

# EVALUATION AND COMPARISON OF THERAPEUTIC EFFICACY OF VARIOUS HORMONAL PROTOCOLS IN ANOESTRUS BUFFALO HEIFERS OF SEMI-ARID REGION

## ABSTRACT

To assess and compare different hormonal protocols' treatment efficacy in anoestrus buffalo heifers in a semi-arid region. Forty buffalo heifers with the history of anoestrus were chosen randomly from the semi-arid region of Banaskanth and divided into four equal groups. Group-I: Heatsynch protocol (n = 10), Group-II Ovsynch protocol (n = 10), Group-III Doublesynch protocol (n = 10) and Group-IV Control (n = 10) are followed by artificial insemination at a predetermined period in treated buffalo heifers and insemination during spontaneous estrus in group-IV buffalo heifers. In Groups I, II, and III, the corresponding percentages of estrus induction were 90, 80, and 80 percent. Major symptoms such as edoema, erythema and dampness of vulva and estrus mucus secretion were present during the expression of estrus. Fifty percent of the buffalo heifers in Group IV displayed estrus at varying intervals. After three consecution services, Group II had the highest overall fertility rate (50%) followed by Group III (40%), Group I (30%) and Group IV (30%). In true anoestrus buffalo heifers, the Heatsynch, Ovsynch, and Doublesynch procedure can be applied with success to induce estrus. Buffalo heifers that follow to the Ovsynch protocol may have a higher conception rate.

**KEYWORDS:** Heatsynch, Ovsynch, Doublesynch, buffalo heifers, anoestrus.

## 1 INTRODUCTION

Buffalo is crucial to the upkeep of a sustainable food production system in emerging nations and India is not an exception to this. 53% of the world's milk produced by buffaloes comes from India (Sethi *et al.*, 2014). Since buffaloes often exhibit both silent and anestrus estrus, buffalo farmers typically have difficulty identifying when their animals are in estrus. Moreover, their incapacity to exhibit clear symptoms of estrus, seasonality, delayed puberty onset and variable duration of estrus impede their improved reproductive control and genetic advancement. Prolonged anestrus and postpartum acyclicity also cause buffalo breeders to suffer significant financial losses.

Anestrus is one of the most commonly occurring reproductive problems in cattle and buffalo of India, affecting livestock productivity and economics to a great extent. It is a functional disorder of the reproductive cycle which is characterized by absence of overt signs of estrus manifested either due to lack of expression of estrus or failure of its detection. In heifers, it poses a herd problem possibly due to low

plane of nutrition, stress of seasonal transition or extremes of climatic conditions. Clinical survey revealed higher incidences of anoestrus and inactive ovaries in buffaloes (55.5 and 19.4%, respectively) than in cows (43 and 17.2%, respectively) (Tanwar *et al.*, 2003). Incidence in India has been reported between 2.13–67.11 and 9.09–82.50 per cent in indigenous cattle and buffaloes (Thakor and Patel, 2013). Incidence in heifers has been reported between 12.37 to 64.66 per cent (Sinha *et al.*, 1987). Variable duration of estrus (4-16 hrs) and difficulty in prediction time of ovulation make AI application in buffaloes difficult (Baruselli, 2001). This consideration indicates need for estrus synchronization using fixed time insemination for implementation of breeding programs in buffaloes (Presicce *et al.*, 2004).

To improve reproductive efficiency, several protocols of estrus induction and ovulation synchronization have been developed. These procedures are based on manipulating the Corpus luteum, either to induce premature luteolysis using prostaglandins or to prolong the luteal phase using progestogens. Estrus synchronization has many advantages and is becoming mandatory in modern animal husbandry practices in indigenous cattle and buffaloes, which are known for anoestrus and silent estrus. Looking to the above fact the comparative study of various synchronization protocols in ovulation was decided. Hence, this study was planned to evaluate the comparative efficacy of heatsynch, ovsynch and doublesynch protocols for fertility enhancement in anestrus buffalo heifers.

