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Evaluation and Comparison of Therapeutic Efficacy of Various Hormonal Protocols in Anoestrus Buffalo Heifers of Semi-Arid Region

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

To assess and compare different hormonal protocols' treatment efficacy in anoestrus buffalo heifers in a semi-arid region. Forty buffalo heifers with 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

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such as edoema, erythema, 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 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. 53% of the world's milk produced by buffaloes comes from India [1]. 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 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, the stress of seasonal transition or extremes of climatic conditions. The 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) [2]. Incidence in India has been reported between 2.13–67.11 and 9.09–82.50 percent in indigenous cattle and buffaloes [3]. Incidence in heifers has been reported between 12.37 to 64.66 percent [4]. Variable duration of estrus (4-16 hours) and difficulty in prediction time of ovulation make AI application in buffaloes difficult [5]. This consideration indicates the need for estrus synchronization using fixed-time insemination for implementation of breeding programs in buffaloes [6].

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 at 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 anestrous buffalo heifers.

2. MATERIALS 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 Anestrous buffalo heifers with an average body condition score between 3 to 5 with 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 was re-examined after 10 days to conformation to the condition.

2.2.2 Grouping of buffalo heifers

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

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

The Selected buffalo heifers of this group were administered with Intramuscular Injection (Inj.)- of 10 µg of GnRH analog 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 $PGF_2\alpha$ 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 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 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^{th} day and second Inj. of 10 µg Buserelin acetate on 9^{th} 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.

2.3 Estrus Induction and Estrus Intensity

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

2.4 Fertility Response

The buffalo heifers which did not show signs of estrus following the treatment were examined rectally for pregnancy diagnosis on 60^{th}-day post-insemination to confirm the pregnancy. The buffalo heifers that did not conceive at the fixed time insemination were re-inseminated up to two subsequent estrus cycles. The conception rate up to 3^{rd} 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 [7].

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 percent (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 Fig. 1.

The recorded estrus intensity at the time of artificial Insemination in Group-I (HeatSynch Protocol) was 90 percent (9/10) induction of estrus with excitement (55.55%), estrus mucus discharge (EMD) (100%), edema, erythema and wetness of vulva (88.88%). The findings (bellowing, excitement, mucus discharge and swollen) of the present study are similar of Mohan et al. [8] in buffaloes following Heatsynch protocol.

Group-II (Ovsynch Protocol) expresses 80percent (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. [9] observed mucus discharge in buffaloes at the time of AI following Ovsynch protocol. Similarly Malik et al. [10] and Yotov et al. [11] also observed 40 and 66.7 percent anestrous buffaloes showing estrus mucus discharge following Ovsynch protocol.

In Group-III (Double Synch Protocol) estrus induction was 80percent (8/10) with excitement (75%), estrus mucus discharge (100%) and edema, erythema and wetness of the vulva (100%). Hoque et al. [12] observed diverse intensities of estrus signs in Ovsynch-treated water buffaloes. They observed Bellowing, Frequent urination, swelling of the 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 percent (5/10) heifer showed estrus at different time 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 percent (9/10) of buffalo heifers showed estrus symptoms at the time of insemination. A similar result was recorded by Buhecha et al. [13] in anestrues buffaloes. However, 100 percent estrus was recorded following heatsynch protocol in buffalo by Ali et al. [14] and Mohan et al. [15]. Kandiel et al. [16] also reported 100 percent estrus induction by Heatsynch protocol in buffalo heifers.

In Ovsynch protocol total of 80 percent (8/10) buffalo heifers showed estrus induction at the time of insemination. A similar result was recorded by Jabeen et al. [17] Savalia et al. [18] and Buhecha et al. [13] in anoestrus buffaloes. Lower estrus response was observed by Kandiel et al. [16] Kalwar et al. [19] and Waqas et al. [20] in buffaloes. However, 89 percent estrus was recorded following Ovsynch protocol in buffalo by Neglia et al. [21] and Paters et al. [22]. The 100 percent estrus induction was recorded following Ovsynch protocol in buffalo by Atanasov et al. [9] and Nakrani et al. (2014). Paul and Prakash, [23] reported 90 percent estrus synchronization in buffalo following Ovsynch protocol.

In Doublesynch protocol total of 80 percent (8/10) of buffalo heifer showed estrus symptoms at the time of insemination. Similar results were recorded by Ozturk et al. [24] in dairy cows. However, 100 percent estrus was recorded following doublesynch protocol in buffalo by Mirmahmoudi and Prakash, [25].

In the control group total of 50 percent (5/10) buffalo heifer showed estrus symptoms at different time intervals within 90 days. Lower estrus response was observed by Parmar et al. [26] Nakrani et al. (2014) and Buhecha et al. [13] in anestrous buffaloes.

