

## CONCEPTION RATE IN POSTPARTUM ANOESTRUS BUFFALOES TREATED WITH DIFFERENT SYNCHRONIZATION PROTOCOLS

Pratipal Singh Kaurav<sup>1,\*</sup>, Suresh Prasad Shukla<sup>1</sup>, Nitin Kumar Bajaj<sup>1</sup>,  
Anil Kumar Singh<sup>2</sup>, Vinay Kumar Manjhi<sup>1</sup> and Ravi Kumar Thakur<sup>1</sup>

### ABSTRACT

Twenty four postpartum anoestrus buffaloes were subjected to treatment with Ovsynch, Heatsynch and CIDR plus Heatsynch. Therapeutic efficacy of different hormonal protocols in terms of oestrus induction efficiency was found to be 66.66, 83.33 and 100% with a mean post treatment oestrus induction intervals of  $19.50 \pm 0.53$ ,  $15.20 \pm 0.61$  and  $17.20 \pm 0.91$  h, while conception rate was recorded as 75.00, 60.00 and 83.33%, respectively in Ovsynch, Heat synch and CIDR plus Heatsynch groups. The mean plasma progesterone concentration (ng/ml) in group Ovsynch, Heatsynch and CIDR plus Heatsynch on day 0 was increased significantly ( $P < 0.01$ ) to day 7 where as the value was decreased on day 9 and day 10, however no significant ( $P > 0.05$ ) difference was recorded in control group. The highest value was found in CIDR plus Heatsynch group on day 7 ( $4.48 \pm 0.05$ ) and lowest value was recorded on day 10 ( $0.53 \pm 0.01$ ).

**Keywords:** *Bubalus bubalis*, buffaloes, postpartum anoestrus buffalo, Ovsynch, heat synch, CIDR, oestrus induction efficiency, oestrus induction

interval, conception rate

### INTRODUCTION

Buffalo is the premier dairy animal in the developing countries of Asia and it is the mainstay of the Indian dairy industry, contributing over 60% of the total milk production (Mondal *et al.*, 2010). Buffaloes not only produce large quantity of milk but also contribute towards meat and drought power. Postpartum fertility is a major factor of economic importance in buffalo reproduction (Kalasariya *et al.*, 2018).

In India, anoestrus is one of the most commonly encountered reproductive problems in buffaloes affecting livestock productivity and dairy economics to a great extent. Productivity of dairy animals largely depends on their reproductive efficiency and is often measured as calf per breeding animal per unit time. Buffaloes are known for their delayed puberty, summer anoestrus and prolonged postpartum anoestrus contributing to its reproductive efficiency. Anoestrus is responsible for a prolonged inter-calving period resulting in great economic loss for the dairy industry (El-

<sup>1</sup>Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Rewa (Nanaji Deshmukh Veterinary Science University, Jabalpur), Madhya Pradesh, India,

\*E-mail: pkaurav12@gmail.com

<sup>2</sup>Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, Rewa (Nanaji Deshmukh Veterinary Science University, Jabalpur), Madhya Pradesh, India

Wishy, 2007).

Several protocols of oestrus induction and ovulation have been tried to induce oestrus with variable success. Hormones like prostaglandins, progestagens and gonadotropin releasing hormone alone and in various combinations have been found to have good therapeutic value to enhance reproductive efficacy in anoestrus buffalo with good nutritional status (Nakrani *et al.*, 2014; Savalia *et al.*, 2014). The variable results obtained following hormonal treatments by different workers may be largely due to different nutritional status, faulty management, ovarian changes endocrine events and even uterine infections.

Use of hormonal protocols during breeding season can be helpful in inducing oestrus and achieving better conception rate with lesser number of services per conception and making acyclic buffaloes to cycle normally, thereby achieving ideal inter-calving interval. This research study was conducted to study induction of ovarian cyclicity and efficacy of different hormonal protocols in postpartum anoestrus buffaloes and compare the level of plasma progesterone concentration in anoestrus and oestrus induced buffaloes.

