

EFFECT OF GONADOTROPIN RELEASING HORMONE (GnRH) PREPARATIONS  
ON INDUCTION OF ESTRUS AND FERTILITY IN BUFFALOES  
UNDER FIELD CONDITIONS IN HARYANA

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**ABSTRACT**

The study was carried out to test the efficacy of Gonadotrophin Releasing Hormone (GnRH) protocols for induction of estrus and fertility in buffaloes. Impact of the GnRH preparations used by the field veterinarians to treat a total of 499 buffaloes with history of anestrus and infertility belonging to different agro-climatic zones of Haryana was analyzed during the period of study. The data of treated buffaloes so obtained was divided into two major groups depending upon the treatment protocol used, viz., GnRH group (n=300) and Controlled Internal Drug Release (CIDR) + GnRH group (n=399). GnRH group animals were subdivided into GnRH alone, GnRH-PG (Prostaglandin F<sub>2α</sub>) and GnRH-PG-GnRH (Ovsynch) protocols. CIDR + GnRH treated animals were categorized into CIDR + GnRH, CIDR + GnRH-PG and CIDR + GnRH-PG-GnRH (Ovsynch) protocols. The overall estrus induction rate was recorded to be 100% in animals treated with Ovsynch, followed by CIDR + GnRH (98.20±1.80%), then CIDR + GnRH-PG (96.87±3.12%) and CIDR + GnRH-PG-GnRH protocols. However, the overall conception rate was observed to be significantly higher in animals of CIDR + GnRH group. It can be concluded that GnRH in combination with Progesterone based CIDR protocol subsequently

improves the estrus induction and pregnancy rates in buffaloes under field conditions.

**Keywords:** anestrus, buffalo, conception rate, estrus, gonadotrophin releasing hormone, GnRH

**INTRODUCTION**

Buffalo is very sensitive to environmental temperature and radiation due to black thick skin and very few sweat glands. Although buffalo is polyestrous animal, however there is distinct seasonal variation in display of estrus, conception rate and calving interval (Singh *et al.*, 2000). During summer, there is reduction in feed intake along with alteration of the profile of reproductive hormones. Lower circulating concentrations of FSH (Razdan *et al.*, 1982), LH (Rao and Pandey, 1982) and progesterone have been detected during summer along with higher prolactin levels (Kaker *et al.*, 1982). This results in weak estrus symptoms during summer months. Also longer inter-calving interval in buffalo due to prolonged postpartum anestrus (Barile, 2005), 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).

Early re-establishment of cyclic ovarian activity after calving is essential because more the estrus cycles a female has before 30 days postpartum, the fewer services per conception are required (Metwelly, 2001). The productive life of a buffalo can be maximized if it is bred within 100-150 days after parturition to produce a calf and start a new lactation every year (Abdalla, 2003). The treatments given in the first month postpartum in order to initiate normal estrus cycles, also improve reproductive performance (Zain *et al.*, 2001). Thus many managemental strategies and hormonal regimens have been administered to stimulate ovulation and resumption of normal cyclicity of anestrus in buffalo during peak breeding and low breeding periods (Singh and Singh, 1986; Aminudeen, 1991; Malik, 2005). These hormones act directly on the reproductive organs or indirectly on the pituitary gland to stimulate the release of naturally occurring hormones, which in turn act on the reproductive organs.

In view of above, the present study was planned in order to test the efficacy of gonadotropin hormone (GnRH) in combination with other hormones for induction of estrus and fertility in anestrus buffaloes.

## MATERIALS AND METHODS

The basic purpose of the study was to evaluate the efficacy of the hormonal preparations being used for treatment of anestrus, induction of estrus and fertility in buffaloes under field conditions. The study constituted the survey of different villages of the selected ten districts of Haryana state (namely Hisar, Sirsa, Fatehabad, Kaithal, Karnal, Panipat, Sonipat, Jind, Rohtak and Bhiwani) known for higher buffalo population

(Livestock Census, 2007) to obtain the information related to different hormonal protocols used for induction of estrus and fertility in buffaloes. At least a ten per cent of total hormonal preparations used by field veterinarians were selected randomly and impact analysis of different hormonal preparations was done.

The hormones were supplied in the field by the Department of Animal Husbandry and Dairying, Haryana. In order to quantify the effect of different hormonal protocols used, data collected was broadly classified into Group I (n=300) and Group II (n=199) with three subgroups in each, based on combination of hormones used.

### Group I: GnRH Group

#### 1. GnRH Alone (n=100)

Injection Receptal® 2.5 ml was administered intramuscularly (I/m) and animals were then observed for heat. This was used in animals with follicular cyst on the ovaries.

