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**Research Article**

# IMPACT OF CIDR IN COMBINATION WITH DIFFERENT HORMONES FOR TREATMENT OF ANESTRUS IN BUFFALOES UNDER FIELD CONDITIONS IN HARYANA

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## ABSTRACT

The aim of the study was to test the efficacy of Controlled Internal Drug Release (CIDR) inserts in combination with different hormones for treatment of anestrus in buffaloes. Impact of the hormonal preparations used by the field veterinarians to treat a total of 729 buffaloes with history of anestrus and infertility belonging to different agro-climatic zones of Haryana was analyzed. The data of treated buffaloes so obtained was divided into six major groups depending upon the treatment protocol used viz., CIDR alone, CIDR-PG, CIDR-PMSG (eCG), CIDR-GnRH, CIDR-GnRH-PG and CIDR-Ovsynch. The overall estrus induction rates of animals treated with different protocols ranged from 66.67 to 98.20%. The animals that expressed estrus, were inseminated with frozen semen and 64.36 to 81.26% animals conceived at 1st service in different treatment groups and upto 7.14% at 2nd service Among all the six combinations, the use of GnRH and $PGF_{2á}$ in combination with progesterone based CIDR showed comparatively better results in terms of estrus induction and pregnancy rates in buffaloes under field conditions.

**Key words**: Anestrus, buffalo, CIDR, heat induction, conception rate

Inspite of their less population, buffalo contributes more than 55% of total milk production. Among buffaloes, Murrah is considered as one of the best milk producer. Haryana is the home tract of Murrah buffalo and holds a special place in milk production. Approximately 80% of total milk in the state comes from the buffaloes alone. Buffalo milk is preferred over the cow milk due to its 100% more fat, high protein and low level of cholesterol. Despite of these merits, buffalo are blamed for slow reproduction, long calving interval, delayed puberty, poor estrus expression and seasonality in breeding and calving (Singh *et al.*, 2000). This low reproductive efficiency is mainly attributed to lower circulating concentration of hypophyseal and gonadal hormones (Madan *et al.*, 1983) and suboptimal functioning of hypothalamo-hypophyseal and gonadal axis (Rao and Shreemannarayan, 1982).

Anestrus is the state of ovarian acylicity, reflected by complete sexual inactivity with manifestation of estrus with the absence of palpable follicular or luteal structures. Various research workers have obtained satisfactory results by the use of different hormonal preparations to stimulate the HPS axis and initiate ovulation and resumption of normal cyclicity of anestrus in buffaloes (Singh and Singh, 1986; Aminudeen, 1991). A variety of progestational compounds have been administered (Malik, 2005) to mimic the luteal function by blocking the release of gonadotrophins from pituitary, so that subsequent withdrawal of these compounds may result in release of gonadotrophins to initiate follicular activity in ovaries with establishment of estrous cycles. Therefore, the present study was planned to compare the efficacy of CIDR in combination with different hormones for induction of estrus in buffaloes under field conditions.

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## MATERIALS AND METHODS

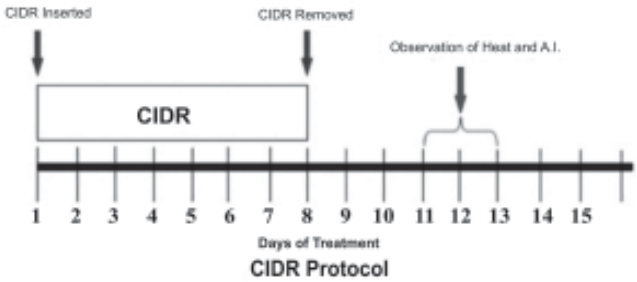
The present study was conducted in Haryana state throughout the year. Ten districts of Haryana state namely Hisar, Sirsa, Fatehabad, Kaithal, Karnal, Panipat, Sonipat, Jind, Rohtak and Bhiwani known for higher buffalo population (Haryana Livestock Census, 2007) were selected for the study. The hormones were distributed by the Haryana Livestock Development Board to the field veterinarians for treatment of reproductive problems so as to enhance the reproductive response of dairy animals. The hormones included Controlled Internal Drug Release (Eazi-breed CIDR® containing 1.38g progesterone, Pfizer Animal Health, Hamilton, New Zealand), inj. Clostenol® (containing 250µg PG, Vetcare, Pune, India), inj. Folligon® (containing

500 I.U. PMSG, Intervet International, The Netherlands), inj. Receptal® VET (containing 0.004mg/ml Buserelin (GnRH analogue), Intervet International, Germany), inj. Folligon® (containing 1000IU serum gonadotropin, Intervet International, The Netherlands) and inj. Chorulon® (containing 1500I.U. hCG, Intervet International, The Netherlands).