## MATERIALS AND METHODS

The present study was carried out on 24 postpartum anoestrus buffaloes located in different villages in and around Rewa district (M.P.) which have not exhibited signs of estrus for 90 days or more postpartum. Buffaloes were divided into treatment and control groups. Eighteen anoestrus buffaloes in the treatment group were further divided into 3 treatment Groups A, B and C with 6 buffaloes in each group. The remaining 6 anoestrus buffaloes were grouped as control

(Control group) in which no treatment was given. In Ovsynch group, animals were given Inj. GnRH analogue (Buserelin acetate 20 µg i.m) on day 0, followed by Inj. PGF2α (Cloprostenol sodium 500 µg i.m) on day 7 and again Inj. GnRH analogue (Buserelin acetate 20 µg i.m) on day 9. In Heatsynch, animals were administered Inj. GnRH analogue (Buserelin acetate 20 µg i.m) on day 0, followed by Inj. PGF2α (Cloprostenol sodium 500 µg i.m) on day 7 and then Inj. estradiol benzoate 1 mg on day 8. In CIDR plus Heatsynch group, animals were administered CIDR (controlled internal drug release device) implant (for 7 days) along with Inj. estradiol benzoate (1mg i.m) on day 0 and the implant was removed on day 7, followed by Inj. PGF2α (Cloprostenol sodium 500 µg i.m) on day 7 and then Inj. estradiol benzoate 1 mg on day 8. Therapeutic efficacies of these regimes were judged on the basis of estrus induction efficiency, estrus induction interval and conception rate after artificial insemination (AI). Pregnancy diagnosis was done by per rectal examination after 60 days following artificial insemination. The effect of different hormonal protocols on the level of plasma progesterone (ng/ml) was studied before start of treatment (day 0) and after start of treatment (day 7, 9 and 10) under Ovsynch, Heatsynch, CIDR plus Heatsynch and control groups.

## RESULTS AND DISCUSSION

The results of oestrus induction efficiency, oestrus induction interval and conception rate in treatment groups anoestrus buffaloes subjected to different hormone protocols is depicted in Table 1.

### Oestrus induction efficiency

In the present study oestrus induction

efficiency was observed in 66.66% buffaloes of Ovsynch group, 83.33% buffaloes of Heatsynch group and 100% buffaloes of CIDR plus Heatsynch group. None of the buffaloes in control group exhibited oestrus.

Oestrus Induction efficiency as observed in Ovsynch group was in close approximation with the findings of Asokan *et al.* (2005), who recorded oestrus in 63.63% buffaloes. Contrary to these findings higher oestrus Induction efficiency was observed by Paul and Prakash (2005) in Murrah buffaloes (90%) and Naikoo (2009) in anoestrus Mehsana buffaloes (100%).

Very low oestrus induction efficiency as compared to present study was recorded by Awasthi *et al.* (2007) as 33.33% in postpartum Mehsana buffaloes and Ali *et al.* (2012) as 50% in postpartum anoestrus buffaloes.

Oestrus induction efficiency as observed in Heatsynch group is in close agreement with Rathore *et al.* (2017) who recorded 86% response in anoestrus buffaloes. Contrary to these findings higher oestrus Induction efficiency observed by Mohan *et al.* (2010) in non-cyclic buffaloes (100%), Kumar *et al.* (2015) in postpartum anoestrus buffaloes (100%) and 91.66% in postpartum anoestrus buffaloes (Buhecha *et al.*, 2016).

Oestrus induction efficiency as observe in CIDR + Heatsynch group is in close agreement with Naikoo (2009) in Mehsana buffaloes and Zaabel *et al.* (2009) in anoestrus buffaloes suffering from ovarian inactivity. In contrary to the present findings, much lower estrus induction response of 58.33% was recorded in buffaloes with CIDR protocol by Ravikumar *et al.* (2009). Ali *et al.* (2012) reported oestrus response of 83.33% in postpartum anoestrus buffaloes using CIDR plus heatsynch.

A cent percent oestrus detection rates as

observed in CIDR group of buffaloes under present study might be attributed to prolonged exogenous progesterone priming from CIDR device with negative feed-back effect on hypothalamo-hypophyseal-gonadal axis favouring GnRH, FSH and LH storage. Its sudden withdrawal in circulatory concentration promotes the release of GnRH as the negative feedback of progesterone, thus stimulating FSH and LH secretion and folliculogenesis and subsequent resumption of ovarian cyclicity (Zaabel *et al.*, 2009).

The possible explanation for higher oestrus induction response in the present study might be due to favourable season and geographical condition. The higher success rate in buffaloes of CIDR + Heatsynch group in the present study might be due to higher sensitivity of buffaloes to progesterone and estrogen.