## **2 MATERIAL AND METHODS**

### **2.1 LOCATION OF THE EXPERIMENTAL AREA**

The present study was carried out at Banaskantha district at Department of Veterinary Gynecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar, Dantiwada, SDAU with Collaboration of All India Co-ordinated Research Project (AICRP) on Nutritional and Physiological Intervention for enhancing reproduction performance in animal, Dept. of Animal Reproduction, Gynecology & Obstetrics, College of Veterinary Science and Animal Husbandry, AAU, Anand.

### **2.2 EXPERIMENTAL PROTOCOL**

#### **2.2.1 Selection of the buffalo heifer**

A total 40 Anestrus buffalo heifers of the average body condition score between 3 to 5 with the history of anestrus were selected from villages of Banaskantha milkshed areas of Gujarat. The buffalo heifers were screened gynaeco-clinically for their reproductive status. Detailed history and rectal palpation findings were recorded. The anestrus buffalo heifers having small, smooth, inactive ovaries with normal genitalia and no palpable CL on either ovary, relaxed uterine horn was selected for study. The reproduction status of their buffalo heifers were re-examined after 10 days for conformation the condition.

#### **2.2.2 Grouping of buffalo heifers.**

All the 40 buffalo heifers were randomly divided in four groups as follow.

#### **2.2.2.1 Group-I (HeatSynch Protocol, n=10).**

The Selected buffalo heifers of this group were administered with Intramuscular Inj. of 10 µg of GnRH analogue i.e. Buserelin Acetate 10 µg (Receptal , @ 2.5 ml, Intervet India Pvt Ltd, New delhi) on day 0, followed by Inj. of 500 µg PGF<sub>2</sub>α analogue i.e. Cloprostenol sodium (Estrumate, @ 2 ml, Intervet India Pvt Ltd) and estradiol benzoate 1 mg I/M (Sigma, USA) on days 7 and 8, respectively, and were fix time artificial inseminated(FTAI) twice at 48 and 60 hrs post-Estradiol injection.

#### **2.2.2.2 Group-II (Ovsynch Protocol, n=10):**

The selected buffalo heifers of this group were administered with Intramuscular Inj. of 10 µg of Buserelin acetate on day 0, Inj. of 500 µg Cloprostenol sodium, on day 7 and second Inj. of 10 µg of Buserelin acetate on day 9 followed by fix time artificial insemination twice at 12 and 24 hrs later.

#### **2.2.2.3 Group-III (Double Synch Protocol, n=10):**

The buffalo heifers of this group were administered with Inj. of 500 µg Cloprostenol sodium on -3 day (3 days before the first injection of GnRH), I/M followed Inj. of 10 µg Buserelin acetate on day 0, Inj. of 500 µg Cloprostenol sodium on 7<sup>th</sup> day and second Inj. of 10 µg Buserelin acetate on 9<sup>th</sup> day, the buffalo heifers were fix time artificial inseminate twice at 12 and 24 hrs later following the second injection of buserelin acetate.

#### **2.2.2.4 Group-IV (Anoestrus control, n=10):**

The selected buffalo heifers of this group were injected with normal saline 5 ml, i.m on days 0, 7 and 9 and were observed for onset of natural estrus and artificial insemination was performed on detection of estrus. The group served as untreated control.

### **2.3 ESTRUS INDUCTION AND ESTRUS INTENSITY**

The buffalo heifers were closely observed for the exhibition of estrus symptoms at the time of fix time insemination. The intensity of induce estrus was recorded based on the expressions of estrus symptoms. Estrus symptoms of group IV were recorded at the spontaneous estrus. The estrus intensity of selected buffalo heifers were monitored on the basis of their behavior changes i.e. bellowing, excitement, off feed, estrus mucus discharge as well as edema, erythema and wetness of vulva.

