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Research Article

Effect of biostimulation and estrus synchronization on estrus response and fertility rate in primiparous and multiparous Kundhi buffaloes

Qudratullah Kalwar[1,4*], Akeel Ahmed Memon[2], Asmatullah Kaka[2], Nazar Ali Korejo[2], Yar Muhammad Jalbani[3,4], Rashid Ali Korejo[4], Tarique Ahmed Khokhar[4], Muhammad Mohsen Rahimoon[4] and Arab Khan Lund[4]

1. Lanzhou Institute of Husbandry and Pharmaceutical Sciences, Chinese Academy of Agricultural Science, Lanzhou 730050, Gansu-China
2. Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam-Pakistan
3. Institude of Quality Standard and Testing Technology for Agro Products-China
4. Shaheed Benazir Bhutto University of Veterinary and Animals Sciences, Sakrand, Sindh-Pakistan

**Corresponding author's email: qudratullahkalwar@gmail.com*

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Abstract

Anestrus and unobserved estrus are one of the crucial managemental lapses in the livestock business. This research was planned to assess the efficiency of different estrus synchronization procedures with bull exposed and with non-bull exposed groups in primiparous and multiparous buffaloes at Kundhi buffalo farm Rorhi and its surroundings. Total 40 (n=20 primiparous, n=20 multiparous) buffaloes were used in this study. The chosen buffaloes were divided into two main groups, A (BE, Bull-Exposed) and group B (NE, Non-Exposed). Based on the treatments A group was further sub-divided into two sub-groups, OvSynch bull-exposed (OBE) A1 and prostaglandin bull exposed (PBE) A2. Also, B group was parted into dual sub-groups Ovsynch non-exposed (ONE) and prostaglandin non-exposed (PNE) group B2. Results of the present study showed that estrus response differed significantly among the primiparous and multiparous Kundhi buffalo in all groups ($P<0.05$). The overall estrus response (50% and 100%) and pregnancy rates (40% and 70%) were higher in both primiparous and multiparous animals of BE groups as compared to primiparous (60% and 70%), and multiparous animals (30% and 60%) of NE groups. The overall estrus, duration was also higher in the primiparous (22 ± 0.66 hours) and multiparous animals (20 ± 2.05 hours) of BE groups, in contrast to 16 ± 0.95 hours and 14 ± 0.84 hours of NE groups. These findings suggested that the use of bull with OvSynch protocol it could efficiently be used to improve the estrus incidence and pregnancy rate in primiparous and multiparous buffaloes as compararion double prostaglandin protocol.

Keywords: Biostimulation; Estrus synchronization; Estrus response; Kundhi buffalo; Pregnancy rate

Introduction

Reproductive performance of an animal is a crucial factor in the livestock business. There are many factors which hinder the reproductive performance of an animal. Amongst these, anestrus or unobserved estrus is the prominent one. In anestrus, there is complete sexual inactivity with no manifestation of heat. The ovary becomes inactive and normal cyclic activities are seen to be immobile in this condition. There are several causes of anestrus, e.g., hormonal imbalance, poor nutrition, acquired abnormalities of the female reproductive system and seasonal changes [1].

Estrus Synchronization (ES) is one of the solutions to overcome these problems. Reproductive performance of female animals can be enhanced by the use of ES. Numerous ES techniques have been developed previosly in animals for the improvement of estrus response and fertility rate [2-4]. In these ES practices progesterone, prostaglandin, GnRH, and estrogens alone or in numerous combinations are used [5, 6]. ES involves manipulating the estrous cycle to cause as many females as possible to enter estrus at a specific time. The first efforts to do so began in the late 1960s by administering oral progesterone and an estrogen injection [7]. Since then numerous estrous synchronization protocols are continued to be developed to facilitate the usage of artificial insemination and for the improvement of the reproductive proficiency of farm animals. Estrus Synchronization and artificial insemination are two of the most significant management practices available to producers to increase the reproductive performance of their animals [8].

Bio stimulation may be defined as a male stimulatory effect on estrus response and ovulation rate in the female via pheromones [9]. Pheromones are defined as airborne chemicals released from the feces, cutaneous glands or from urine, that are sensed by the respiratory or olfactory systems that cause endocrine response and behavioral changes [10]. In male animals, pheromones are largely released from urine and they affect on hypothalamic-pituitary-adrenal (HPA) activity of female animals [11, 12]. It has been stated by exposing the cows to bulls (biostimulation) before, during, and after a GnRH-based ES protocol following fixed timed artificial insemination improved pregnancy rate in bovine animals [13, 14]. However, information related to the effects of bull exposure combination with estrus synchronization in primiparous and multiparous Kundhi buffaloes is scarce. Therefore, the current research was established, with the goal to evaluate the bio-stimulatory effect of bulls to improve the estrus response & fixed-timed artificial insemination pregnancy rate in primiparous and multiparous Kundhi buffaloes using different estrous synchronization protocols.