#### 2. GnRH- PG Protocol (n=153)

The first injection of Receptal® 2.5ml was administered I/m on day 1 of treatment. Injection Clostenol® 2 ml was administered I/m 7 days after the Receptal® injection. Animals were observed for estrus and inseminated 12 h after onset of estrus.

#### 3. GnRH- PG- GnRH / Ovsynch Fixed Time Artificial Insemination Protocol (Ovsynch FT AI) (n=47)

The first injection of Receptal® 2.5 ml I/m was administered on the first day of treatment followed by injection Clostenol® 2 ml I/m after 7 days. A second injection of Receptal® 2.5 ml was administered I/m 48 h after Clostenol® injection. All the animals were inseminated either at the time of second injection of Receptal® (0 h) or 12 h later.

## Group II: CIDR + GnRH Group

### 1. CIDR- GnRH protocol (n=58)

CIDR device was inserted on the first day of treatment and was removed on day 7. Heat was observed for 2-6 days and insemination was done 12 hours after observing heat. Injection Receptal® 2.5 ml was injected I/m at the time of AI.

### 2. CIDR- GnRH – PG protocol (n=111)

On the first day of treatment, CIDR was inserted and injection Receptal® 2.5 ml was injected I/m. On day 7, the CIDR was removed and injection Clostenol® 2 ml was administered I/m. The animals were inseminated 12 h after heat observation.

### 3. CIDR- GnRH - PG- GnRH / CIDR Ovsynch FT AI protocol (n=130)

On the first day of treatment, the CIDR was inserted intravaginally and injection Receptal® 2.5 ml was administered I/m. On day 7, CIDR was removed and injection Clostenol® 2 ml was given I/m. Injection Receptal® 2.5 ml was administered I/m 48 h after Clostenol injection and insemination by clock was followed between 0 and 12 h after GnRH injection.

Estrus induction rate, conception rate/ pregnancy rate at 1<sup>st</sup> service (induced heat), conception rate/ pregnancy rate at 2<sup>nd</sup> service (spontaneous heat) and overall pregnancy rate were recorded in order to evaluate the response of different hormonal treatment protocols used. Comparative study of the treatment regimes was carried out for comparing their effectiveness and efficacy for estrus induction and successful conception. The data obtained in the study was statistically analyzed using Duncan's multiple range test for comparing means in an analysis of variance ( $p=0.05$ ) to draw the scientific inferences.

## RESULTS AND DISCUSSION

Postpartum anestrus in buffaloes is responsible for long calving intervals (Borghese *et al.*, 1994). A variety of hormones are being used to treat reproductive disorders and to regulate the estrus cycle for timed breeding in buffaloes. These hormones act directly on the reproductive organs or indirectly on the pituitary gland to stimulate the release of naturally occurring hormones, which in turn act on the reproductive organs. Different hormonal protocols give satisfactory pregnancy rates, which are comparable to those achieved in animals inseminated at natural estrus (De-Rensis and Lopez, 2007).

A total of 499 buffalo with history of anestrus and infertility belonging to different agro-climatic zones of Haryana were subjected to different hormonal treatment protocols. The treated buffaloes were divided into two groups: GnRH group and CIDR + GnRH group with three sub groups in each. In the present investigation with the use of GnRH alone protocol, estrus induction and conception rate were observed to be  $90.53 \pm 3.61\%$  and  $61.06 \pm 7.12\%$  respectively (Table 1). Previously, Ramoun *et al.* (2012) observed 60% and 30%, estrus induction and conception rate, respectively with the administration of this protocol. They also suggested that in order to get better results with GnRH, the nutritional requirement of animal should be fulfilled for proper development of follicles for action of these hormones.

With the use of GnRH-PG protocol, estrus induction and overall pregnancy rates were observed  $81.03 \pm 7.92\%$  and  $62.68 \pm 7.78\%$ , respectively (Table 1). GnRH treatment would enhance ovulation and subsequent PGF<sub>2 $\alpha$</sub>  treatment would induce luteolysis of CL and later on, ovulation. Hafez and Hafez (2000) suggested that

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

Group	Name of Hormonal Protocol Used	No. of animals treated	Animals Induced to Estrus		Conception at Induced Estrus		Conception at 2 <sup>nd</sup> Estrus		Overall Pregnancy	
			n	(%Mean± SE)	n	(%Mean± SE)	n	(%Mean± SE)	n	(%Mean± SE)
GnRH Group	GnRH Alone	100	91	90.53±3.61 <sup>a</sup>	58	61.06±7.12	0	0.00±0.00	58	61.06±7.12 <sup>b</sup>
	GnRH-PG	153	120	81.03±7.92 <sup>ab</sup>	86	58.95±7.27	5	3.73±2.31	91	62.68±7.78 <sup>b</sup>
	Ovsynch FT AI	47	47	100.00±0.00 <sup>a</sup>	29	66.23±8.68	2	1.79±1.79	31	68.02±8.59 <sup>ab</sup>
CIDR+GnRH Group	CIDR-GnRH	58	54	98.20±1.80 <sup>a</sup>	42	81.26±8.46	0	0.00±0.00	42	81.26±8.46 <sup>a</sup>
	CIDR-GnRH-PG	111	100	96.87±3.12 <sup>a</sup>	78	74.19±9.13	2	7.14±7.14	80	81.33±11.01 <sup>a</sup>
	CIDR-Ovsynch	130	124	95.74±2.87 <sup>a</sup>	87	71.65±7.95	1	2.78±2.78	88	74.43±9.16 <sup>ab</sup>