### **2.4 FERTILITY RESPONSE**

The buffalo heifers which did not show signs of estrus following the treatment were examined per rectally for pregnancy diagnosis on 60<sup>th</sup> day post insemination to confirm the pregnancy. The buffalo heifers which did not conceive at the fixed time insemination were re-inseminated up to two subsequent estrus cycles. The conception rate up to 3<sup>rd</sup> insemination was recorded.

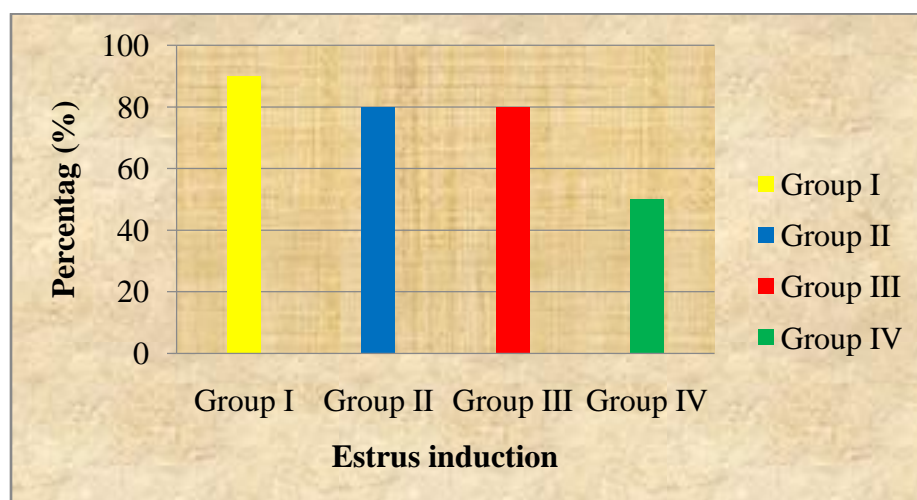
### **2.5 STATISTICAL ANALYSIS OF DATA**

The data collected were suitably tabulated and analyzed following standard statistical method shown by Steel and Torrie (1981).

## **3 RESULTS AND DISCUSSION**

### **3.1 INDUCTION AND INTENSITY OF ESTRUS**

When the records of all 40 buffalo heifers were pooled it was observed that 75 per cent (30/40 buffalo heifer) heifers showed induction of estrus. The induction of estrus along with the intensity in respect to the expression of estrus signs like bellowing, excitement, off feed, estrus mucus discharge and edema, erythema and wetness of vulva were recorded. The details of observation (Induction of estrus (%)) are depicted in figure 1.



**Fig. 1: Induction of estrus (%) in the buffalo heifers treated with different treatment and control group.**

The recorded estrus intensity at the time of artificial Insemination in Group-I (HeatSynch Protocol) was 90 per cent (9/10) induction of estrus with excitement (55.55%), estrus mucus discharge (EMD) (100%), edema, erythema and wetness of vulva (88.88%). The finding (bellowing, excitement, mucus discharge and swollen) of present study are similar of Mohan *et al.* (2009) in buffaloes following Heatsynch protocol.

Group-II (Ovsynch Protocol) express 80per cent (8/10) induction of estrus with excitement (87.50 %), bellowing (11.1 %), estrus mucus discharge (100%) and edema, erythema and wetness of vulva (100%). Atanasov *et al.* (2011) observed mucus discharge in buffaloes at the time of AI following Ovsynch protocol. Similar Malik *et al.* (2011) and Yotov *et al.* (2012) also observed 40 and 66.7 per cent anestrous buffaloes showing estrus mucus discharge following Ovsynch protocol.

In Group-III (Double Synch Protocol) estrus induction was 80per cent (8/10) with excitement (75%), estrus mucus discharge (100%) and edema, erythema and wetness of vulva (100%). Hoque *et al.* (2014) observed diverse intensity of estrus signs in Ovsynch treated water buffaloes. They observed Bellowing, Frequent urination, swelling of vulva and pink colored vestibule along with uterine tone at time of insemination in all the buffaloes at induce estrus with different protocols like modified Ovsynch and Double-ovsynch.

