



Short Communication

Improvement of Fertility Rate Using Ovsynch and Biostimulation Protocols with Timed Artificial Insemination in Thari Cattle

Ghulam Nabi Dahiri¹, Akeel Ahmed Memon², Qudratullah Kalwar^{3*}, Asmatullah Kaka², Waseem Ali Vistro⁴, Yar Muhammad Jalbani³, Kiran Nazish³ and Jai Parkash Kolhi⁵

¹Directorate of Animal Breeding, Hyderabad

²Department of Animal Reproduction, Sindh Agriculture University, Tandojam

³Shaheed Benazir Bhutto University of Veterinary and Animal Sciences, Sakrand, Sindh

⁴Department of Anatomy and Histology, Sindh Agriculture University, Tandojam

⁵Department of Animal Products Technology, Sindh Agriculture University, Tandojam

Ghulam Nabi Dahiri, Akeel Ahmed Memon and Qudratullah Kalwar contributed equally to this work.

ABSTRACT

The study was carried out to compare efficacy of estrus synchronization protocols with or without biostimulation in Thari cattle. A total of forty Thari cattle maintained at semi intensive management conditions at Thari Cattle Farm Nabisir Road, Distt: Umerkot were used in the study. The selected animals were divided into four groups to observe estrus signs and artificial insemination. Animals of group B and D were treated with OvSynch (GnRH at day 0 followed by PGF2 α day 7 and 2nd GnRH at day 9) for estrus synchronization but D was bio-stimulated and B was not bio-stimulated. Animals in group A and C were treated with 2ml normal saline on day 0, 7 and 9 of the treatment. Group C was biostimulated and A was not biostimulated. The results showed that estrus response (60 %) and pregnancy rate (50%) was higher, onset of estrus was earlier (54.89 ± 1.20), duration of estrus was long (23.17 ± 1.20) in group D as compare to other groups. It was concluded in the present study that the biostimulation with ovsynch protocol can effectively be used to induce cyclic activities and increase estrus response and fertility rate in Thari cattle.

Article Information

Received 20 September 2018

Revised 26 October 2020

Accepted 24 November 2020

Available online 01 March 2022
(early access)

Published 23 September 2022

Authors' Contribution

GND, AAM, QK and AK conceived and designed the study and performed the experiments. WAV, YMJ, KN and JPK help in revision of manuscript. GNB, AAM and QK wrote the manuscript.

Key words

Thari cattle, Estrus synchronization, Estrus response, Pregnancy rate

Reproductive efficiency of dairy animals has been decreasing in the past 50 years. Reduced reproductive efficiency not only frustrate the dairy producers and their consultants but also substantially reduce the dairy farm profitability. Several approaches have been applied to improve reproductive efficiency of dairy animals and it has been found that the use of hormones for induction of estrus in anestrus and/or post-parturient animals effectively reduce calving interval thus improving reproductive performance (Mehmood *et al.*, 2012; Tenhagen *et al.*, 2005). Induction of estrus in a group of animals or bringing a group of females into estrus (heat) at a predetermined time with the use of hormones is called estrus synchronization (ES).

ES involves the manipulation of the estrus cycle in order to cause as many females as possible to enter estrus at a specific time. The first efforts to do so began in the late 1960's by administering oral progestins and an estrogen injection (Wiltbank and Kasson, 1968). Since then numerous ES protocols continue to be developed in order to facilitate the use of artificial insemination (AI) and improve the reproductive efficiency of farm animals. Utilizing AI and ES offers many benefits to farmers. AI allows access to elite genetics that would not otherwise be available for use. Using AI in combination with ES can improve the reproductive efficiency and management. ES and AI are two most important management practices available for producers to increase reproductive performance of their animals (Jabeen *et al.*, 2012; Roy and Prakash, 2009).

Furthermore, it has been reported that fixed-timed AI pregnancy rate and overall AI pregnancy rate of bovine animals improved by exposing the cattle to bulls (biostimulation) before, during, and after a GnRH-based ES protocol (Berardinelli *et al.*, 2007; Tauck and Berardinelli, 2007). Biostimulation may be defined as the stimulatory

* Corresponding author: qudratullahkalwar@gmail.com
0030-9923/2022/0006-2981 \$ 9.00/0



Copyright 2022 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

effect of a male on estrus and ovulation through genital stimulation, olfactory pheromones, or other less well defined external cues such as tactile, visual and auditory (Chenoweth, 1983). However, very little information is available on the combined use of biostimulation with ES protocols in Thari cows. Hence, this study was intended with the aim to determine the biostimulatory effect for improvement of the synchronization of estrus and fixed-timed AI, pregnancy rates of Thari cows, with use of Ovsynch synchronization protocol.