Materials and methods

Animals and their management

Total 40 (n=20 primiparous, n=20 multiparous) buffaloes were used in this study. These animals were raised on semi intensive managemental circumstances at Kundhi Farm Rohri and its surroundings. Before the start of experiment all the buffaloes were confirmed nonpregnant by rectal palpation.

Experimental design

The chosen buffaloes were divided into two main groups, A (BE, Bull-Exposed) and group B (NE, Non-Exposed). Based on the treatments A group was further sub-divided into two sub-groups, OvSynch bull-exposed (OBE) A1 and in prostaglandin bull exposed (PBE) A2. Similarly, B group was parted into two sub-groups ovSynch non-exposed (ONE) and prostaglandin non-bull exposed (PNE) group B2.

Sub-group-A-1.OvSynch Bull Exposed (OBE) group n=10

Estrus Synchronization was implemented with the OvSynch protocol, in which 02 ml GnRH on day 0 and 9th and 05ml PGF2α was injected on day 7, and also these buffaloes were exposed to an aproned bull two times a day i.e. at 6 am and 6 pm for half an hour, from the starting of experiment to artificial insemination.

Sub-group-A2. Prostaglandin F2α Bull-Exposed (PBE) group n=10

In this group double PGF2α was injected at the interval of 11-days. After injection of PGF2α, these animals were also exposed to an intact, aproned bull for 30 minutes, two times in a day (6am, and 6pm) from the starting of treatment till artificial insemination.

Sub-group-B-1.OvSynch Non Bull Exposed (ONE) group n=10

Synchronization was performed in these animals with a similar procedure, as used in the animals of group A-1. But the animals of this group were not exposed to bull from the start of experiment till artificial insemination.

Sub-group-B-2.Prostaglandin F2α Non-Exposed (PNE) group n=10

The treated animals were not exposed to the bull, but synchronization was performed with a similar method, as recived by subgroup B2 animals.

Estrus detection

Group A-1 and B-1 animals were observed after the injection of 2ndGnRH for behavioral changes to confirm the heat. However, animals of group A-2 and B-2 were watched closely after 2ndPGF2α inoculation to check the estrus. In group A1 and B1 artificial insemination was performed two times i.e. 12 and, 24 hours after the last gonadotropin inoculation. While artificial insemination was done after 72 and 96 hours in A2 and B2 group, following second prostaglandin shot by frozen thawed semen got from Directorate of Animal Breeding, Livestock Department Government of Sindh.

Statistical analysis

Results were calculated by using graph pad instate 3.05 versions of the statistics through Chi square test and ANOVA. The significant was considered to be at $P<0.05$.

Results

The current research was carried out to evaluate with or without biostimulation the effectiveness of different protocols of estrus synchronization (ES), in primiparous and multiparous Kundhi buffaloes. The outcomes are existing in the following tables.

Occurrence of estrus

The effect of estrus synchronisation with and without bull exposure on the appearance of estrus in OvSynch and double prostaglandin F2α groups in different parities of Kundhi buffaloes are defined in (Table 1). Intensity of estrus significantly ($P<0.05$) differed among the primiparous and multiparous Kundhi buffaloes in all groups. Furthermore, higher estrus response (100%) was detected in all multiparous animals, while primiparious animals showed (60%) and (40%) in OBE and ONE group respectively, Likewise, OvSynch non-exposed treatment induces higher estrus response in multiparous animals as compare to primiparous respectively. Overall estrus response (Table 2) was observed higher in the primiparous and multiparous animals of BE, (50%), (100%), groups as compared to NE (60%), (70%) groups respectively.