Whereas in group-IV (control anestrous buffalo heifer) 50 per cent (5/10) heifer showing estrus at different time interval. i.e 20 days (2 buffalo heifer), 35 days (1 buffalo heifer) and 55 days (2 buffalo heifer) post last injection of NS (Normal saline) with expressions of excitement (100%), bellowing (60%), estrus mucus discharge (100%) and edema, erythema and wetness of vulva (100%).

In Heatsynch protocol total 90 per cent (9/10) buffalo heifer showed estrus symptoms at the time of insemination. Similar result was recorded by Buhecha *et al.* (2016) in anestrous buffaloes. However, 100 per cent estrus was recorded following heatsynch protocol in buffalo by Ali *et al.* (2012) and Mohan *et al.* (2014). Kandiel *et al.* (2012) also reported 100 per cent estrus induction by Heatsynch protocol in buffalo heifers.

In Ovsynch protocol total 80 per cent (8/10) buffalo heifers showed estrus induction at the time of insemination. Similar result was recorded by Jabeen *et al.* (2012), Savalia *et al.* (2014) and Buhecha *et al.* (2016) in anoestrus buffaloes. Lower estrus response was observed by Kandiel *et al.* (2012), Kalwar *et al.* (2015) and Waqas *et al.* (2016) in buffaloes. However, 89 per cent estrus was recorded following Ovsynch protocol in buffalo by Neglia *et al.* (2003) and Paters *et al.* (2003). The 100 per cent estrus induction was recorded following Ovsynch protocol in buffalo by Atanasov *et al.* (2011) and Nakrani *et al.* (2014). Paul and Prakash, (2005) reported 90 per cent estrus synchronization in buffalo following Ovsynch protocol.

In Doublesynch protocol total 80 per cent (8/10) buffalo heifer showed estrus symptoms at the time of insemination. Similar results were recorded by Ozturk *et al.* (2010) in dairy cow. However, 100 per cent estrus was recorded following doublesynch protocol in buffalo by Mirmahmoudi and Prakash, (2012).

In control group total 50 per cent (5/10) buffalo heifer showed estrus symptoms at different time interval within 90 days. Lower estrus response was observed by Parmar *et al.* (2012), Nakrani *et al.* (2014) and Buhecha *et al.* (2016) in anestrous buffaloes.

In This study maximum estrus induction was obtained in Heatsynch group followed by Ovsynch and Doublesynch group. The higher success rate in Buffalo heifer observed in Heatsynch group using estradiol benzoate could be due to the higher sensitivity of buffalo heifer to this treatment.