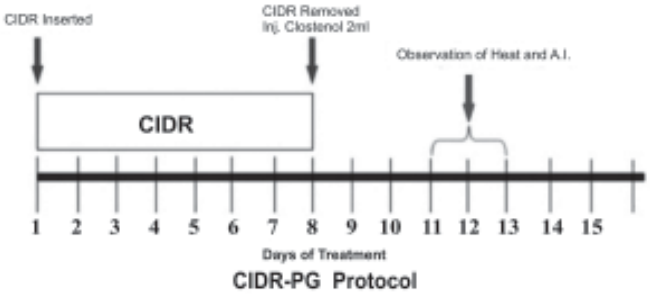
The study constituted survey of 3-5 villages of the concerned districts in the state of Haryana to obtain the information related to different hormonal protocols used for induction of estrus and fertility. All the anestrus animals in the present study were confirmed for their reproductive status by rectal examination of ovaries on two occasions at eleven days interval to find out presence or absence of corpus luteum (CL) or any other structure. Prior to the hormonal treatment, mineral-mixture was given and deworming was performed and then treated with different hormonal preparations. At least, 10% of total hormonal preparations used by field veterinarians for management of reproductive conditions in buffaloes in these ten districts were selected randomly.

A total of 729 buffaloes with history of anestrus and infertility belonging to different agro-climatic zones of Haryana were subjected to various hormonal treatment protocols by field veterinary doctors. In order to quantify the effect of different hormonal protocols used, data collected was classified into following six groups based on combination of hormones used:

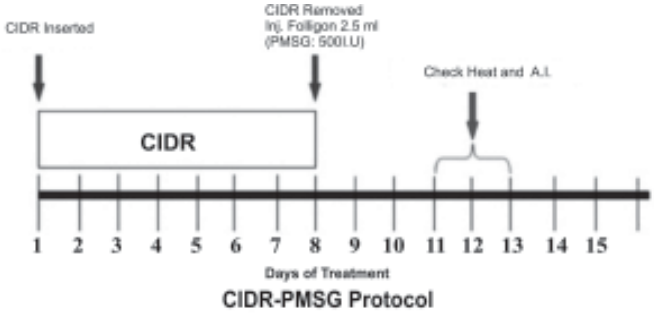
**CIDR Alone Protocol:** CIDR device was inserted into the vagina of anestrus animals on day 1 of treatment and removed on day 8. Animals were observed for estrus for 2-6 days post insertion and were inseminated on the observed heat.



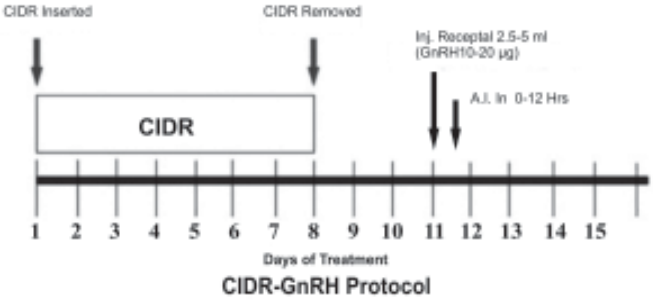

**CIDR-PG Protocol:** CIDR device was inserted into the vagina of animal on day 1 of the treatment and removed on day 8. An injection Clostenol® 2ml (PG) was given intramuscularly (I/m) at day 8 i.e. at the time of removal of CIDR. Heat was observed for 2-6 days and artificial insemination was performed at 12 h after detection of the estrus.



