Journal of Animal Research: v.12 n.2, p. 273-277. April 2022

DOI: 10.30954/2277-940X.02.2022.17

Inclusion of Antioxidants in Ovsynch Protocol for Improving Fertility in Pubertal Anestrus Buffalo Heifers

Pooja Gayke[1], W.A.A. Razzaque[1]*, Anil Patil[1], S.S. Ramteke[1], N.Z. Gaikwad[1], S Sajid Ali[2] and Suresh Jadhav[1]

[1]College of Veterinary and Animal Sciences, MAFSU, Udgir Dist. Latur, Maharashtra, INDIA
[2]College of Veterinary and Animal Sciences, MAFSU, Parbhani, Maharashtra, INDIA

**Corresponding author: WAA Razzaque; E-mail: waquarahmed@mafsu.in*

Received: 10 Feb., 2022 **Revised:** 17 March, 2022 **Accepted:** 20 March, 2022

ABSTRACT

Present work was carried out to study the effect of hormonal regimens, together with vitamin E and selenium on *estrus* induction and fertility response in pubertal anestrus buffalo heifers. A total of 24 buffalo heifers were selected and equally divided into two groups. Heifers in Group I were treated with Ovsynch protocol, whereas Group II heifers were treated with Ovsynch plus antioxidants (Vitamin E and Selenium) at a dosage rate of 1 ml/50 kg body weight. In both treatment groups, the response to *estrus* induction was 100 per cent. Time required for onset of *estrus* ranged between 40 to 65 and 40 to 62 hrs. with an average of 50.17±2.66 and 47.83±1.92 hrs. In Group I and Group II, the duration of *estrus* was 21.42±0.62 hours and 22.17±0.66 hours, respectively. Intensity of *estrus* was Intense, intermediate and weak in group I as 33.33, 41.66 and 25.00 per cent, while in group II as 41.66, 50.00 and 8.33 per cent, respectively. First service conception rate in group I treated with Ovsynch protocol was 33.33% and in group II treated with Ovsynch protocol along with antioxidant was 41.66% and similarly overall pregnancy rate in group I and II was 58.33% and 66.66% respectively.

HIGHLIGHTS

- Inclusion of antioxidants in Ovsynch protocol improves fertility in pubertal anestrus buffalo heifers.
- Higher first service conception rate is achieved by including Vitamin E and Selenium in Ovsynch protocol.

Keywords: Pubertal anestrus buffalo heifers, Vitamin E and Se, Ovsynch, Oxidative stress

In India, the livestock industry is the most important source of income in rural areas. Due to its multifaceted utility and important role in Indian economy buffalo is regarded as BLACK GOLD of India. The rural economy in India suffers as a result of a higher prevalence of low reproductive performance in buffaloes (Kumar *et al.,* 2009). In order to achieve more young ones in a lifetime along with the challenging constraint of higher age at first calving and pubertal anestrus needs technical intervention for augmenting fertility. Low plane of nutrition, stress of season or extreme climatic conditions poses a herd problem in general and heifers in particular. Expression of overt signs of estrus is greatly affected by heat stress in buffaloes (Kumar *et al.,* 2014). Anestrus buffaloes treated with various hormonal regimens had partial success in terms of ovulation synchronization and first service conception rate (Ghuman *et al.,* 2009). In buffalo heifers, *estrus* synchronization has proven to be beneficial in resolving the problem of anestrus (Kaliannan *et al.,* 2018) helping in breaking the length of prolonged non-productive phase of the life and efficiently boosting the reproductive function. The Ovsynch protocol, which consists of two injections of gonadotropic releasing hormone (GnRH) paired with a single administration of prostaglandin F2 alpha (PGF2α) and fixed-time artificial insemination (FTAI), is the most

How to cite this article: Gayke, P., Razzaque, W.A.A., Patil, A., Ramteke, S.S., Gaikwad, N.Z., Ali, S.S. and Jadhav, S. (2022). Inclusion of Antioxidants in Ovsynch Protocol for Improving Fertility in Pubertal Anestrus Buffalo Heifers. *J. Anim. Res.,* **12**(02): 273-277.

Source of Support: None; **Conflict of Interest:** None

efficient alternative option for overcoming the challenge of sub *estrus* and anestrus in buffalo heifers. Vitamin E, along with Selenium, is a powerful lipid-soluble chain-breaking antioxidant that inhibits peroxidation reactions by scavenging oxygen radicals and terminating free radical chain reactions thereby protecting the biological membrane from damage (Kassab *et al.,* 2020). Vitamin E and Selenium are the utmost needed constituent of the antioxidant defense mechanism having important role in reproductive performance (Maraba *et al.,* 2018). Vitamin E intake promotes fertility by improving steroid synthesis and release, follicle development and ovulatory *estrus* symptoms (Srivastava, 2008).

The purpose of this study was to determine the efficacy of hormonal therapy in pubertal anestrus buffalo heifers utilizing injections of gonadotropin releasing hormone (GnRH), prostaglandin F2α (PGF2α), vitamin E and Selenium.

