

RESEARCH ARTICLE

Estrus intensity scoring and conception rate in postpartum anestrus buffaloes using estrus induction protocols

Renuka Mishra¹(✉), Nitin Kumar Bajaj², Satya Nidhi Shukla³, Madhuri Dhurvey⁴, Abhishek Bisen⁵, Pushpendra Maravi⁶ and Shashank Vishvakarma⁷

Received: 21 July 2023 / Accepted: 11 December 2023 / Published online: 23 August 2024
© Indian Dairy Association (India) 2024

Abstract: The onset of cyclicity of oestrus cycle and initiation of ovulation during postpartum period in buffaloes constitute a major problem and results into long postpartum anestrus and delayed breeding with consequent serious economic losses in the milk production and efficient reproduction. Effective treatment of postpartum anestrus in buffaloes using estrus induction protocols can efficiently improve their milk productivity. The objective of this study was to compare the estrus intensity score and conception rate in postpartum anestrus buffaloes using estrus induction protocols. Twenty four postpartum buffaloes (5-10 years age) reared under field conditions with the history of not showing signs of estrus for 60 days or more postpartum were considered to be affected with anestrus. These buffaloes were examined per rectally twice at 10 days apart to confirm ovarian activity and genital status. These animals were randomly divided into 4 groups (03 treatment and 01 control group) and each group having 06 anestrus buffaloes. Group I, II and III were treated with CIDR plus Ovsynch, CIDR plus Cosynch and eCG plus Cosynch protocols, respectively, while the control group (Group IV) animals were not given any treatment. The mean weighted oestrus intensity score and conception rate for group I, II, III and IV were 71.33±3.04, 67.66±3.04, 78.33±5.85, 65.00±5.00 and 50.00, 33.33, 66.67 and 33.33 per cent, respectively. It can be concluded that addition of eCG to a GnRH and prostaglandin based protocol (eCG+Cosynch) substantially improved the estrus intensity and conception rates in postpartum anestrus buffaloes.

Keywords: Postpartum anestrus, estrus intensity score, conception rate, estrus induction protocols

Introduction

Buffalo is regarded as a shy / poor breeder with delayed onset of puberty, long postpartum ovarian quiescence, poor signs of estrus and long inter-calving intervals (Brar and Nanda, 2004). In Indian climatic conditions, postpartum acyclicity is the most common single cause of infertility in rural buffaloes (Iyer, 1978). Incidence of anestrus varies from herd to herd and under different managemental conditions. Silent ovulation (lack of overt signs of estrus) and unobserved estrus (poor estrus detection efficiency) can greatly increase the incidence of anestrus.

Although signs of estrus normally are less pronounced in buffaloes, the majority of farmers detect estrus based on visual observation. When presented for AI, clinicians often base their diagnosis of estrus in buffaloes on transrectal evaluation of uterine tone, palpation of an ovulatory sized follicle and cervical status and visualization of the ovulatory follicle and/or uterus. Singh and Kharche (1985) devised a score card system with 75 and 70 as maximum weight score, respectively, to categorise the buffaloes into intense, intermediate and weak on the basis of oestrus behaviour pattern and status of reproductive organ.

Estrus induction protocol using various hormones with fixed-time artificial insemination (FTAI) have been tried for the treatment of anestrus with variable success rates (Kumar et al. 2014).

The present investigation was planned to evaluate the oestrus intensity scoring of modified Ovsynch and modified Cosynch protocols in postpartum anestrus buffaloes.

