

**Short Communication**

**CONCEPTION RATE AFTER ESTRUS SYNCHRONIZATION AND BIOSTIMULATION DURING LOW BREEDING SEASON IN BUFFALOES**

M. R. Kayani, M. Anwar\*, S. M. H. Andrabi and A. Ghaffar

PARC Institute of Advanced Studies in Agriculture, NARC, Park Road, Islamabad

\* Corresponding Author email: manwar\_94@yahoo.com

**ABSTRACT**

Estrus synchronization and timed artificial insemination can be used to carry AI to small farmers. Present study compared two estrus synchronization protocols during low breeding season and recorded estrus incidence and conception rate after timed insemination in buffaloes that were exposed to a bull (BE, biostimulated) round the clock or not exposed to a bull (BN, not biostimulated). Fifty non pregnant, healthy Nili Ravi buffaloes received controlled intravaginal drug release (CIDR) cattle inserts (containing 1.38 gm progesterone) for 7 days for estrus synchronization. Animals also received GnRH or No GnRH injection on day of CIDR insertion. Inseminations were made with frozen thawed buffalo semen at 58 hour after CIDR removal. Heat incidence did not differ among the treatment groups ( $P>0.05$ ). Conception rate was higher in GnRH + BE group (87.5%) than that in GnRH + BN (37.5%) and No GnRH + BN (0.0%) groups ( $P<0.05$ ). Conception rate did not differ between GnRH + BE (87.5%) and No GnRH + BE (60.0%) groups ( $P>0.05$ ). It was concluded that bull exposure may be used to attain an improved conception rate in buffaloes synchronized for heat with CIDR during low breeding season.

**Key words:** Estrus synchronization, biostimulation, artificial insemination, buffaloes, low breeding season.

**INTRODUCTION**

Artificial insemination (AI) is the method of choice for farmers to improve genetic quality of their dairy cattle. The advantages of AI have led to the replacement of natural service throughout the commercial dairy populations of the world, and in some countries like Denmark and Netherlands coverage of AI is 100% (Cunningham, 1999). However in Pakistan, out of 15.56 and 15.16 million breed able buffaloes and cows, respectively, AI is practiced only in 7.1% and 11.1 % animals, respectively (Anonymous, 2006) due to various reasons like non availability of facility in remote areas, small size of livestock holdings and lack of expertise. Bringing a large number of animals in estrus at the same time (estrus synchronization) and timed insemination can be used to spread the technology in such situations. In addition it has been recorded that buffaloes have a seasonal breeding behavior; they are polyestrous and are sexually less active in the summer (Ahmad *et al.*, 1980). Estrus induction with hormones may also be used to overcome seasonality of breeding in buffaloes. Prostaglandins in combination with prostaglandin F<sub>2</sub> have been used for estrus synchronization in buffaloes with conception rate varying from 0% to 80% during low breeding season (Singh, 2003; Naseer *et al.*, 2011; Naseer *et al.*, 2013). It has been observed that the presence of male has stimulatory effect on estrus and ovulation induction through pheromones and other external cues (biostimulation) (Berardinelli *et al.*, 2007). Conception rate after estrus synchronization protocol in cows was

improved by biostimulatory effect of bull (Berardinelli *et al.*, 2007). Studies on use of biostimulation alone or in combination with estrus synchronization in buffalo are meager. So the present study compared two estrus synchronization protocols with or without biostimulation and recorded estrus incidence and conception rate after timed insemination in buffaloes during low breeding season (summer).

**MATERIALS AND METHODS**

**Animals and their management:** Fifty healthy and non pregnant buffaloes in good body condition were used in the study. These buffaloes were owned by 16 farmers around Dadyal, District Mirpur (Azad Jammun and Kashmir). Average ( $\pm$  SD) livestock holding with these farmers was  $10.06 \pm 10.55$  buffaloes (range 2-40) and  $2.38 \pm 1.20$  cows (range, 0-4). The buffaloes included in the study were in their 1<sup>st</sup> to 5<sup>th</sup> lactation, and had last calved  $7.62 \pm 3.85$  months ago (range, 3-18 months). Animals were maintained on green fodder (Oats and Wheat plants ad lib.), wheat straw (4-6 kg / animal / day), and concentrate (4-5 kg / day). Some of the farmers (4/16, 25%) also offered Barseem along with Oats and wheat plants. In addition grazing on natural grasses was practiced by 75% of the farmers. Animals had free access to rock-salt. Water was offered twice a day (8 am and 5 pm). Animals were washed and the yards were cleaned twice a day. Animals were kept in open yards during day time and in semi open sheds at night hours. Animals were vaccinated against Hemorrhagic septicemia, Foot and

Mouth Disease, and Black Quarter Disease twice a year. De-worming was practiced once a year.