MATERIALS AND METHODS

The research was carried out at the College of Veterinary and Animal Sciences, Udgir, in the Department of Animal Reproduction, Gynaecology and Obstetrics. Twenty-four anestrus buffalo heifers, aged 3 years and above, were selected. The animals were selected based on their reproductive history and gynaeco-clinical examination and were equally divided in 2 treatment groups. Group **I,** 12 heifers were administered Inj. 20 µg GnRH (Buserelin acetate, 5 ml I/m.) on day 0, Inj. $PGF_2\alpha$ 500 µg (Cloprostenol sodium 2 ml I/m.) on day 7, Inj.20 µg GnRH (Buserelin acetate, 5 ml I/m.) on day 9 and fixed time AI was done. Group II**,** 12 heifers were administered Inj.20 µg GnRH (Buserelin acetate, 5 ml I/m.) on day 0, Inj. $PGF_2\alpha$ 500 µg (Cloprostenol sodium 2 ml I/m.) on day 7 and Inj. Vit. E and Se @ 1 mg/50 kg body weight S/c on day 7, Inj.20 µg GnRH (Buserelin acetate, 5 ml I/m.) on day 9 and fixed time AI was done on day 10, Inj. Vit E and Se @ 1 mg/50 kg body weight S/c on day 14.

STATISTICAL ANALYSIS

The data generated on onset of *estrus*; duration of *estrus* was subjected to student's "t" test using SPSS software ver. 24.

RESULTS AND DISCUSSION

The *estrus* induction response, time required for onset of *estrus*, duration of *estrus* and intensity of *estrus* in treated pubertal anestrus buffalo heifers using two hormonal protocols is presented in Table 1.

All the heifers (100 %) treated for anestrus in the two groups responded to the treatment and expressed *estrus*. Response (100 %) to the treatment protocol for induction of *estrus* is clearly suggests that GnRH caused the release of FSH and LH from the pituitary. The LH stimulated the ovulation of the dominant follicle if any present on the ovary along with this FSH stimulated for new follicular wave. On day 7, therapy with prostaglandins causes CL to fade and progesterone levels to drop. The second dose of GnRH triggered another LH surge and ovulation 24 to 32 hours later. The present finding for *estrus* induction response is in agreement with Kaliannan *et al.* (2018) and Phogat *et al.* (2018) who reported 100 per cent response in buffalo heifers treated with Ovsynch treatment protocol. Similar finding for *estrus* induction response was reported by Alyas *et al.* (2013); Nakrani *et al.* (2014); Alyas *et al.* (2015); Kumar *et al.* (2015); Ahmed *et al.* (2016); Biradar *et al.* (2016); Manokaran *et al.* (2018); Dhami *et al.* (2019) and Mujawar *et al.* (2019) who all reported (100%) *estrus* induction response using Ovsynch protocol in anestrus buffaloes. Time required for the onset of *estrus* in buffalo heifers subjected to two treatments for Group I and Group II ranged between 40 to 65 hours and 40 to 62 hours, respectively. The average time needed for the start of *estrus* in Group I and II buffalo heifers was 50.17±2.66 and 47.83±1.92 hrs., respectively. Time required for onset of *estrus* was numerically less in Group II as compared to Group I however ANOVA revealed that there was non-significant difference between two treatment groups. Duration of *estrus* in buffalo heifers in group I ranged between 18-25 hrs. and the mean duration of *estrus* was 21.42±0.62 hrs. In group II it ranged between 20-26 hrs. and the mean duration of *estrus* was 22.17±0.66 hrs. ANOVA suggested that there was no statistically significant difference in *estrus* duration between the two treatment groups. The present finding for duration of *estrus* in treated buffalo heifers is in accordance to the earlier reports of Kaliannan *et al.* (2018) they observed that the average duration of *estrus* in buffalo heifers was

Table 1: Time required for onset, duration and intensity of *estrus* in treated pubertal anestrus buffalo heifers

Group	No. of animals treated	Estrus induction response (%)	Time required for onset of *estrus* (hrs.)	Duration of *estrus* (hrs.)	Intensity of *estrus* (%)		
					Intense	Intermediate	Weak
Group I (Ovsynch)	12	100.00	50.17±2.66	21.42±0.62	33.33	41.66	25.00
Group II (Ovsynch + Antioxidant)	12	100.00	47.83±1.92	22.17±0.66	41.66	50.00	8.33

Table 2: Conception rate in buffalo heifers treated with two different treatments

Treatment group	No. of Buffalo heifers treated	Buffalo heifers responded (%)	Conception rate in first service (%)	Conception rate subsequent service (%)	Overall Conception rate (%)
Group I	12	100.00	33.33	37.50	58.33
Group II	12	100.00	41.66	42.85	66.66