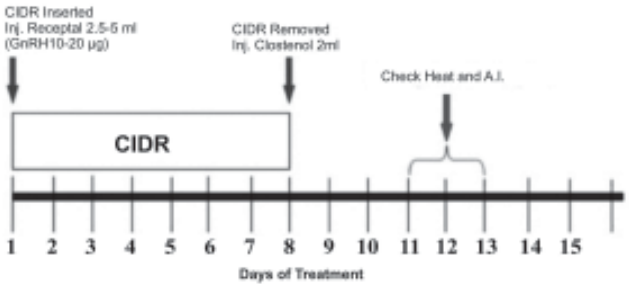

**CIDR- PMSG (eCG) Protocol:** CIDR device was inserted into the vagina of animal on day 1 and removed at day 8. A freshly reconstituted Folligon® 2.5 ml (PMSG) was administered I/m on day 8 i.e. at the time of CIDR removal. Animals were artificially inseminated at the detected oestrus.




**CIDR- GnRH Protocol:** CIDR device was inserted on day 1 of treatment and was removed on day 8. Heat was observed for 2-6 days post-insertion and AI was done 12 h after observing heat. Inj. Receptal 2.5 ml (GnRH) was injected I/m at the time of AI.

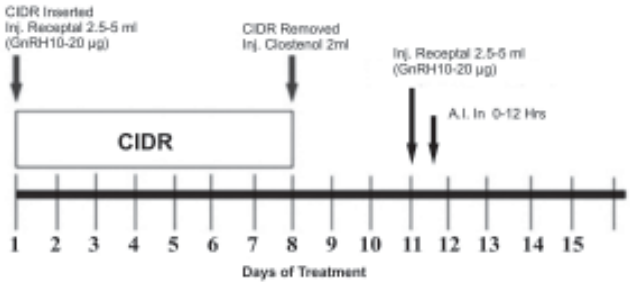



**CIDR- GnRH – PG Protocol:** On the first day of treatment, CIDR was inserted and inj. Receptal® 2.5 ml (GnRH) was injected I/m. On day 8, the CIDR was removed and inj. Clostenol® 2 ml (PG) was administered I/m. The animal was inseminated 12 h after heat observation.




CIDR - GnRH-PG Protocol

**CIDR- GnRH - PG- GnRH / CIDR-GnRH FT AI / CIDR Ovsynch Protocol:** On day 1, the CIDR was inserted intravaginally and Inj. Receptal® 2.5 ml (GnRH) was administered I/m. On day 8, CIDR was removed and inj. Clostenol® 2 ml (PG) was given I/m. Inj. Receptal® 2.5 ml (GnRH) was administered I/m on day 11 and AI by clock was followed between 0 and 12 h after GnRH injection.




CIDR-GnRH Fixed Time AI/CIDR-Ovsynch Protocol

To evaluate the response of different hormonal treatment protocols, the parameters used were: Estrus induction rate, Conception rate/Pregnancy rate at 1st service (induced heat), Conception rate/Pregnancy rate at 2nd service (Spontaneous heat) and Overall pregnancy rate. Comparative study of all the treatment regimens was carried out for comparing their effectiveness and efficacy for estrus induction and successful conception. The data obtained in the study was statistically analyzed to draw the scientific inferences. Duncan's multiple range test was employed for making all possible pair wise comparison.

## RESULTS AND DISCUSSION

Of the 729 buffaloes subjected to various hormonal treatment protocols, 682 animals (93.55%) showed estrus response. The animals conceived at first estrus and subsequent estrus were 474 (65.02%) and 27 (3.70%), respectively (Table 1). On comparing the data, it was observed that all the treatment protocols showed good response in terms of induction of estrus in anestrus buffaloes. Maximum estrus induction rates were observed in CIDR-GnRH group (98.2%), followed by CIDR-PG (97.36%), CIDR-GnRH-PG (96.87%), CIDR-Ovsynch (95.74%), CIDR alone (94.25%) and CIDR-PMSG (66.67%) groups, respectively. Among these groups, CIDR-PMSG group animals showed minimum response for the induction of estrus and differed significantly ($P \leq 0.05$) from other groups.