### **Oestrus induction interval**

Buffaloes of Ovsynch group exhibited oestrus between 18-24 h with a mean post treatment oestrus induction interval of  $19.5 \pm 0.53$  h. Buffaloes of Heatsynch group exhibited oestrus between 13 to 17 h with a mean post treatment oestrus induction interval of  $15.2 \pm 0.61$  h, while buffaloes of CIDR plus Heatsynch group exhibited oestrus between 19 to 22 h with a mean post treatment oestrus induction interval of  $17.2 \pm 0.91$  h.

The results are in close approximation with findings of Ali *et al.* (2012) who could induce oestrus in buffaloes in 13 to 24 h when treated with Ovsynch protocol, 7 to 19 h when treated with Heatsynch and 10 to 23 h when treated with CIDR protocol and Naikoo (2009) who could induce oestrus in 10 to 20 h when treated with Ovsynch protocol and 14 to 20 h when treated with CIDR protocol.

The possible explanation for shorter

oestrus induction interval in the present study may be due to sensitivity of buffaloes for estrogen. Amer (2008) found that cows treated with estradiol cypionate had a peak preovulatory concentration of estradiol and thus better expression of oestrus.

### Conception rate

The conception rate observed in induced oestrus buffaloes after artificial insemination was recorded to be 75% in Ovsynch group (3/4) and 60% in Heatsynch group (3/5), while, 83.33% in CIDR plus Heatsynch group (5/6), respectively.

In the present study the conception rate is quite similar to that reported by Nakrani *et al.* (2014) who observed 73.33% conception rate in anoestrus buffaloes when treated with Ovsynch protocol. However, comparatively lower conception rate reported by Parmar (2013) as 57.14% in anoestrus buffaloes when treated with Ovsynch protocol. Higher conception rate as compared to that observed in present study was reported by Thorat *et al.* (2014) in Marathwadi buffaloes (85.71%).

Conception rate in the present study in Heatsynch group (60%) are higher than earlier report by Mohan *et al.* (2010) who reported conception rate of 40% in winter season and Ali *et al.* (2012) who reported conception rate 50% for buffaloes treated with Heatsynch protocol. Very low conception rate was also reported by Buhecha *et al.* (2016) in buffaloes treated with Heatsynch protocols (25%) and Rathore *et al.* (2017) in anoestrus buffaloes and delayed pubertal heifers (18%) under field conditions following FTAI.

Conception rate of 83.33% was recorded in CIDR plus Heatsynch group anoestrus buffaloes. The findings are quite in approximation with those of Singh and Kumar (2006) who reported 77% conception rate in 10 heifers and 40 buffaloes when treated with CIDR. Contrary to the present finding,

comparatively higher overall conception rates were reported by Singh (2003) as 90% in buffaloes during the non-breeding season.

In present finding, overall conception rate of 83.33% was observed which was higher than that reported by earlier researchers as 60% in postpartum anoestrus buffaloes (Ali *et al.*, 2012) and 70 to 80% in anoestrus buffaloes and crossbreed cows (Savalia *et al.*, 2013). However, the other workers documented much lower overall conception rate of 12.65% in Iranian buffaloes during low breeding season (Ekrami *et al.*, 2008) and 31.80% in postpartum anoestrus buffaloes (Azawi *et al.*, 2012) from abroad probably due to different agro-climatic and nutritional conditions.

### Plasma progesterone concentration (ng/ml)

The results of mean plasma progesterone concentration (ng/ml) obtained in control and different treatment group anoestrus buffaloes at different days of study are depicted in Table 2.

The plasma progesterone profile was studied in postpartum anoestrus buffaloes before start of treatment (day 0) and after start of treatment (day 7, 9 and 10) under Ovsynch group A, Heatsynch group, CIDR plus Heatsynch group and control group. The mean plasma progesterone (ng/ml) concentration were low on day 0 in Ovsynch, Heatsynch and CIDR plus Heatsynch ( $0.62 \pm 0.01$ ,  $0.59 \pm 0.01$  and  $0.59 \pm 0.02$ , respectively) groups, suggestive of the fact that most of the buffaloes were not having any functional luteal structure on their ovaries and were in true anoestrus. These levels subsequently elevated significantly ( $P < 0.01$ ) to the peak values on day 7 ( $3.59 \pm 0.042$ ,  $2.29 \pm 0.02$  and  $4.48 \pm 0.05$ ), particularly in CIDR plus Heatsynch and Ovsynch protocols, *i.e.* just before implants were removed. There after the mean plasma progesterone concentration dropped suddenly and

significantly to basal level on day 9 ( $0.75\pm 0.01$ ,  $0.54\pm 0.01$  and  $0.84\pm 0.01$ ) due to effect of  $\text{PGF}_{2\alpha}$  injection and day 10 ( $0.53\pm 0.01$ ,  $0.72\pm 0.01$  and  $0.80\pm 0.01$ ) in Ovsynch, Heatsynch and CIDR plus Heatsynch, respectively. However, in control group the values on day 0 did not increased significantly ( $P>0.05$ ) on day 7, 9 and 10.