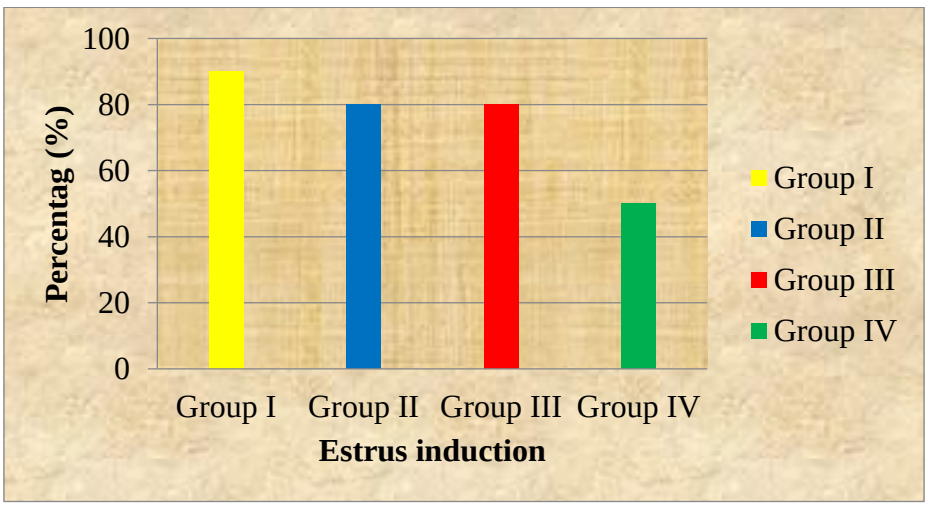

Fig. 1. Induction of estrus (%) in the buffalo heifers treated with different treatment and control group

In 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 fixed time and the control group was inseminated at spontaneous estrus by the semen of a buffalo bull. The buffalo heifers that failed to conceive at 1st insemination were re-insemination for two subsequent estrus. The fertility response of buffalo heifer is shown in Table 1 and Fig. 2.

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

In this study 30 percent Fertility response was achieved by using Heatsynch protocol in anestrous buffalo heifers. A similar conception rate was obtained by Mohan et al. [15] using Heatsynch protocol during the summer and winter seasons in anestrous buffaloes. An almost similar conception rate was obtained by Buhecha et al. [13] in buffaloes using Heatsynch protocol.

Fertility response obtained was 50 percent following Ovsynch protocol in anestrous buffalo heifers. Similar results were obtained by Berber et al. [27] and Naikoo et al. [28] in buffalo. However, contrary to the findings lower conception rate was recorded in post-partum anestrous buffaloes [9] and Buhecha et al., 2016). The higher conception rate following Ovsynch protocol was recorded by Kandiel et al. [16] Nakrani et al. [29] and Savalia et al. [18] in an anestrous buffaloes.

In Doublesynch protocol obtained fertility response was 40 percent in anestrous buffalo heifers. A higher conception rate was obtained by Mirmahmoudi and Prakash, [25] in anestrous buffaloes. Ozturk et al. [24] reported 72 percent conception rate in anestrous dairy cow following Doublesynch protocol.

Table 1. Fertility response of buffalo heifers following different treatments

Group	Conception/service			Overall	Percent (%)
	1st	2nd	3rd		
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

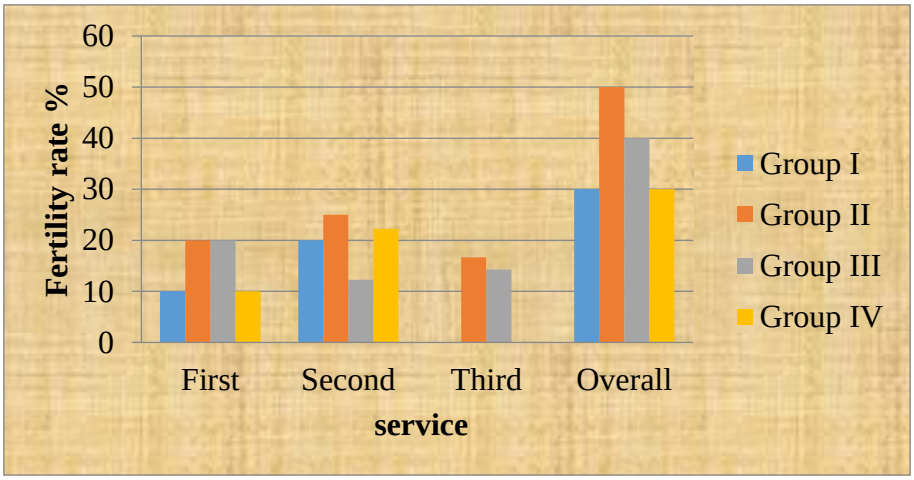

Fig. 2. Fertility rate (%) in buffalo heifers treated with hormonal protocol and control group

In control group overall fertility response was 30 percent in anestrous buffalo heifers. Contrary to the present study higher conception rate (Nakrani et al., 2014 and Buhecha et al., [13] in anestrous buffaloes and a lower conception rate (10.00%) in buffaloes Yotov et al., [11] was observed.

4. 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, edema, the 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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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