Estrus duration

The effect of synchronization of estrus with bull exposed and non-bull exposed on duration of oestrus in OvSynch & double PGF2α groups in different parities Kundhi buffaloes are presented in (Table 3). Duration of estrus significantly ($P<0.05$) differed amongst the groups. Overall estrus length was higher in the primiparous (22 ± 0.663 hours) and multiparous animals (20 ± 2.05 hours) of bull exposed groups, as compared

to the primiparous (16 ± 0.954 hrs), and multiparous animals (14± 0.841 hrs) of non-bull exposed groups (Table 4). Furthermore, longer estrus duration was prominent in primiparous and multiparous animals of OvSynch bull-exposed group (23 ± 0.577 hours), (20 ± 2.059 hours) as compared to PGF2α bull-exposed (21 ± 1.000 hours), (18 ± 0.632 hours), Ovsynch non- exposed (16 ± 1.763 hours), (15 ± 1.291 hours) and PGF2α non-bull-exposed (16 ± 1.154 hours), (14 ± 1.154 hours) groups.

Pregnancy rate

The results of synchronization of estrus with bull exposed and non bull exposed on pregnancy rate following OvSynch and double prostaglandin F2α in primiparous and multiparous Kundhi buffaloes are precised in (Table 5). These results revealed that pregnancy rate significantly (P<0.05) vary amongst all the groups. While overall pregnancy rate was greater in the primiparous (40%) and multiparous (70%) animals of bull exposed (BE) group as compared to non-exposed (NE) (30%) & (60%) groups (Table 6). Furthermore, higher pregnancy rate was identified in the primiparous and multiparous animals of OvSynch bull exposed group (40%) & (80%) as compared to prostaglandin F2α bull exposed (40%),(60%) group. However in primiparous and multiparous animals of OvSynch non-exposed (40%), (60%) and prostaglandin F2α non-bull exposed group were (20%) & (60%) respectively.

Table 1. Outcomes of estrus synchronisation with bull exposed (BE) and non bull exposed (NE) on the estrus response in OvSynch & double PGF2α groups in primiparous and multiparous Kundhi buffaloes

Groups	Parity	Estrus Response (%)	
		OvSynch	Double PGF2α
BE	Primiparious	3/5 (60%)c	2/5 (40%)c
	Multiparious	5/5 (100%)a	5/5 (100%)a
NE	Primiparious	3/5 (60%)c	3/5 (60%)b
	Multiparious	4/5 (80%)b	3/5 (60%)b

Dissimilar superscript within the similar column represents significant results, P= 0.049

Table 2. Sound effects of estrus synchronization with bull exposed (BE) and non bull exposed (NE) on the estrus response in OvSynch & double PGF2α groups in primiparous and multiparous Kundhi buffaloes

Groups	Parity	Overall Estrus Response (%)
BE	Primiparious	5/10 (50%)d
	Multiparious	10/10 (100%)a
NE	Primiparious	6/10 (60%)c
	Multiparious	7/10 (70%)b

Dissimilar superscript within the similar column represents significantly results, P = 0.045

Table 3. The effect of synchronization of estrus with bull exposed (BE) and non-bull exposed (NE) on duration of estrus in OvSynch & double PGF2α groups in primiparous and multiparous Kundhi buffaloes

Groups	Parity	Estrus duration (hours)	
		Ovsynch	Double PGF2α
BE	Primiparious	23 ± 0.577 hours[a]	21 ± 1.000 hours[a]
	Multiparious	20 ± 2.059 hours[b]	18 ± 0.632 hours[b]
NE	Primiparious	16 ± 1.763 hours[c]	16 ± 1.154 hours[c]
	Multiparious	15 ± 1.2911hours[c]	14 ± 1.154 hours[d]

Dissimilar superscript within the similar column represents significantly results, P=0.005

Table 4. The effect of synchronization of oestrus with bull exposed (BE) and non-bull exposed (NE) on overall duration of estrus in OvSynch & double PGF2α groups in primiparous & multiparous Kundhi buffaloes

Groups	Parity	Over all Estrus duration (hours)
BE	Primiparious	22 ± 0.663 hours[a]
	Multiparious	20 ± 2.05 hours[b]
NE	Primiparious	16 ± 0.954 hours[c]
	Multiparious	14 ± 0.841 hours[d]

Dissimilar superscript within the similar column represents significant results, P=0.039

Table 5. The results of synchronization of estrus with bull exposed and non bull exposed on the pregnancy rate fallowing ovSynch and double prostaglandin F2α in primiparous and multiparous Kundhi buffaloes

Groups	Parity	Pregnancy rate (%)	
		OvSynch	Double PGF2α
BE	Primiparious	2/5 (40%)c	2/5 (40%)c
	Multiparious	4/5 (80%)a	3/5 (60%)a
NE	Primiparious	2/5 (40%)c	1/5 (20%)c
	Multiparious	3/5 (60%)b	3/5 (60%)b

Dissimilar superscript within the similar column represents significant results, P=0.049

