ($P<0.05$); the difference being non-significant between BP and BN groups. Number of buffaloes pregnant as a result of this service was also significantly higher in BE and BP groups than that in BN group ($P<0.05$); the difference being non-significant between BE and BP groups.

**Table 1:** Reproductive performance of anestrus buffaloes in bull exposed (BE), bull partially exposed (BP) and bull non exposed (BN) groups over 60 days of treatment period during low breeding season

| Parameter | BE (N=20) | BP (N=20) | BN (N=20) |
|---|---|---|---|
| No. of buffaloes that expressed heat and were served over 60 days of period (%) | 12 (60)[a] | 8 (40)[a] | 1 (5)[b] |
| Interval from start of experiment to service (days) (mean±SEM) | 26.3±4.9[a] | 37.0±3.7[b] | 40.0±0.4[b] |
| No. of pregnant buffaloes at day 60 after service (%) | 8 (40)[a] | 4 (20)[a] | 0 (0)[b] |

Values bearing different superscripts in a row differ significantly (P<0.05).

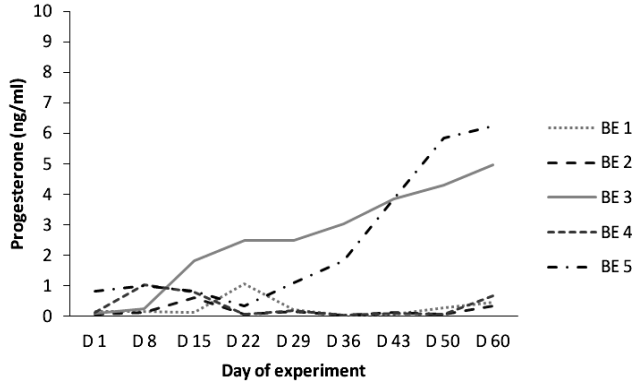


**Fig. 1:** Plasma progesterone concentration in 5 buffaloes of bull exposed (BE) group. Buffaloes BE1, BE2, BE4 had $P_4$ levels below 1 ng/mL, and were not served. Buffalo BE3 and BE5 depicted $P_4$ rise at day 15 and day 34.

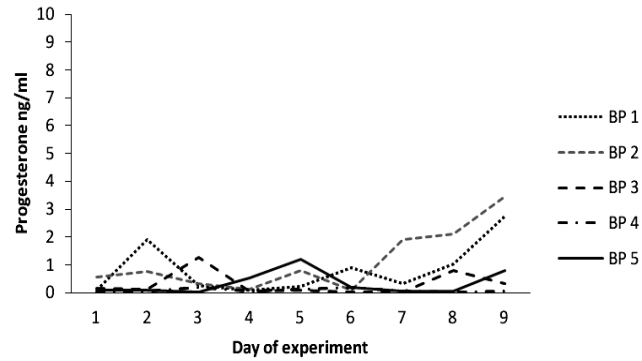


**Fig. 2:** Plasma progesterone concentration in 5 buffaloes of bull partial exposed (BP) group. Buffalo BP1, BP 2 depicted $P_4$ rise at day 43 and 50 of experiment while buffaloes BP3, BP4 and BP5 showed $P_4$ levels below 1 ng/mL over 60 days.

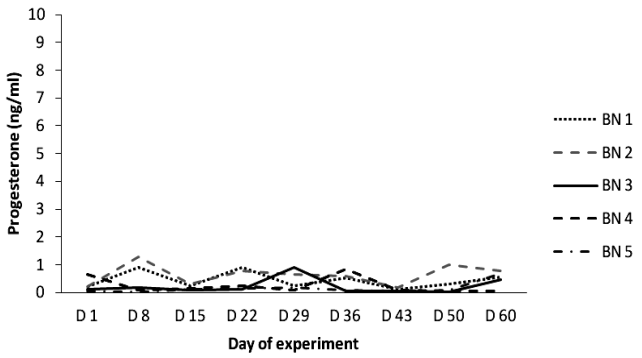


**Fig. 3:** Plasma progesterone concentration in 5 buffaloes of bull non exposed (BN) group. All the Buffaloes had $P_4$ level below 1ng/mL over 60 days.