Materials and Methods

Selection of animal

The present research work was carried out in different villages of Jabalpur (M.P.). Twenty four postpartum buffaloes (5-10 years age) reared under field conditions with the history of not showing signs of estrus for 60 days or more postpartum were considered

Department of Veterinary Gynaecology and Obstetrics, C.V.Sc. & A.H., N.D.V.S.U., Jabalpur (M.P)

Renuka Mishra (✉)

Department of Veterinary Gynaecology and Obstetrics, C.V.Sc. & A.H., N.D.V.S.U., Jabalpur (M.P)

Email: renukamishra0001@gmail.com

to be affected with anestrus. These buffaloes were examined per rectally twice at 10 days apart to confirm ovarian activity and genital status. These animals were randomly divided into four groups *i.e.* three treatment group and one untreated control group (n=06 animals/group) and were dewormed. The animals of treatment groups were subjected to various synchronization protocols (Table 1) after 07 days of deworming as follows:

Application and removal of CIDR

The CIDR is made of a silicone rubber impregnated with progesterone and molded over nylon spine that is T- shaped. The wings of the CIDR fold upon themselves when placed in the applicator. The buffaloes were restrained in trevis and CIDR was inserted into the vagina. Extra nylon tail attached to the end of the CIDR was cut to prevent pulling out by neighboring animals. The CIDR was removed by pulling out the nylon tail that is exposed from the reproductive tract.

Estrus detection and breeding

Detection of oestrus was carried out twice daily (morning and evening) by visual observations. Buffaloes at induced oestrus were bred by artificial insemination (AI) on the fixed day of treatment protocols. The observations regarding induction and intensity of estrus after the end of treatment were recorded.

Estrus intensity score

Table 1: Treatment protocols for different groups

Groups (n=06 animals /group)	Treatment regimen	Schedule
I (Modified Ovsynch protocol/ CIDR + Ovsynch)	Inj. GnRH (Buserelin acetate 20µg, I/M) + CIDR Implant Inj. PGF _{2α} (Cloprostenol 500µg, I/M) + Removal of CIDR Implant Inj. GnRH (Buserelin acetate 10µg, I/M) FTAI	Day 0 Day 07 Day 09 Day 10 (24 hrs. after 2 nd GnRH injection)
II (Modified Cosynch/ Cosynch)	Inj. GnRH (20µg, I/M) + CIDR Implant Inj. PGF _{2α} (Cloprostenol 500 µg, I/M) + Removal of CIDR implant Inj. GnRH (Buserelin acetate 10µg, I/M) + FTAI	Day 0 Day 07 Day 09
III (Modified Cosynch eCG+Cosynch)	Inj. eCG (400 IU, I/M) Inj. GnRH (Buserelin acetate 20µg, I/M) Inj. PGF _{2α} (Cloprostenol 500 µg, I/M) Inj. GnRH (Buserelin acetate 10µg, I/M) + FTAI	Day -3 (3 days prior to day 0) Day 0 Day 07 Day 09
IV (Untreated Control)	No treatment	AI on estrus

It was numerically scored on the basis of a proper weightage given for each of the parameter comprising estrus changes in the external and internal genitalia and behavioural expression. The estrus intensity was classified into intense, moderate and weak grade. Animals failed to exhibit signs of estrus were considered as silent estrus. For this purpose, the score card device of Sirmour (1999) was used.

Statistical analysis

The data was analysed on R platform (R Core Team, 2018) using “dplyr” library. Data from different experiments were presented as Mean ±SE. The pair-wise comparison of means was carried out using Fisher’s multiple comparison test as per standard statistical method described by Snedecor and Cochran (1994). The difference at pd’0.05 was considered to be satisfactory significances. The results of oestrus induction were expressed in percentage.

Results and Discussion

Oestrus Induction Efficiency

In response to different treatment protocols, oestrus was observed in 100.00(06/06) per cent postpartum buffaloes in group I, II and III. In control group (group IV), 33.33 per cent of postpartum buffaloes with anestrus showed oestrus signs (Table 2).

Oestrus induction efficiency obtained in treatment group I (100 per cent) in present study was in corroboration with the findings of Mujawar et al. (2019) in postpartum Marathwadi buffaloes, Kundulkar et al. (2016) in postpartum buffaloes and Baruselli et

al. (2007) in anestrus buffaloes. However, oestrus induction efficiency obtained in present study were higher than that reported by Azawi et al. (2012) in northern Iraqi buffaloes, Vikash et al. (2014) in anestrus buffaloes of Haryana (95.74 per cent) and Ghallab and Nosier (2016) in Egyptian buffaloes (80 per cent).