Materials and methods

Forty Thari cattle of 1st to 4th parity maintained on semi intensive management conditions at Thari Cattle Farm Nabisir Road, Distt. Umerkot and its surroundings were used in the study. Before the start of experiment rectal palpation was done to confirm non-pregnant females. The feeding and drinking was done according to routine practices of the farm.

The females were grouped into four groups each of 10: (i) Group-A (non-treated without biostimulation) in which animals received 2ml sodium chloride (NaCl) on 0, 7 and 9th of the experiment and were not exposed to bull. (ii) Group-B (Ovsynch without biostimulation n=10) in which animals were administered with 2ml of GnRH (Dalmarelin, Fatro-Italy) on day 0, on day 7 5ml PGF2 α (Lutalyse[®], Pfizer-USA) and 2nd injection of 2ml GnRH (Dalmarelin, Fatro-Italy) on day 9. Females were not exposed to bull. (iii) Group-C (non-treated with biostimulation) in which females were injected 2ml of normal saline on day 0, 7 and 9 of the synchronization and females were exposed to biostimulation for half an hour daily (6 am and 6 pm) from day 0 of synchronization to artificial. (iv) Group-D (Ovsynch with biostimulation) in which females were injected with Ovsynch protocol which involves administration of 2ml GnRH (Dalmarelin, Fatro-Italy) on day 0, injection of 5ml PGF2 α (Lutalyse[®], Pfizer-USA) on day 7 and a 2nd injection of 2ml GnRH (Dalmarelin, Fatro-Italy) at day 9. Females were also exposed to biostimulation for half an hour, daily (6 am and 6 pm) from day 0 of synchronization to AI.

Females of all groups were watched daily for heat from the start of the treatment (day 0). All females were inseminated after the last injection (day 9) following protocols of injection at 12 and 24 h after onset of estrus with semen received from Directorate of Animal Breeding (DAB), The following parameters were recorded in all groups. (i) Onset of estrus were watched closely (in females of all groups) after 2nd injection of PGF2 α for behavioral signs of heat. The major behavioral signs were observed including mounting, bellowing, sniffing, restlessness and micturition. The animals were inseminated artificially at 12 and 24 h, after third injection of GnRH in group D and

B. However, females of A and C group were artificially inseminated twice 72 and 96 h after the PGF_{2 α} injection. (ii) Duration of estrus was recorded by observing estrus signs visually from onset of estrus signs till cessation of estrus signs. (iii) Fertility/ Pregnancy rate was determined by per rectum palpation at 2 months post AI.

Statistical package Graph pad instate 3.05 versions of was used to analysis the data. Analysis of variance (ANOVA). To determine significant Difference between means of groups' chi square test was used. Significant level was considered at P<0.05.

Results

The effect of ovsynch protocol with or without biostimulation on estrus response, onset of estrus, estrus duration and fertility rate of Thari cattle is shown in Table I. Estrus response was significantly different among the groups (P<0.05). Ovsynch bull-exposed group showed higher estrus response as compared to other groups. In addition to that multiparous animals significantly showed better estrus response as compared to primiparous Thari cattle in all groups (Table I). The onset of estrus was earlier with ovsynch biostimulated group than non-treated non-exposed, and control group. Similarly onset of estrus was lower in than ovsynch bostimulated group (54.89 \pm 1.20) and higher in control group (60 \pm 0.00), respectively (Table I). Moreover, onset of estrus differ significantly among bull (exposed and non-exposed) group (P<0.05) and primiparous animals showed earlier onset of estrus as compare to multiparous animals (Table I). The estrus duration did not differ significantly among ovsynch bull exposed and non-exposed the group (P<0.05). While duration of estrus was higher with ovsynch bostimulated group than non-treated non-exposed, and control groups. However, primiparous animals showed longer estrus duration as compare to multiparous animals (Table I). Pregnancy rate significantly differed among the groups (P<0.05). Ovsynch bull-exposed group showed higher pregnancy rate as compare to other treated and control groups (Table I). In addition to that multiparous animals showed significantly higher pregnancy rate as compare to primiparous Thari cattle in all groups (Table I).

Discussion

ES technique is applied to reduce the problems of silent heat and anestrus problems in cattle. Hormones like gonadotropin releasing hormone prostaglandin F2 α and analogues are being used for ES (Khumran *et al.*, 2012). The ovsynch synchronization protocol is the most popular ES protocol which consists of series of GnRH -PGF2 α -GnRH injection treatments since last decade and provides satisfactory fertilization to timed AI (Jabeen *et al.*, 2012).

Table I. The effect of ovsynch protocol with or without biostimulation on estrus response, onset of estrus, estrus duration and on number of pregnancies in Thari cattle.