Mean conception rate at 1st AI during estrus was 81.26% in animals treated with CIDR-GnRH protocol. The number of animals conceived with other treatment protocols varied from 64.36% to 74.19%. The conception rate at 1st AI in CIDR alone and CIDR-PG group were 64.36% and 64.86%, respectively, which were lower as compared to other groups. Conception rate at 2nd estrus was observed to be higher, although statistically non-significant in CIDR-GnRH-PG group (7.14%) as compared to other groups. The overall pregnancy rate was the highest in CIDR-GnRH-PG group (81.33%) followed by CIDR-GnRH group (81.26%).

CIDR alone came to the forefront throughout the world for estrus synchronization, increased pregnancy rates and the treatment of postpartum anestrus in cattle (Macmillan and Peterson, 1993) and in buffaloes (Andurkar *et al*., 1997; Singh, 2003a; Malik, 2005). In the present study, estrus induction was found to be 94.25% with the use of CIDR alone protocol. These results were higher than those reported by Singh (2003b) and Nayak *et al*. (2009). As after CIDR removal, the exhibition of ovulatory estrus in the responded buffaloes was observed which suggested that the increased circulatory concentration of progesterone had sensitized the hypothalamic-pituitary system (Singh 2003a, b). Following termination of progesterone therapy (after CIDR removal) the rapid drop in circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone was abolished, followed by FSH and LH release with subsequent resumption of ovarian cyclicity (Zerbe *et al*., 1999). Likewise, progesterone increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of estrus (Fabre-Nys and Martin, 1991). In present study the pregnancy

**Table 1**
**Comparison of different hormonal treatment protocols for induction of estrus and fertility in buffaloes**

| Hormonal protocol | No. of animals treated | Estrus interval (days) | Animals induced to estrus n (% Mean±SE)* | Conception at induced estrus n (% Mean±SE) | Conception at 2nd estrus n (% Mean±SE) | Overall pregnancy n (% Mean±SE) |
|---|---|---|---|---|---|---|
| CIDR alone | 266 | 3-5 | 246 (94.25±4.33[a]) | 162 (64.36±6.54[a]) | 14 (4.86±4.14[a]) | 176 (69.22±5.84[a]) |
| CIDR-PG | 157 | 3-5 | 152 (97.36±1.26[a]) | 99 (64.86±5.19[a]) | 10 (4.03±3.54[a]) | 109 (68.89±3.89[a]) |
| CIDR-PMSG | 7 | 3-5 | 6 (66.67±33.33[b]) | 6 (66.67±33.33[a]) | 0 (0.00±0.00[a]) | 6 (66.67±33.33[a]) |
| CIDR-GnRH | 58 | 3-5 | 54 (98.20±1.80[a]) | 42 (81.26±8.46[a]) | 0 (0.00±0.00[a]) | 42 (81.26±8.46[a]) |
| CIDR-GnRH-PG | 111 | 3-5 | 100 (96.87±3.12[a]) | 78 (74.19±9.13[a]) | 2 (7.14±7.14[a]) | 80 (81.33±11.0[a]) |
| CIDR-Ovsynch | 130 | 0-1 | 124 (95.74±2.87[a]) | 87 (71.65±7.95[a]) | 1 (2.78±2.78[a]) | 88 (74.43±9.16[a]) |

*Means in a column with different superscripts are significantly different ($P<0.05$).

rates in CIDR treated buffaloes were found to be high (69.22%). Similar findings have been reported earlier (Baruselli *et al.,* 2004; Naseer *et al.,* 2011).

In CIDR-PG protocol, reasonably good results in heat induction (97.36%) were recorded. CIDR+PG combination with $PGF_{2\alpha}$ was more effective than CIDR alone in terms of exhibition of estrus and conception rate. This can be explained by the fact that $PGF_{2\alpha}$ increases pituitary responsiveness to GnRH in the postpartum cow. Hence, the released GnRH after CIDR removal effectively stimulates the pituitary gonadotropins with subsequent estrus induction in anestrus buffaloes. Singh (2003a, b) did experiments by taking buffaloes with smooth ovaries and after administration of CIDR insertion for 8 day and 14 day continuously, got 33% and 83% heat induction results, respectively. In our study, we did not distinguish animals on the basis of their ovary structure that may be the cause for better response in heat induction. In this study a conception rate of 68.89% was observed, which is in agreement with previous study (Zaabel *et al.,* 2009) who also got 43% and 85% conception rates with administration of CIDR for 7 and 14 days, respectively.