Oestrus induction efficiency obtained in treatment group II (100.00%) in present study were similar to the findings of Patil et al. (2020) in postpartum anestrus buffaloes and Sah et al. (2019) in anestrus cows. It was higher in present study as compared to the study of Kumar et al. (2016) using cosynch protocols (81.30%) in postpartum anestrus buffaloes.

Oestrus induction rate of 100.00 per cent was observed in CIDR + Ovsynch group of buffaloes in 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. 2018).

Oestrus induction efficiency obtained in treatment group III (100.00%) in present study were higher than that observed by Dhaka et al. (2019) in postpartum Murrah buffaloes (86.60 per cent).

Rathore et al. (2017) in their study reported lower oestrus response (90.00 and 88.00%) than present study by eCG + CIDR and Ovsynch+eCG in postpartum anestrus Nili-Ravi buffaloes.

Suboptimal functioning of the hypothalamus–pituitary–gonadal (HPG) axis and low FSH and LH peaks is related to postpartum anestrus in the buffalo cow under tropical and subtropical conditions. In another study of Murugavel et al. (2009), eCG subsequent to 13 day of progesterone treatment increased plasma concentrations of LH and FSH and enhanced the LH peak during estrus in non-cyclic buffalo heifers. Thus, administration of eCG probably helps complete the recovery of HPG axis function already stimulated by the progesterone treatment.

The variation in results could be due to the reproductive status or stage of oestrus cycle at the beginning of the protocol, in addition variations in nutrition, managerial practices, breed, parity, age and geographical condition.

In control group animals 33.33 per cent animals showed oestrus induction which can be attributed to utero-ovarian massage during palpation resulting in enhancement of blood supply to ovary and uterus, activation of intra-ovarian factors increases the availability of hormones and growth factors; stimulation of local oxytocin production by the ovaries which consequently influence local blood circulation and luteolysis, if CL is present (Mwaanga et al. 2010).

Oestrus intensity score

Group wise total oestrus intensity score in postpartum anestrus buffaloes of different treatment and control group is depicted in table 3.

Statistical analysis revealed non-significant variation ($p \geq 0.05$) was observed between the different groups for oestrus intensity.

Table 2: Estrus induction efficiency and conception rate to various hormone protocols in control and different treatment groups

Groups (N=06/Group)	Estrus induction efficiency (per cent)	Conception rate (per cent)
Group I (CIDR+Ovsynch)	100.00 (6)	50.00 (3)
Group II (CIDR+Cosynch)	100.00 (6)	33.33 (2)
Group III (eCG+Cosynch)	100.00 (6)	66.67 (4)
Group IV (Untreated control)	33.33 (2)	33.33 (2)

Figures in parenthesis indicate number of animals

Table 3: Group wise total oestrus intensity score in postpartum anestrus buffaloes of different treatment and control group

Group	Name of group	Oestrus intensity Score	
		Range	Mean±SE
I	CIDR+Ovsynch (06/06)	64-84	71.33±3.04
II	CIDR+Cosynch (06/06)	60-88	67.66±4.20
III	eCG+Cosynch (06/06)	58-92	78.33±5.85
IV	Control (02/06)	60-70	65.00±5.00
Overall (20/24)		58-92	70.58±2.89

Figures in parenthesis indicate no. of buffaloes

Table 4: Group wise oestrus intensity at induced/observed oestrus in postpartum anestrus buffaloes

Group	Name of group	Oestrus intensity Score (per cent)			Total
		Intense	Moderate	Weak	
I	CIDR+Ovsynch (n=06)	16.67 (01)	50.00 (03)	33.33 (02)	06
II	CIDR+Cosynch (n=06)	16.67 (01)	16.67 (01)	66.67 (04)	06
III	eCG+Cosynch (n=06)	66.67 (04)	00.00 (00)	33.33 (02)	06
IV	Control (n=02)	00.00 (00)	50.00 (01)	50.00 (01)	02
	Total	30.00 (06)	25.00 (05)	45.00 (09)	20