Table 6. Effect of estrus synchronization with and without bio-stimulation on overall pregnancy rate in primiparous and multiparous Kundhi buffaloes

Groups	Parity	Overall pregnancy rate (%)
BE	Primiparious	4/10 (40%)c
	Multiparious	7/10 (70%)a
NE	Primiparious	3/10 (30%)d
	Multiparious	6/10 (60%)b

Dissimilar superscript within the similar column represents significant results, P=0.038

Discussion

Reproductive insufficiency remains one of the significant hitches to the dairy farmers, which reduces the production. Efforts are being made by researchers to improve the reproductive performance of the females **[15]**. Several approaches have been applied to achieve the maximum reproductive performance, and it has been found that the use of hormones effectively reduces calving interval, and bring animals in estrus at the desired time thus improving reproductive performance **[16, 17]**. In all groups, multiparous animals showed higher estrus responses as compared to primiparous animals, and estrus response differed significantly among the groups (P<0.05). These findings are in disagreement with Khanh *et al*. **[11]** who reported that the response of estrus did not significantly differed between the primiparous and multiparous cows while they found higher estrus response in multiparous animals as compared to primiparous animals. Our findings are better than Ghosh et al. **[8]** who reported 38.9% estrous response in heifers and 77.7% in multiparous cattle. Some factors could be familiar to this outcome; different synchronization treatments may induce different physiological responses and

could show a significant variation in results. In the present research prostaglandin and OvSynch method of estrus synchronization were used whereas Ghosh *et al*. [8] used ovSynch synchronization treatment Khanh *et al*. [11] used controlled internal drug releasing device (CIDR) estrus synchronization treatment. In both cases estrus response and estrus duration, the effect may also be differed due to the difference in the species. Cows respond to treatment might be in a different manner as compared to buffaloes.

Estrus duration was higher in the primiparous and multiparous animals of both treated bull-exposed groups as compared to non-exposed groups. These results are in agreement with Khanh *et al*. [11] for the primiparous cows but not for multiparous. However, in another study by *Flores et al.* [18] who reported that reported the primiparous cows represent low estrus duration as compared to multiparous cows. The difference observed between the present study, and the above mentioned two studies may be due to the different breeds and environment. In different environmental condition and in the breeds the esrus response varies. In current findings, duration of estrus was apparent in primiparous (22±0.663 hours) and multiparous (20 ± 2.05 hours) animals of bull-exposed group followed by primiparous ((16 ± 0.954 hours) and multiparous (14 ± 0.841 hours) animals non-bull exposed group respectively. Our results of estrus duration are greater than those stated by Roeflos *et al*. [19] who declared that duration of estrus in primiparous is 13.6 hours and in multiparous animals 10.8 hours. These alterations in findings may be due to different environmental conditions, type of housing, how estrus was detected, and the frequency of handling.

In all groups, a significantly higher pregnancy rate was noted in multiparous animals as compared to primiparous animals. The present findings are similar to the Khanh *et al.* [11] who observed a higher pregnancy rate in multiparous animals as compared to primiparous animals. But they reported the non-significant difference in pregnancy rate among the primiparous and multiparous cows. Murugavel *et al.* [20] reported 57.6% conception rate in multiparous cows and 40.6% in primiparous with progesterone based estrus synchronization treatment. While in current study high conception rate was observed in multiparous buffaloes (80%) and (60%), while in primiparous (40%) conception rate was observed in case of OvSynch bull exposed and prostaglandin F2α bull exposed group respectively.

Conclusion

This study concluded that the use of bull with OvSynch protocol of estrus synchronization could efficiently be used to improve the response of estrus and fertility rate in primiparous and multiparous Kundhi buffaloes as compare to prostaglandin group.

Authors' contributions

Conceived and designed the experiments: Q Kalwar, AA Memon & A Kaka, Performed the experiments: Q Kalwar & AA Memon, Analyzed the data, YM Jalbani, RA Korejo & TA Khokhar, Contributed reagents/ materials/ analysis tools: MM Rahimoon, NA Korejo & AK Lund, Wrote the paper: Q Kalwar & AA Memon.