Parameters	Groups			
	A	B	C	D
No. of animals showed estrus response	01	05	02	06
Onset of estrus (hours)	60 ± 0.00 ^d	56 ± 0.80 ^b	59 ± 1.00 ^c	54.89 ± 1.20 ^a
Estrus duration (hours)	18 ± 0.00 ^a	22.05 ± 0.60 ^a	21 ± 1.500 ^a	23.17 ± 1.20 ^a
Number of pregnancies	00	04	01	05
No. of animals showed estrus response	Primiparous	5/0	5/2	5/0
	Multiparous	5/1	5/3	5/2
Onset of estrus (hours)	Primiparous	0.0+0.00	56 +0.50 ^b	0.0±0.00
	Multiparous	60+0.00	56 +0.50 ^b	59 ±1.00
Estrus duration (hours)	Primiparous	0.0+0.00 ^c	22.00±0.00 ^a	0.0 ±0.00 ^c
	Multiparous	18 + 0.00 ^a	21±1.108 ^a	21 ±1.500 ^a
Number of pregnancies	Primiparous	5/0	5/1	5/0
	Multiparous	5/0	5/3	5/1

Different superscripts within the column shows significant difference $p < 0.05$

This protocol is being used in buffaloes with satisfactory pregnancy rates (Chaikhun *et al.*, 2010). Biostimulation or effect of a male via genital stimulation, olfactory pheromones, or other less defined external sign such as tactile, visual and auditory have been used to induce estrus (Chenoweth, 1983). In the current study response of estrus was significant in the cattle with ovsynch and biostimulated group than non-treated non-exposed, and control groups. Roy and Prakash (2009) reported 18% reduction in anestrus rate with synchronization and biostimulation. Estrus response was higher with ovsynch with biostimulation (60%) than ovsynch non-exposed to biostimulation (50%). However, difference was non-significant among groups ($P > 0.05$). These results are in agreement with the findings of Berardinelli *et al.* (2001). They reported that the response of estrus was more in postpartum cattle exposed to biostimulation during synchronization of estrus than non biostimulated. Ahmed *et al.* (2011) reported 90% of estrus response in cattle exposed to biostimulation than 65% females without biostimulation. Similarly, Alberio *et al.* (1987) also reported 67.9% estrus response with biostimulation than 32.7% without biostimulation.

In the current study multiparous animals showed significantly higher estrus response as compared to primiparous Thari Cattle in all groups. All multiparous animals showed significantly better estrus responses as compared to primiparous animals among the groups ($P < 0.05$). Contrary to this, Khanh *et al.* (2012) observed non-significant difference among the primiparous and multiparous cattle, however, estrus response was higher

in multiparous than primiparous animals. Similarly, Ghosh *et al.* (2012) reported lower estrus response in primiparous (38.9%) than in multiparous (77.7%) cattle. Number of factors could be affecting this outcome such as synchronization protocols may have induced difference in physiological response and played a major role in variation among observations. In the present study ovsynch method of ES were used whereas Khanh *et al.* (2012) used progesterone supplementation in the form of controlled internal drug releasing device (CIDR) for ES. In this study estrus duration was higher with ovsynch biostimulation than non-treated without biostimulation, and control groups. This finding lies in range with Khanh *et al.* (2012). However, Flores *et al.* (2006) observed higher duration of estrus ($P < 0.05$) in multiparous than primiparous cattle. The difference observed between present study and above two studies may be due different breeds and environmental condition which induce variation response among synchronization treatments. In the current study the duration of estrus were 23 and 22 h in primiparous and multiparous animals, respectively. This was higher than results by Roelofs *et al.* (2005). They reported 13.6 and 10.8 h duration of estrus in primiparous and multiparous females, respectively. The difference in results may be due to difference in housing, handling and environmental condition.

In this current study pregnancy rate was significantly higher in ovsynch with biostimulations group 50% as compared to ovsynch without bio-stimulations group (40%). Tauck and Berardinelli (2007) found 59% and 37% fertility rate with biostimulation and without biostimulation

in cattle, respectively. These findings support the result of current study. In the present study 50% and 40% pregnancy rate were recorded with biostimulation and without biostimulation. These findings are lower than those reported by Purabi *et al.* (2011). Ahmed *et al.* (2011) found 81.82%, 66.7% and 40.0%, 33.7% conception rate with biostimulation and without biostimulation group, respectively. The difference in the results may be due to difference in environment, breed and ES protocol.