Weekly plasma progesterone levels in buffaloes in BE, BP and BN groups are shown in Fig. 1, 2 and 3, respectively. Progesterone levels remained below 1ng/mL over 60 days treatment period in three out of five animals sampled in BE group, indicating non-functional ovaries. In two out of five animals sampled, progesterone rise was observed on day 15 and day 29 respectively and it did not decline in later samplings until day 60 indicating ovulation and presence of a CL. Both the animals showed heat, they were served and found pregnant as a result of this service indicating that the progesterone plateau was due to CL of pregnancy. Progesterone levels remained below 1ng/mL over 60 days treatment period in three out of five animals sampled in BP group, indicating non-functional ovaries. In two out of five animals sampled, progesterone rise was observed on day 43 and day 50 respectively. Both the animals showed heat, they were served and found pregnant. Progesterone levels remained below 1ng/mL over 60 days treatment period in all the five animals sampled in BN group, indicating non-functional ovaries. None of the five buffaloes of BN group showed heat over this 60 day period.

## DISCUSSION

The present work demonstrates positive effect of biostimulation (by bull exposure) on estrus expression, ovarian activity and conception rate in anestrus buffaloes during the low breeding season. Biostimulation by bull exposure represents one of the possible management tools to overcome this problem. In the present study, the anestrus buffaloes that were also passing through peak summer (June, July), when exposed to a bull 24 h a day, showed estrus expression in 60% cases followed by 40% estrus incidence in animals exposed to bull for 1 h per day and only 5% in non exposed group. Presence of males has also been found to enhance the estrus expression in Murrah buffaloes in winter (Gokuldas *et al*., 2010) and early postpartum period (Barman *et al*., 2011). A significantly lower number of days from start of experiment to standing heat in bull exposed group than bull partial exposed and non-exposed animals was an indication that a longer bull exposure per day might result in a stronger biostimulatory effect and an earlier ovarian stimulation in anestrus buffaloes even during peak summer. Similar observations have been reported in Murrah buffaloes during winter season (Gokuldas *et al*., 2010). It is known that resumption of ovarian cycling activity is characterized by the release of GnRH from the hypothalamus in low amplitude, high frequency temporal release pattern (Wright *et al*., 1992), resulting in release of low amplitude, high frequency pulses of LH that help in maturation of Graffian follicle and stimulates ovulation. Delayed resumption of ovulation in cattle, is invariably due to a GnRH-mediated lack of LH pulse frequency (Crowe *et al.*, 2014). Unlike small ruminants, aspects of biostimulation such as the behavioral and pheromonal signal and its translation into a hormonal response remain unclear in buffaloes. Further work is required to elucidate the biostimulatory effect on hormone secretion and mechanism of response to male effect in buffaloes.

The enhanced conception rate noted in the present study might not be directly linked with biostimulation but be just an outcome of higher estrus activity in the bull exposed groups. However, a recent report shows that bull exposure gave an improved conception rate in buffaloes synchronized for heat with an intra-vaginal drug release (CIDR) device during low breeding season (Kayani *et al.*, 2017).

Progesterone profile has been considered a valuable tool for determination of ovarian activity in buffaloes (El-Wishy, 2007, Ullah *et al*., 2010) like that in cattle. The progesterone profile of five buffaloes per group in the present study supported the visual observations in three groups. All the five buffaloes in non-exposed group showed a progesterone level below 1ng/mL during 60 days of sampling period, indicating non-functional ovaries and the depth of anestrus over summer. The profile in partial exposed group showed a revival of ovarian function in two out of five (40%) animals after >40 days of bull exposure for 1 h daily. Both the parameters matched with the group values of visually monitored estrus activity and interval to heat from start of experiment.

**Conclusions:** Biostimulation by bull exposure for 24 h/day accelerated the resumption of postpartum ovarian activity, with a significantly higher estrus expression.

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**Authors contribution:** NSZ and MA conceived and designed the project. NSZ executed the experiment and performed progesterone assay. Both authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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