References

1. Ahmad S, Kumar H, Singh G & Patra MK (2011). The administration of GnRH plus PGF_2 alpha synchronizes the estrous in anestrous crossbred cows expose to bull urine. *Ind J Vet Res* 20(1): 42-45.
2. Baruselli PS, Reis EL, Marques MO, Nasser and LF & Bo GA (2004). The use of the hormonal treatments to improve reproductive performance of anestrous beef cattle in a tropical climate. *Anim Repro Sci* 82-83: 479-486.
3. Bridges GALA, Helser DE, Grum, Mussard ML, Gasser CL and Day M L (2008). Decreasing the interval between GnRH and PGF2α from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy

rates in beef cows. *Theriogenol* 69: 843–851.
4. Claro IJO, SáFilho G, Peres RF, Aono FH, Day ML & Vasconcelos JL (2010). Reproductive performance of prepubertal Bos indicus heifers after progesterone basedtreatments. *Theriogenol* 74: 903-911.
5. Brito LFC, Satrapa R, Marson EP & Kastelic JP (2006). Efficacy of PGF2α to Synchronize Estrus in water buffalo cows (*Bubalus bubalis*) is dependent upon plasma progesterone concentration, corpus luteum size, and ovarian follicular status before treatment. *J Anim Sci* 44: 154-159.
6. Cerri RLJE, Santos SO, Juchem K, Galvao N & Chebel RC (2004). Timed artificial insemination with estradiol cypionate or insemination estrus in high-producing dairy cows. *J Dairy Sci* 87: 3704–3715.
7. Wiltbank JN & Kasson CW (1968). The synchronization of estrus in Cattle with an oral progestational agent and an injection of estrogen. *J Anim Sci* 27(1): 113-116.
8. Ghosh T, Paul AK, Talukder AK. Alam MGS and Bari FY (2012). Use of Two Estrus Synchronization Protocols and Their Success on Pregnancy in Zebu and Crossbred Heifers at Char Areas of Bangladesh. *J Embr Trans* 27(4): 223-228.
9. Chenoweth PJ (1983). Reproductive management procedures in control of breeding. *Anim prod Aust* 15: 28.
10. Rekwot PI., Ogwy D & Oyedipe E (2000). Influence of bull stimulation, season and parity on the resumption of ovarian activity of zebu (Bos indicus) cattle following parturition. *J Anim Rep Sci* 63: 1-11.
11. Khanh NP, Roshina Y, Ornar MA, Dhaliwal GD, Wahid H, Kumaran AM, Yap KC, Fahmi M & Azmil A (2012). The effect of biostimulation on estrus behaviour, ovulation time and conception rate in primiparous and multiparous beef cows. *J of Vet and Anim Adv* 11(3): 404-411.
12. Mora OA & Sanchez-Criado JE (2004). Involvement of the cortical adrenal hormones in the pheromonal restoration activity in ageing rats. *Life Sci* 74: 3285-90.
13. Berardinelli JG, Joshi PS and Tuck SA (2007). Conception rates to artificial insemination in primiparous suckled cows exposed to the biostimulatory effect of bulls before, and during a gonadotropin-releasing hormone-based estrus synchronization protocol. *J Anim Sci*. 85: 848-852.
14. Tauck SA, Olsen JR, Wilkinson JRC & Berardinelli JG (2010). Duration of daily bull exposure on the resumption of ovulatory activity in postpartum, primiparous, suckled beef cows. *Anim Repro Sci* 118(1): 13–18.
15. Singh S, Wani NA & Maurya SN (2003). Use of different hormones for the treatment of postpartum anestrous in buffaloes under field condition. *Ind J Anim Sci* 73: 894-896.
16. Mehmood MU, Mehmood S, Riaz A, Ahmad N & Sattar A (2012). The super-ovulatory response in summer anestrus buffaloes and cattle treated with Estrus Synchronization protocol. *Anim and Plant Sci* 22: 888-893.
17. Neglia G, Gasparrini B, Palo RD, Rosa CD & Zicarelli L (2008). Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. *Anim Repro* 45: 120-125.
18. Flores R, Looper ML, Kreider DL, Post NM & Rosenkrans CF (2006). Estrous behaviour and initiation of estrous cycles in postpartum Brahman-influenced cows after treatment with progesterone and prostaglandin F2α. *J Anim Sci* 84: 1916–1925.
19. Roeflos J, VanEerdenburg FJCN, Soede NM & Kemp B (2005).Various behavioural signs of estrus and their relationship with time of ovulation in dairy cattle. *Theriogenol* 63: 1366-1377.
20. Murugavel K, Antoine D, Raju MS & Lo´pez-Gatius F (2009). The effect of the addition of equine chorionic gonadotropin to a progesterone-based estrous synchronization protocol in buffaloes (*Bubalus bubalis*) under tropical conditions. *Theriogenol* 71: 1120–1126.