Figures in parenthesis indicate no. of buffaloes

Table 5: Oestrus intensity at induced oestrus in postpartum anestrus buffaloes

Character	Intense	Moderate	Weak
Range	80-92	68-74	58-64
Mean weighted score	86.66±1.97	70.80±1.02	62.22±0.77
Conception rate (%)	66.67	80.00	50.00

In group III administration of PMSG three days before first GnRH resulted in development of follicle which was luteinized and transformed in to CL by first GnRH which acted as source of progesterone (Dhaka et al. 2019). The amount of progesterone secreted by this CL was sufficient enough to induce heat symptoms and additional supplementation of progesterone to improve heat intensity is not required in eCG plus Cosynch protocol. However, in other treatment groups intra vaginal device (CIDR) performed the same function by providing exogenous progesterone. So, non-significant variation ($p > 0.05$) was observed between the different groups for oestrus intensity.

Per cent distribution of buffaloes exhibited intense, moderate and weak oestrus in each treatment group is presented in the table 4.

Oestrus intensity score of buffaloes in intense, moderate and weak oestrus intensities at induced/observed oestrus are depicted in table 5. Difference in the oestrus intensity score between treatment groups clearly indicates the influence of CIDR plus Ovsynch, CIDR plus Cosynch and eCG plus Cosynch protocol at induced oestrus with different oestrus intensities. The highest oestrus intensity was observed in eCG plus Cosynch compared to other groups might be due to the effect of exogenous eCG, which sensitizes the hypothalamus receptors for the estrogen. The FSH and LH like effects of eCG is implemented through its attachment to FSH and LH receptors in theca and granulosa cells (Murphy, 2012). Higher estradiol production by larger follicles in this group mainly responsible for behavioral signs of oestrus (Nayak et al. (2009).

Non-significant ($p \geq 0.05$) variation between oestrus intensity score at induced/observed oestrus. As far as the conception rate based on the intensity of oestrus response is concerned, the results of this study revealed that conception rate was higher in animals showing moderate heat signs (80.00%) than intense (66.67%) and weak heat sign (50.00%). Sirmour (1999) studied oestrus intensity in 34 crossbred heifers and reported that 26, 7 and 1 heifers were in intense, moderate and weak oestrus (76.47, 20.59 and 2.94 per cent), respectively and Ali et al. (2012) studied oestrus intensity in 18 anestrus buffaloes treated with Ovsynch, Heatsynch and CIDR protocol and reported that 09, 03 and 02 buffaloes show intense, moderate and weak oestrus (64.28, 21.43 and 14.28 per cent), respectively. The high conception rate in moderate and intense estrus intensity animals may be attributed to optimum level of estrogen which might have been sufficient enough to induce LH surge for ovulation.

Conclusion

It can be concluded that addition of eCG to a GnRH and prostaglandin based protocol (eCG+Cosynch) substantially improved the estrus intensity in postpartum anestrus buffaloes.

Acknowledgement

Authors are thankful to Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India for providing the financial support to the research work.

References

- Ali R, Shukla SP, Nema SP (2012) Hormonal induction of ovarian cyclicality and conception rate in postpartum anestrus buffaloes. Indian J Field Veterinarian 7(4): 44-46