The present study revealed the highest mean progesterone concentration ( $4.48\pm 0.05$ ) at day 7 in CIDR plus Heatsynch might be due to continuous release of the exogenous progesterone from the progesterone molded silastic coil inserted in the anterior vagina of the buffaloes, while in the Ovsynch and Heatsynch protocol groups the rise in progesterone concentrations recorded on day 7 might be due to luteinization of some of the growing follicle and/or ovulation of dominant follicle and formation of CL under the influence of GnRH. Moreover, the exogenous estradiol benzoate (1 mg) used on day 8 in Heatsynch and CIDR plus Heatsynch protocols, probably helped by positive feedback effect on pituitary and hypothalamus in triggering the ovulatory LH surge and thereby better synchronized the ovulation timing in treated animals (Mirmahmoudi *et al.*, 2014).

Present findings on levels and trend of plasma progesterone in buffaloes under different oestrus induction protocols on different days closely corroborated with the earlier reports (Savalia *et al.*, 2014; Nakrani *et al.*, 2014). However, with respect to effect of Heatsynch protocol higher progesterone concentration on day 7 are comparable with findings of Buhecha *et al.* (2016) who reported progesterone concentration as  $2.0\pm 0.32$  ng/ml.

The result of the present study suggested that estimation of the plasma progesterone levels by ELISA is helpful tool to detect the current reproductive/cyclical status of the animals and to diagnose early pregnancy with reasonable accuracy

in buffaloes after AI.

## CONCLUSION

It could be concluded that the Ovsynch, Heat synch and CIDR plus Heat synch can be applied in postpartum anoestrus buffaloes for induction of ovarian cyclicity and obtain acceptable conception rate. Amongst the different hormone protocols, CIDR plus Heatsynch proves to be excellent in oestrus induction (100%) with conception rate of 100%. Plasma progesterone was found as a marker for monitoring the functional status of corpus luteum and diagnostic tool for identifying ovarian condition.

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Table 1. Response to various hormone protocols in control and different treatment groups of buffaloes.

S. No.	Character	Ovsynch Group (n=06)	Heatsynch Group (n=06)	CIDR+ Heatsynch Group (n=06)	Control Group (n=06)
1	Oestrus induction efficiency (%)	66.66 (04)	83.33 (05)	100 (06)	0.00 (0)
2	Oestrus induction interval (h.)	19.5±0.53 (04)	15.2±0.61 (05)	17.2±0.91 (06)	0.00 (0)
3	Conception rate (%)	75 (03)	60 (03)	83.33 (05)	0.00 (0)

Figures in parentheses indicate number of buffaloes.

Table 2. Plasma progesterone concentration (ng/ml) in control and different treatment groups.

Groups	Days				(P-Value)
	Day 0	Day 7	Day 9	Day 10	
Ovsynch Group (n=06)	0.62 <sup>ap</sup> ±0.01	3.59 <sup>bp</sup> ±0.04	0.75 <sup>cp</sup> ±0.01	0.72 <sup>cp</sup> ±0.01	P<0.01
Heatsynch Group (n=06)	0.59 <sup>ap</sup> ±0.01	2.29 <sup>bq</sup> ±0.02	0.54 <sup>cq</sup> ±0.01	0.53 <sup>cq</sup> ±0.01	P<0.01
CIDR + Heatsynch Group (n=06)	0.59 <sup>ap</sup> ±0.02	4.48 <sup>br</sup> ±0.05	0.84 <sup>cr</sup> ±0.01	0.80 <sup>cr</sup> ±0.01	P<0.01
Control Group (n=06)	0.59 <sup>ap</sup> ±0.02	0.59 <sup>as</sup> ±0.02	0.61 <sup>as</sup> ±0.01	0.61 <sup>as</sup> ±0.01	P>0.05

Mean±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (P<0.05).



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