In the current study 60% conception rate was observed in multiparous cattle in ovsynch with bio-stimulation and ovsynch without biostimulations, while in primiparous these values were 40% and 20%, respectively with ovsynch with biostimulations and ovsynch without biostimulation. The present results are in line with those of Khanh *et al.* (2012) who observed higher pregnancy rate in multiparous animals compared to primiparous animals. However, the differences were non-significant. Murugavel *et al.* (2009) observed 57.6% conception rate in multiparous cattle and 40.6% in primiparous cattle with progesterone based ES protocol.

Conclusions

It can be concluded from the present study that: The estrus response and pregnancy rate was higher in bull exposed (with biostimulation) groups as compare to non-bull exposed (without biostimulation) groups.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

- Ahmad, S., Kumar, H., Singh, G., and Patra, M.K., 2011. *Ind. J. Vet. Res.*, **20**: 42-45. <https://doi.org/10.1186/1297-9716-42-20>
- Alberio, R.H., Shiersmann, G., Carou, N. and Mestre, J., 1987. *Anim. Reprod. Sci.*, **14**: 263-272. [https://doi.org/10.1016/0378-4320\(87\)90016-9](https://doi.org/10.1016/0378-4320(87)90016-9)
- Berardinelli, J.G., Anderson, K., Robinson, B. and Adair, R., 2001. *Proc. Western Sect. Am. Soc. Anim. Sci.*, pp. 52.
- Berardinelli, J.G., Joshi, P.S. and Tuck, S.A., 2007. *J. Anim. Sci.*, **85**: 848-852. <https://doi.org/10.2527/jas.2005-763>
- Chaikhun, T., Tharasanit, T., Rattanatep, J., De Rensis, F. and Techakumphu, M., 2010. *Theriogenology*, **74**: 1371–1376. <https://doi.org/10.1016/j.theriogenology.2010.06.007>
- Chenoweth, P.J., 1983. *Anim. Prod. Aust.*, **15**: 28.
- Flores, R., Looper, M.L., Kreider, D.L., Post, N.M. and Rosenkrans, C.F., 2006. *J. Anim. Sci.*, **84**: 1916–1925. <https://doi.org/10.2527/jas.2005-692>
- Ghosh, T., Paul, A.K., Talukder, A.K., Alam, M.G.S. and Bari, F.Y., 2012. *J. Embr. Tran.*, **27**: 223-228. <https://doi.org/10.12750/JET.2012.27.4.223>
- Jabeen, S., M. Anwar., S.M.H., Rabi, A., Mehmood, S., Murtaza and Shahab, M., 2012. *Pak. Vet. J.*, **33**: 221-224. <https://doi.org/10.1007/s00292-012-1694-5>
- Khanh, N.P., Roshina, Y., Ornar, M.A., Dhaliwal, G.K., wahid, H., Kumran, A.M., Yap, K.C., Fahmi, M. and Azmil, A., 2012. *J. Vet. Anim. Adv.*, **11**: 404-411. <https://doi.org/10.3923/javaa.2012.404.411>
- Khumran, A.M., Roshina, Y., Arif, M.O., Wahid, H., Dhaliwala, G., Khanh, N.P., Yap, K.C., Fahmi, M. and Azmil, M.E., 2012. *J. Anim. Vet. Adv.*, **11**: 3561-3567. <https://doi.org/10.3923/javaa.2012.3561.3567>
- Mehmood, M.U., Mehmood, S., Riaz, A., Ahmad, N. and Sattar, A., 2012. *J. Anim. Pl. Sci.*, **22**: 888-893.
- Murugavel, K., Antoine, D., Raju, M.S. and Lo'pez-Gatius, F., 2009. *Theriogenology*, **71**: 1120–1126. <https://doi.org/10.1016/j.theriogenology.2008.12.012>
- Purabi, B., Yadav, M.C., Kumar, H. and Meur, S.K., 2011. *Orig. Article Buff. Bull.*, **30**:11-12.
- Roeflos, J., Van Eerdenburg, F.J.C.N., Soede, N.M. and Kemp, B., 2005. *Theriogenology*, **63**: 1366-1377. <https://doi.org/10.1016/j.theriogenology.2004.07.009>
- Roy, K.S. and Prakash, B.S., 2009. *Trop Anim. Hlth. Prod.*, **41**: 677-687. <https://doi.org/10.1007/s11250-008-9241-3>
- Tauck, S.A. and Berardinelli, J.G., 2007. *J. Anim. Sci.*, **85**: 1669-1674. <https://doi.org/10.2527/jas.2006-849>
- Tenhagen, B.A., Kuchenbuch, S. and Heuwieser, W., 2005. *Reprod. Domest. Anim.*, **40**: 62-67. <https://doi.org/10.1111/j.1439-0531.2004.00557.x>
- Wiltbank, J.N. and Kasson, C.W., 1968. *J. Anim. Sci.*, **27**: 113. <https://doi.org/10.2527/jas1968.271113x>