- Azawi OI, Ali MD, Oday SA, Salih A, Al-Hadad AS, Mouayad, SJ (2012) Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes. *Vet World* 5(4): 201-205.
- Baruselli PS, Carvalho NAT, Gimenes LU, Crepaldi GA (2007) Fixed-time artificial insemination in buffalo. *Ital J Anim Sci* 6: 107-118
- Brar PS, Nanda AS (2004) Impact of conventional managemental practices on reproductive performance of rural buffaloes. *Indian J Anim Sci* 25(2): 94-96
- Dhaka AP, Phogat JB, Singh S, Pandey AK, Sharma K, Kumari S (2019) Efficacy of modified co-synch plus protocol with or without progesterone device for estrus induction and conception rate in Murrah buffaloes under field conditions during summer season. *Buffalo Bull* 38(2): 353-361
- Ghallab RS, Noseir WM (2016) Comparative efficiency of different CIDR protocols for treatment of postpartum anestrous in Egyptian buffaloes. *Alex J Vet Sci* 49(2): 149-156
- Kumar L, Phogat JB, Pandey AK, Phulia SK, Kumar S, Dalal J (2016) Estrus induction and fertility response following different treatment protocols in Murrah buffaloes under field conditions. *Vet World* 9(12): 1466-1470
- Kumar PR, Singh SK, Kharche SD, Govindaraju CS, Behera BK, Shukla SN, Kumar H, Agarwal SK (2014) Anestrus in cattle and buffalo: Indian perspective. *Adv Anim Vet Sci* 2(3): 124-138
- Kundulkar AD, Ingawale MV, Deshmukh SG, Hajare SW, Ingole RS (2016) Absence of potential benefit of progesterone priming during ovsynch protocol in postpartum anestrous buffaloes near the end of breeding season. *Indian J Anim Repro* 37(2): 61-62.
- Mujawar AS, Razzaque WAA, Ramteke SS, Patil AD, Ali SS, Bhikane AU, Khan MA, Mogal IR (2019) Estrus induction and fertility response in postpartum anestrous marathwadi buffaloes using hormonal protocol along with vitamin E and selenium. *Int J Livest. Res* 9(3): 289-296.
- Murphy, BD (2012) Equine chorionic gonadotropin: an enigmatic but essential tool. *Anim. Reprod.*, 9: 223-230.
- Murugavel K, Antoine D, Raju MS, Lopez-Gatius F (2009) The effect of addition of equine chorionic gonadotropin to a progesterone based estrous synchronization protocol in buffaloes (*Bubalis bubalis*) under tropical conditions. *Theriogenology* 71: 1120-1126.
- Mwaanga ES, Janowski T, Zdunczyk S, Simukoko H (2010) Ovarian massage: a simple, but useful tool to manage ovarian acyclicity in dairy cows. *Bull Anim Health Prod Afr* 58: 294-295
- Nayak V, Agrawal RG, Shrivastava OP, Thakur MS (2009) Induction of estrus in true anestrous buffaloes using Crestar implant alone and in combination with PMSG. *Buffalo Bull* 28(2): 51-54
- Patil SK, Tandle MK, Bijurkar RG, Patil NA, Kumar D, Kulkarni S, Naveen Kumar S (2020) Efficacy of Co-Synch Plus CIDR Oestrus Synchronization Protocol on Fertility in Anestrous Cows and Buffaloes. *Int J Curr Microbiol Appl Sci* 9(11): 3488-3495
- Rathore R, Sharma RK, Phulia SK, Mudgal V, Jerome A, Ghuman SPS, Singh I (2017) Comparative efficacy of oestrus synchronization protocols in buffalo (*Bubalus bubalis*). *Trop Anim Health Prod* 49(7): 1377-1382
- R Core Team (2018) R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing
- Sah AK, Pandeya YR, Pathak LR, Gautam G (2019) Controlled Internal Drug Release (CIDR) Based Hormonal Protocols Effect upon Estrus Response and Pregnancy Outcome in Anestrous Cows. *Nepalese Vet J* 36: 46-52
- Sirmour SK (1999) Therapeutic and biochemical studies in anestrous cross bred heifers. M.V.Sc. thesis (Gynaecology and Obstetrics), Jawahar Lal Nehru Krishi Vishwa Vidhyalay, Jabalpur (M.P.)
- Snedecor GW, Cochran WG (1994) *Statistical Methods*, 8th Edn., The Iowa state university press, Ames, Iowa, USA, pp. 491
- Singh MM, Kharche K (1985) Sexual behavior and reproductive efficiency of cross bred cows. *Livestock Advisor* 10(4): 9-13
- Vikash VM, Malik RK, Singh P (2014) Impact of CIDR in combination with different hormones for treatment of anestrous in buffaloes under field conditions of Haryana. *Haryana Vet* 53: 28-33
- Zaabel SM, Hegab AO, Montasser AE, El-Sheikh H (2018) Reproductive performance of anestrous buffaloes treated with CIDR. *Anim Reprod* 6(3): 460-464