**Estrus synchronization Treatment:** Each buffaloes received an intravaginal “Controlled internal drug release device” (CIDR<sup>®</sup>, Pfizer New Zealand Ltd, Auckland) containing 1.38 gram progesterone. On the day of CIDR insertion (Day 0), the animals were assigned into one of two experimental groups i.e. animals receiving or not receiving a GnRH analogue (Lecirelin, 50 µg, Dalmarelin, Fatro, Italy). Buffaloes either received biostimulation by exposure to bull (BE) round the clock or were not exposed to bull (BN) since more than a year. Distribution of animals in to these four groups is shown in Table 1.

All the animals received Prostaglandin F2 analogue (Cloprostenol 150 µg, Dalmazin, Fatro, Italy) on day 6. CIDR was removed on day 7, exactly 24 hours after cloprostenol administration. Heat detection started 12 hours after CIDR removal and observations were recorded at 12 hour interval. Timed insemination (TAI) was done 58 hours after CIDR removal using frozen thawed semen from a bull of proven fertility. All the inseminations were made by the same technician. Animals were carefully watched for heat / repeat breeding from day 18 – 25 after TAI. Pregnancy test was performed 45 days after TAI by palpation per rectum.

Heat signs recorded were mucus discharge, swelling of vulva, bellowing, hyperemic vestibule, Flehman response by bull, stand to be mounted by bull (last two signs recorded only in buffaloes exposed to bull).

**Statistical Analysis:** Estrus incidence and conception rate was compared among four groups of animals using Chi Square test.

## RESULTS AND DISCUSSION

**Estrus incidence and synchrony:** A total of 48 (96%) buffaloes were observed in heat until 48 hours after CIDR removal. Heat incidence was similar among four treatment groups ( $P > 0.05$ ; Table 2). Standing heat was observed in 73.9% animals (17/23) in bull exposed animals at 48 hours after CIDR removal (i.e. 72 hours after PGF2a injection). A high incidence of heat (96%) as well as synchrony of heat observed after synchronization treatment shows that CIDR was efficient in manipulating hypothalamo-ovarian activity during low breeding season in buffalo irrespective of biostimulation or GnRH. However it is worth mentioning that animals used in the study received good quality ad lib feed and were in good body condition. Also the animals were washed twice in a day to keep them cool. Similarly, Singh *et al.* (2003) reported that 83% buffaloes were in heat by using CIDR

in combination with prostaglandin. However, in this study, CIDR was placed intra vaginally either for 10 or 12 or 14 days, and prostaglandin was injected either on day 8 or 10 or 12 post placement of CIDR. Our findings are also in line with Zaabel *et al.* (2009) who reported that 100% anestrus Egyptian buffaloes resumed cyclicity after treatment with CIDR. However, they used GnRH along with CIDR for estrus synchronization. Naseer *et al.* (2011, 2013) reported an estrus response (93 and 85%, respectively) in buffaloes with CIDR based protocol during low breeding season, and these findings are comparable with that of present study. A lower heat incidence (50%) was found by Mehmood *et al.* (2012) in summer anestrus buffaloes by using CIDR for 7 days and an injection of prostaglandin on day 6 post CIDR placement. However the number of animals was too low in this study to draw a solid conclusion.

**Conception rate:** Conception rate in four groups of buffaloes has been presented in Table 2. Highest conception rate was observed in bull exposed groups whether receiving or not receiving GnRH (87.5 and 60.0% respectively). Conception rate between these two groups did not differ significantly ( $P > 0.05$ ). Conception rate was significantly higher in the BE group receiving GnRH than that in BN groups receiving or not receiving GnRH ( $P < 0.05$ ). So there is indication that presence of bull (or biostimulation) can improve conception rate in buffaloes treated with CIDR during low breeding season. Biostimulation is thought to increase the success of estrus synchronization protocols by inducing an ovulatory response in non-cycling females (Patterson *et al.*, 2010). Similar observation has been reported in cows by Berardinelli *et al.* (2007) who observed that conception rate after estrus synchronization protocol in cows was improved (57.6%) by biostimulatory effect of bull than for non biostimulated cows (35.6%). So the possibility exists that biostimulation may not only affect resumption of ovulatory activity in postpartum cows, but may also directly influence the ovary or physiology of reproductive tract (Berardinelli *et al.*, 2007). Roelofs *et al.* (2007) observed that exposing dairy cows in early postpartum to a bull for 8 h significantly increased basal and average LH concentration and pulse frequency. It is concluded that use of CIDR along with biostimulation may improve conception rate in Nili Ravi buffaloes after timed artificial insemination, however there is a need to further investigate mechanism of improved pregnancy rate after biostimulation both in cows and buffaloes.