23.7±1.18 hours. Duration of *estrus* recorded during the present study was higher than the earlier reports and hence not in agreement with. Porto-Filho *et al.* (2014); Layek *et al.* (2013) and Velladurai *et al.* (2014) reported the mean duration of *estrus* was 11.0±5.3, 16.67±2.22 and 168 to 18.2 hrs respectively in postpartum buffaloes. Deka *et al.* (2018) reported the average duration of *estrus* was 19.41 ± 0.97 hrs. in buffalo cows. This variation may be due to difference in hormonal protocols, age, breed, parity, body condition and geographical condition and season of the experiment. Observation of displayed symptoms of *estrus* with estimated intensity would be helpful for proper timing of artificial insemination (AI) and achieving optimal conception rate Garcia *et al.* (2011). On the basis of the score achieved related to manifestation of various signs and symptoms by an individual animal intensity of *estrus* was divided into three categories viz. intense, intermediate and weak *estrus*. Intensity of *estrus* in Group I was intense, intermediate and weak in 4 (33.33%), 5 (41.66%) and 3 (25.00%), respectively. However, intense and intermediate intensity of *estrus* 5 (41.66%) and 6 (50.00%), respectively was comparatively higher in Group II buffalo heifers also 1 (8.33%) buffalo heifer manifested weak *estrus*. Intensity of *estrus* varies during different season as it is highest during winter followed by monsoon and least during summer season. Kaliannan *et al.* (2018) reported the intensity of *estrus* as intense in 20.00%, intermediate in 60.00% and weak *estrus* in 20.00 % buffalo heifers. Velladurai *et al.* (2014); Alyas *et al.* (2015); Kumar *et al.* (2015) and Ahmed *et al.* (2016) reported the intensity of *estrus* as intense in 16.67 to 20.00%, intermediate in 50.00 to 66.67% and weak *estrus* in 20.00 to 33.33 per cent buffaloes. Gupta *et al.* (2015) reported *estrus* intensity as excellent, good, fair and poor in 20, 10, 20 and 10 per cent buffaloes, respectively treated with Ovsynch protocol. Mujawar *et al.* (2019) reported *estrus* intensity as intense, intermediate and weak in 12.5, 75 and 12.5 per cent buffaloes, respectively. The difference in the intensity of *estrus* may possibly be due to effect of season, environment and management factors.

Farmers were advised to observe all the animals being inseminated and to report if any of them return to *estrus*. They were regarded to be conceived based on no return to *estrus* and on or after day 42 of insemination, which was further verified by per rectal examination on day 60. In group I and group II, 4 (33.33%) and 5 (41.66%) buffalo heifers, respectively conceived at first service. Those who did not conceive during the first *estrus* began cycling and were inseminated again during the second *estrus*. In group I and group II, 3 (37.5%) and 3 (42.85%) conceived out of 8 and 7 buffalo heifers, respectively during subsequent *estrus*. The overall pregnancy rate in group I and II was 7/12 (58.33%) and 8/12 (66.66%), respectively. Higher incidence of pubertal anestrus, silent or delayed ovulations, not conceiving at first service after treatment and delay in age at first calving Gokuldas *et al.* (2010) leads to heavy economic loss to the buffalo industry. In present study, the results obtained in terms of first service conception and conception during subsequent service is in agreement with the earlier reports of Hussein *et al.* (2016); Derar *et al.*

(2018); Kaliannan *et al.* (2018) and Phogat *et al.* (2018) who reported 40%, 62.5%, 30% and 20% conception rate in buffalo heifers. Rathore *et al.* (2017) and Sharma *et al.* (2017) reported 36.36% and 23.1% conception rate respectively in buffaloes treated with the Ovsynch protocol. Alyas *et al.* (2013); Prasad *et al.* (2019) and Tiwari *et al.* (2019) reported 66.67, 60 and 66.7 per cent conception rate in anestrus and repeat breeder buffaloes treated with Ovsynch protocol. Warriach *et al.* (2008) reported 55.5 and 30.4 per cent, conception rate in high and low breeding season in synchronized buffaloes, respectively treated with Ovsynch protocol. Qureshi *et al.* (2010) reported the conception rate in control group was 45% and in treated group with vit E and Se was 66% in dairy buffaloes. Wani *et al.* (2018) and Mujawar *et al.* (2019) reported 66.66 and 75.00 per cent conception rate in buffaloes treated with Ovsynch plus antioxidant. Erythrocytes synthesize nitric oxide enzymatically, using L-arginine as substrate, just like endothelial cells Kleinbongard *et al.* (2006) and this dilatation of blood vessels supplying to uterus and other reproductive tract causes increase in the tonicity of uterine horn which favors the movement of gametes towards the site of fertilization and successful conception.

CONCLUSION

Estrus can be effectively induced by using Ovsynch and Ovsynch plus antioxidant protocol in buffalo heifers. Intensity of *estrus* in buffalo heifers treated with hormonal protocol with antioxidant was better than buffalo heifers treated with hormonal protocol. More number of heifers settled to first service and in subsequent service when treated with Ovsynch protocol along with antioxidant as compared to Ovsynch protocol without antioxidant.

ACKNOWLEDGEMENTS

We are thankful to Associate Dean, College of Veterinary and Animal Sciences, Udgir, for providing the required facilities and assistance in carrying out this research.

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