Means with different superscripts are significantly different (P<0.05).

the administration of PGF<sub>2α</sub> in early postpartum period would reduce the incidence of subclinical uterine infection and hasten the return to a suitable uterine environment for fertilization and pregnancy. Administration of PGF<sub>2α</sub> at 7-10 days postpartum was effective in facilitating the uterine involution and resumption of ovarian cyclicity and improving reproductive performance (Noakes *et al.*, 2001). The results of present study are in harmony with Hammam *et al.* (2009) and Yendraliza *et al.* (2011) who observed 60% and 100% estrus induction rates and 67% and 100% pregnancy rates respectively.

A 100% induction of estrus in treated animal was observed by the use of Ovsynch FT AI protocol (Table 1). Such a higher percentage of estrus induction might be due to the reason that GnRH – PGF<sub>2α</sub>-GnRH (Ovsynch) protocol synchronizes follicular development, luteal regression and time of ovulation, thus permitting fixed time AI after the second GnRH administration (Azawi *et al.*, 2012). Conception rate obtained in our study (68.02±8.59 %) with Ovsynch protocol treatment in buffalo is considered high when compared to other similar studies conducted by several workers such as Irikura *et al.* (2003); Mialot *et al.* (1999) and Baruselli *et al.* (1999) who found lower conception rates in buffalo were 27.2, 36.1 and 42.4%, respectively. The differences in conception rates in the present study as compared to the others could be because buffaloes in our study were not specifically selected as true anestrus and also the breeding season favors the higher estrus induction and conception rates in buffaloes.

Using CIDR-GnRH protocol for treatment, estrus induction rate was observed to be 98.20±1.80% with a conception rate of 81.26±8.46% (Table 1). CIDR causes an increased circulatory concentration of progesterone resulting in increased sensitivity of hypothalamus to estrogen

with subsequent increase in the intensity of estrus (Azawi *et al.*, 2012). The conception rate in present study is higher than 20% and 66.5% reported by Azawi *et al.* (2012) and Hammam *et al.* (2009) in summer season respectively. The differences in conception rate between the present study as compared to others, could be due to the reason that the animals were treated during their normal breeding season (autumn-winter).

In the animals treated with CIDR-GnRH-PG combination, estrus induction of 96.87±3.12% and pregnancy rate of 81.33±11.01% was observed (Table 1). 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 (Naseer *et al.*, 2011). The results of this study are in agreement with the results obtained in beef cattle (Martinez *et al.*, 2011), dairy cows (Thatcher *et al.*, 2006) and buffaloes (Azawi *et al.*, 2012).

The estrus induction rates using CIDR-Ovsynch FT AI protocol were observed to be 95.74±2.87% in the present study (Table 1). These results are in agreement with Baruselli *et al.* (2007) who also observed that 100% estrus induction rates could be achieved in breeding season by combining CIDR with Ovsynch protocol in anestrus buffaloes. The main action of GnRH used at the start of progesterone treatment in order to synchronize emergence of a new cohort of follicles (Rhodes *et al.*, 2003). Additionally, second injection of GnRH has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of animals, resulting in elevated concentrations of progesterone in anestrus buffaloes. GnRH synchronizes the development and occurrence of follicles and resulted in more homogenous follicular development. It also induces ovulation or luteinization of dominant follicle in non cyclic

animals. However, the induced ovulation in non cyclic animals stimulated luteal tissue development and function resulting in the occurrence of cyclic activity (Bao *et al.*, 2003). Inserting CIDR at the initial GnRH injection of the Ovsynch program (Ovsynch plus CIDR) and then removing the CIDR and injecting PGF<sub>2α</sub> has been demonstrated to improve pregnancy rates in lactating buffaloes (Ravikumar *et al.*, 2011). The conception rate in our study was observed to be 74.43±9.16% 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.

Thus in the present study, it was observed that Ovsynch FT AI protocol produced maximum estrus induction rate in anestrus buffaloes under field conditions. However, inclusion of a CIDR device in combination with GnRH resulted in comparable estrus induction rate along with higher conception rate in postpartum anestrus buffaloes.

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