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~~ a 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 anestrous 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 anestrous and postpartum acyclicity also cause buffalo breeders to suffer significant financial losses.

Anestrous 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~~ that is characterized by the 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, thestress of seasonal transition, or extremes of climatic conditions. ~~Clinical~~ The clinicalsurvey 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~~percent in indigenous cattle and buffaloes (Thakor and Patel, 2013). Incidence in heifers has been reported between 12.37 to 64.66 percent (Sinha *et al.,* 1987). Variable duration of estrus (4-16 hours) and difficulty in prediction time of ovulation make AI application in buffaloes difficult (Baruselli, 2001). This consideration indicates the need for estrus synchronization using ~~fixed time~~fixed-time insemination for the 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~~ atthe 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 anestrous 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 ~~b~~Buffalo ~~h~~Heifer

A total of 40 Anestrous buffalo heifers with ~~of~~an~~the~~ average body condition score between 3 to 5 with ~~the~~ a history of anestrous were selected from villages of Banaskantha milk-shed areas of Gujarat. The buffalo heifers were screened gynaeco-clinically for their reproductive status. Detailed history and rectal palpation findings were recorded. The anestrous 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~~ was re-examined after 10 days ~~for~~ toconform~~ation~~to the condition.

2.2.2 Grouping of ~~b~~Buffalo~~H~~~~h~~eifers.

Comment [A1]: Percent should not be spilted

Comment [A2]: Define the full meaning of CL before abbreviating

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

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

The Selected buffalo heifers of this group were administered with ~~Intramascular~~ Intramuscular Injection (Inj.)~~.~~ of 10 µg of GnRH ~~analogue~~ analogi.e. Buserelin Acetate 10 µg (Receptal , @ 2.5 ml, Intervet India Pvt Ltd, New delhi) on day 0, followed by Inj. of 500 µg $PGF_2\alpha$ 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~~fixed 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 Intramascular 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~~ 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 µgBuserelin acetate on day 0, Inj. of 500 µg Cloprostenol sodium on 7th day and second Inj. of 10 µgBuserelin acetateon 9th 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 an untreated control.

2.3ESTRUS INDUCTIONAND ESTRUS INTENSITY

The buffalo heifers were closely observed for the exhibition of estrus symptoms at the time of ~~fix~~ fixedtime insemination. The intensity of ~~induce~~ induced 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~~ was monitored ~~on the basis of~~based on their behavior changes i.e. bellowing, excitement, ~~off feed~~off-feed, estrus mucus discharge as well as edema, erythema, and wetness of the vulva.

2.4FERTILITY RESPONSE

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

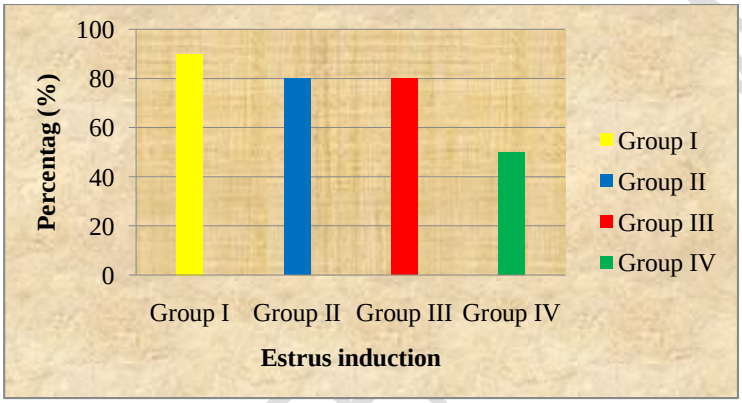

Fig. 1: Induction of estrus (%) in the buffalo heifers treated with different treatment and control group.

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

~~Group II~~Group II(Ovsynch Protocol) ~~express~~ expresses80per 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~~ SimilarlyMalik *et al.* (2011) and Yotov *et al.* (2012) also observed 40 and 66.7 ~~per cent~~percent 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 the vulva (100%). Hoque *et al.* (2014) observed diverse ~~intensity~~ intensitiesof estrus signs in ~~Ovsynch~~

~~treated~~Ovsynch-treated water buffaloes. They observed Bellowing, Frequent urination, swelling of the vulva, and ~~pink colored~~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~~group IV (control anestrous buffalo heifer) 50 per cent (5/10) heifer ~~showing~~ showedestrus at different time ~~interval~~intervals. 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 of 90 ~~per cent~~percent (9/10) of buffalo ~~heifer~~ heifersshowed estrus symptoms at the time of insemination. ~~Similar~~ A similarresult was recorded by Buhechae*t al.* (2016) in ~~anestrues~~anestrusanestrusbuffaloes. However, 100 ~~per cent~~percent 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~~percent estrus induction byHeatsynch protocol in buffalo heifers.