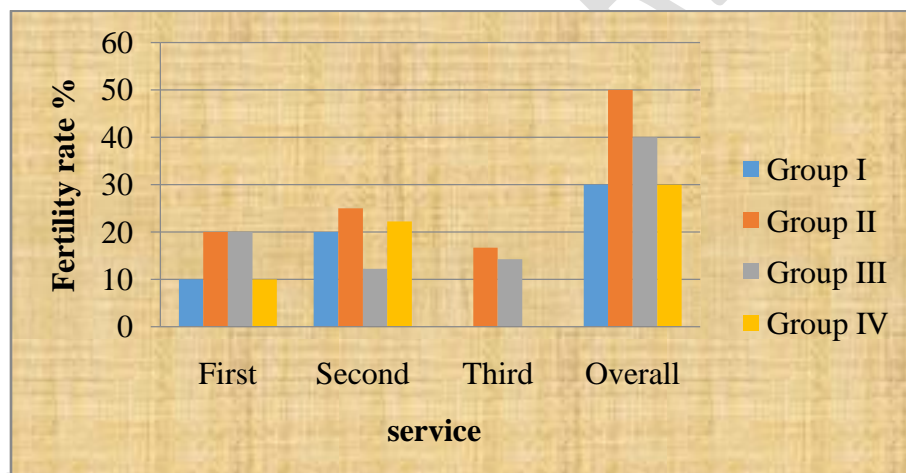
### **3.2 FERTILITY RESPONSE**

The buffalo heifers of treatment group were inseminated at fix time and the control group was inseminated at spontaneous estrus by semen of buffalo bull. The buffalo heifers which failed to conceive at 1<sup>st</sup> insemination were re-insemination for two subsequent estrus. Fertility response of buffalo heifer showed in table 1 and figure 2.

**Table 1: Fertility response of buffalo heifers following different treatments.**

Group	Conception/service			Overall	Per cent (%)
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>		
Group-I (n=10)	1(10.00%)	2(22.22%)	-	3/10	30.00
Group-II (n=10)	2 (20.00%)	2 (25.00%)	1 (16.66%)	5/10	50.00
Group-III (n=10)	2(20.00%)	1(12.50%)	1(14.28%)	4/10	40.00
Group-IV (n=10)	1(10.00%)	2(22.22%)	-	3/10	30.00

The recorded overall Fertility response of group-I, II, III and IV was 30.00, 50.00, 40.00 and 30.00 per cent, respectively. The 1<sup>st</sup> insemination conception rate of group I, II, III&IV was 10.00, 20.00, 20.00 and 10.00 per cent, respectively. Whereas the similar fingers for 2<sup>nd</sup> insemination conception rate was 22.22, 25.00, 12.50 and 22.22 per cent, respectively. Following the third insemination recorded conception rate was 0, 16.66, 14.28 and 0 per cent, respectively in group I, II, III and IV.



**Fig. 2: Fertility rate (%) in buffalo heifers treated with hormonal protocol and control group.**

In this study 30 per cent Fertility response was achieved by using Heatsynch protocol in anestrus buffalo heifers. Similar conception rate was obtained by Mohan *et al.* (2009) using Heatsynch protocol during summer and winter season in anestrus buffaloes. Almost similar conception rate was obtained by Buhecha *et al.* (2016) in buffaloes using Heatsynch protocol.

Fertility response obtained was 50 per cent following Ovsynch protocol in anestrus buffalo heifers. Similar results were obtained by Berber *et al.* (2002), Naikoo *et al.* (2010) in buffalo. However, contrary to the findings lower conception rate was recorded in post-partum anestrus

buffaloes (Atanasov *et al.*, 2011 and Buhecha *et al.*, 2016). The higher conception rate following Ovsynch protocol was recorded by Kandiel *et al.* (2012), Nakrani *et al.* (2014) and Savalia *et al.* (2014) in anestrus buffaloes.

In Doublesynch protocol obtained Fertility response was 40 per cent in anestrus buffalo heifers. Higher conception rate was obtained by Mirmahmoudi and Prakash, (2012) in anestrus buffaloes. Ozturk *et al.* (2010) reported 72 per cent conception rate in anestrus dairy cow following Doublesynch protocol.

In control group overall Fertility response was 30 per cent in anestrus buffalo heifers. Contrary to the present study higher conception rate (Nakrani *et al.*, 2014 and Buhecha *et al.*, 2016) in anestrus buffaloes and Lower conception rate (10.00%) in buffaloes (Yotov *et al.*, 2012) was observed.

## CONCLUSIONS

According to the study's findings, true anoestrus buffalo heifers can be successfully induced to undergo estrus using the Heatsynch, Ovsynch, and Doublesynch methodology. The most noticeable symptoms of induced estrus in buffalo heifers were erythema, edoema, wetness of vulva, and estrus mucus secretion. In buffalo heifers, comparatively improved fertility response can be obtained by using the Ovsynch protocol followed by Doublesynch, and Heatsynch protocols.

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