It was concluded that bull exposure may be used to attain an improved conception rate in buffaloes synchronized for heat with CIDR during low breeding season.

**Table 1. Distribution of buffaloes in different treatment groups.**

Treatment on day 0 in addition to CIDR insert	Buffaloes Exposed to bull (BE) (No.)	Buffaloes Not exposed to bull (BN) (No.)	Total
GnRH	8	16	24
No GnRH	15	11	26
Total	23	27	50

**Table 2. Conception rate in buffaloes after estrus synchronization and biostimulation during low breeding season.**

Treatment	Estrus incidence		Buffaloes pregnant	
	No. in heat / Total	(%)	No. pregnant / Total	(%)
BE and GnRH	7/8	87.5 <sup>a</sup>	7/8	87.5 <sup>a</sup>
BE and No GnRH	14/15	93.3 <sup>a</sup>	9/15	60.0 <sup>ab</sup>
BN and GnRH	16/16	100 <sup>a</sup>	6/16	37.5 <sup>b</sup>
BN and No GnRH	11/11	100 <sup>a</sup>	0/11	0.0 <sup>c</sup>

Values with different superscripts in a column differ significantly ( $P < 0.05$ ).

**Acknowledgements** This research work was funded by Pakistan Agriculture Research Council's ALP project entitled "Improving reproductive efficiency of Sahiwal cows and Nili-Ravi buffaloes through estrus synchronization and timed artificial insemination at NARC (AS125)". Help of Mr. Muhammad Iqbal Khan in the field work is gratefully acknowledged.

## REFERENCES

- Ahmad, N., R.A. Chaudhry and B.B. Khan (1980). Effect of month and season of calving on the length of subsequent calving interval in Nili-Ravi buffaloes. *Anim. Reprod. Sci.* 3: 301–306.
- Anonymous (2006). Pakistan Livestock Census, Statistics Division, Govt. Pakistan., Islamabad.
- Berardinelli J.G., P.S. Joshi and S.A. Tauck (2007). Conception rates to artificial insemination in primiparous, suckled cows exposed to the biostimulatory effect of bulls before and during a gonadotropin-releasing hormone-based estrus synchronization protocol. *J. Anim. Sci.* 85: 848–852.
- Cunningham, E. P. (1999). The application of biotechnologies to enhance animal production in different farming systems. *Livest. Prod. Sci.* 58: 1–24.
- Naseer, Z., E. Ahmad, J. Singh and N. Ahmad (2011). Fertility following CIDR based synchronization regimens in anoestrous Nili-Ravi buffaloes. *Reprod. Dom. Anim.* 46: 814–817.
- Naseer, Z., E. Ahmad, N. Ullah, M. Yaqoob and Z. Akbar (2013). Treatment of anestrous Nili-Ravi buffaloes using eCG and CIDR protocols. *Asian. Pac. J. Reprod.* 2: 215–217.
- Mehmood M.U., S. Mehmood, A. Riaz, N. Ahmad and A. Sattar (2012). Superovulatory response in summer anestrous buffaloes and cattle treated with estrus synchronization protocol. *The J. Anim. Plant Sci.* 22: 888–893.
- Patterson, D.J., D.A. Mallory, J.M. Nash, and M.F. Smith (2010). Estrus synchronization protocols for heifers. In: *Proc. 10th Applied Reproductive Strategies in Beef Cattle.* 28–29, San Antonio, Texas, USA: 74–111.
- Roelofs, J.B., N.M. Soede, S.J. Dieleman, W. Voskamp-Harkema and B. Kemp (2007). The acute effect of bull presence on plasma profiles of luteinizing hormone in postpartum, anoestrous dairy cows. *Theriogenology* 68: 902–907.
- Singh, C. (2003). Response of anestrous rural buffaloes (*Bubalus bubalis*) to intravaginal progesterone implant and PGF2 injection in summer. *J. Vet. Sci.* 4: 137–141.
- Zaabel, S.M. A.O. Hegab, A.E. Montasser and H. El-Sheikh (2009). Reproductive performance of anestrous buffaloes treated with CIDR. *Anim. Rep.* 6: 460–464.