In Ovsynch protocol total of 80 ~~per cent~~percent (8/10) of buffalo heifers showed estrus induction at the time of insemination. ~~Similar~~ A similarresult was recorded by Jabeen *et al.* (2012), Savalia *et al.* (2014), and Buhechae*t 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~~percent estrus was recorded following Ovsynch protocol in buffalo by Neglia *et al.* (2003) and Paterse*t al.* (2003). The 100 ~~per cent~~percent estrus induction was recorded following Ovsynch protocol in buffalo by Atanasov *et al.* (2011) and Nakranie*t al.* (2014). Paul and Prakash, (2005) reported 90 ~~per cent~~percent estrus synchronization in buffalo following Ovsynch protocol.

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

In the control group total of 50 ~~per cent~~percent (5/10) buffalo heifer showed estrus symptoms at different time ~~interval~~ intervalswithin 90 days. Lower estrus response was observed by Parmar *et al.* (2012), Nakranie*t al.* (2014), and Buhechae*t al.* (2016) in anestrous buffaloes.

In ~~T~~this study maximum estrus induction was obtained in the 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 the treatment group were inseminated at ~~fix~~ fixedtime and the control group was inseminated at spontaneous estrus by the semen of a buffalo bull. The buffalo heifers ~~which~~ thatfailed to conceive at 1st insemination were re-insemination for two subsequent estrus. ~~Fertility~~ The fertilityresponse of buffalo heifer ~~showed~~ is shownin table 1 and figure 2.

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

Group	Conception/service			Overall	Per cent (%)
	1st	2nd	3rd		
Group-I (n=10)	1(10.00%)	2(22.22%)	-	3/10	30.00
~~Group-II~~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~~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~~percent, respectively. The 1st insemination conception rate of group I, II, III&IV was 10.00, 20.00, 20.00 and 10.00 ~~per cent~~percent, respectively. Whereas the similar fingers for 2nd insemination conception rate ~~was~~ were22.22, 25.00, 12.50, and 22.22 ~~per cent~~percent, respectively. Following the third insemination recorded conception rate was 0, 16.66, 14.28, and 0 ~~per cent~~percent, respectively in group I, II, III, and IV.

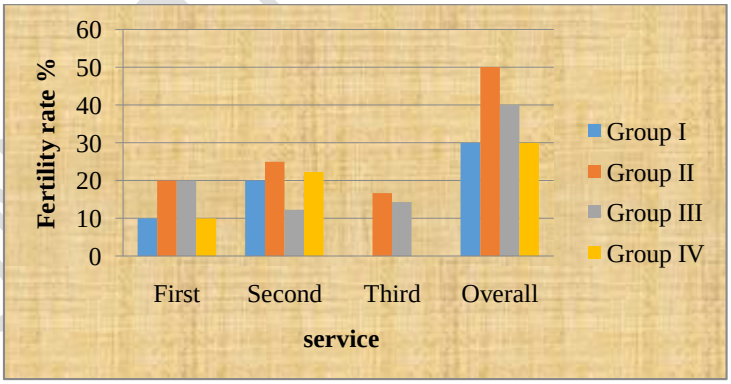

Fig. 2: Fertility rate (%) in buffalo heifers treated with hormonal protocol and control group.

In this study 30 ~~per cent~~percent~~F~~fertility response was achieved by using the Heatsynch protocol in anestrous buffalo heifers. ~~Similar~~ A

similarconception rate was obtained by Mohan *et al.* (2009) using the Heatsynch protocol during the summer and winter ~~season~~ seasonsin anestrous buffaloes. ~~Almost~~ An almostsimilar conception rate was obtained by Buhecha*et al.* (2016) in buffaloes using the Heatsynch protocol.

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

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

In the control group, overall ~~F~~fertility response was 30 ~~per cent~~percent in anestrous buffalo heifers. Contrary to the present study higher conception rate (Nakrani*et al.*, 2014 andBuhecha*et al.,* 2016) in anestrous buffaloes and a ~~l~~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, ~~e~~doema~~e~~dema, the wetness of the 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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