In the present study, 66.67% animals were induced to estrus by CIDR-PMSG protocol. The results of the present investigation on induction of estrus by using CIDR and followed by PMSG are in close agreement with those reported by De-Rensis *et al.* (2005) and Dodamani *et al.* (2011). However, Andurkar and Kadu (1997) and Bahga *et al.* (1997) reported an estrus response between 23 to 83%. Removal of a CIDR results in a rapid decline in systemic progesterone concentration over a 12-24 h interval in intact cows (Perry *et al.,* 2004) which allows for the occurrence of estrus quite rapidly after CIDR removal. The eCG (PMSG) along with Crestar may be useful as eCG leads to induction of behavioral estrus signs (Singh *et al.,* 2004). Beneficial effect of eCG supplementation at the time of implant removal has also been reported by Rao and Rao (1979) as it enhances the ovarian stimulation that may result in earlier onset and tighter synchrony of estrus. The pregnancy rates in present investigation were 66.67%. These results are in agreement with the earlier reports (Honparkhe *et al.,* 2008; Murugavel *et al.,* 2009; Azawi *et al.,* 2012) who also reported 50%, 40% and 75% pregnancy rates, respectively with a CIDR regimen alongwith 1000 I.U. eCG on device removal. This confirms the hypothesis that some amount of progesterone is necessary to sensitize the hypothalamic-hypophyseal system as it plays a negative role on its withdrawal by day 7 or 9 as the case may be. The results of our study on induction of cyclicity by this treatment resulted in 66.6% estrus response which is higher than Younis *et al. (*1996) who reported 45.5% induction of cyclicity in buffaloes.

In CIDR-GnRH protocol treatment estrus induction rate was found around 98.20%. The role of GnRH administration at the time of AI was to increase the pregnancy rate through hastening ovulation of the preovulatory follicle. A conception rate of 81.26% was observed with CIDR-GnRH protocol in the present study. Previously a lot of work has been done on this aspect but results in the present study are better than others (Azawi *et al.,* 2012) as these authors used same protocol in summer season and got 20% and 66.5% conception rates, respectively. The differences in conception rate could be due to the reason that more number of animals were treated during their normal breeding season (autumn-winter) in the present study.

In the animals treated with CIDR-GnRH-PG combination, estrus induction rate of 96.87% and pregnancy rate of 81.33% was observed. The results indicated that administration of GnRH after removal of CIDR showed tighter synchrony in estrus response and tended to increase the pregnancy rate in anestrus buffaloes. The present investigation has also shown that the use of CIDR together with GnRH and $PGF_{2á}$ treatment was able to induce fertile estrus in non-cycling postpartum buffaloes. The results of this study are in agreement with the results obtained in buffaloes (Azawi *et al.*, 2012).

The estrus induction rates with CIDR-Ovsynch protocol were 95.74% in the present study. These results are in agreement with Baruselli *et al.* (2007) who showed that 100% estrus induction rates may be achieved in breeding season by combining CIDR with Ovsynch protocol in anestrus buffaloes. The conception rate in CIDR-Ovsynch group was 74.43% when Ovsynch protocol was supplemented with progesterone. Baruselli *et al.* (2007) and Azawi *et al.* (2012) observed 57.5 and 32% overall pregnancy rates, respectively. A very low conception rate of 4.7% has also been reported by De-Rensis *et al.* (2005) after synchronized ovulation with Ovsynch in non-cyclic buffaloes but conception rates were significantly increased to 30% when CIDR was also combined with Ovsynch treatment. Ravikumar *et al.* (2007) also reported that inclusion of a CIDR device with Ovsynch protocol might be an ideal strategy for dealing with postpartum anestrus buffaloes.

On comparing the different hormonal treatments, it can be concluded that the use of GnRH and $PGF_{2á}$ in combination with progesterone based CIDR protocol subsequently may improve the estrus induction and conception rates in buffaloes under field conditions.

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