RESEARCH ARTICLE

Induction of Estrus in Postpartum Anestrus Crossbred Cattle

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Abstract

The present experiment was designed for estrus induction in post-partum anestrus cattle. A total of 120 cows were examined for reproductive status, and 30 were incorporated in induction, including 24 anestrus and 6 normal cyclic cows. The cows were assigned to 5 groups (6 animals per group). The group-1 cows were induced using Ovsynch protocol, while group-2 cows were inserted TRIU-B (958 mg progesterone) intra-vaginal and were inseminated at a fixed time. The group-3 cows were supplemented with a standard chelated mineral mixture of 40 g per-oral daily for 45 days and were inseminated on observed estrus. Group-4 included normal cyclic untreated cows inseminated at estrus. The group-5 included untreated anestrus control cows. Estrus induction response was 100% in groups 1 and 2, 66.66%, and 0.00% in groups 3 and 5, respectively. The conception rate in groups 1, 2, 3, 4 and 5 were 33.33%, 50.00%, 75.00%, 83.33% and 0.00%, respectively. The mean serum progesterone in different groups increased non-significantly from days 0 to 21 post-insemination. All groups' mean serum calcium increased significantly from day 0 to day 21. However, the mean serum phosphorus showed no such significant differences between and within the groups. It was noticed that the overall Ca:P ratio was disturbed (Ca being on the lower side) in the animals under study, which might be attributed to the animals' anestrus condition. In conclusion, the Ovsynch, TRIU-B, and standard mineral mixture proved useful for induction of estrus and ovulation and enhancement of pregnancy rate in post-partum anestrus cows.

Keywords: Anestrus, Chelated minerals, Conception, Cows, Ovsynch, Triu-B.

Ind J Vet Sci and Biotech (2022): 10.48165/ijvsbt.18.4.05

Introduction

The Indian subcontinent possesses the largest livestock population in the world, and the livestock sector acts as a vital source of the Indian economy as well as an important contributing factor to agriculture. Anestrus is one of the commonly encountered reproductive problems in Indian cattle, affecting livestock productivity and economics to a great extent (Bhoraniya *et al.,* 2012; Naikoo *et al.,* 2016, 2021). It is a multi-causative associated problem, such as inadequate nutrition, environmental stress, uterine pathology, and improper management practices (Kumar *et al.,* 2014). Chelated mineral mixture supplementation with or without bypass fat has also been shown to improve nutritional status and reproductive performance in anestrus and repeat breeding dairy animals (Dhami *et al.,* 2019; Joshi *et al.,* 2020).

Similarly, Hussain et al. (2020) recorded overall conception rates of 83.33, 66.66, and 83.33 % in anestrus Sahiwal cows treated with Lugols iodine, XXX herbal bolus, and modified ovsynch protocol, respectively, compared to 0.00% in the untreated control group. Fixed time artificial insemination (FTAI) using Ovsynch, controlled intra-vaginal drug release (CIDR) and Ovsynch-CIDR combined treatment results in effective induction of cyclicity and pregnancy rates in true acyclic post-partum cows (Bhoraniya *et al.,* 2012; Velladurai *et al.,* 2014; Naikoo *et al.,* 2016, 2021). Therefore, keeping in view the importance of anestrus and its negative impact on reproduction, the present study was designed to induce estrus in post-partum anestrus cattle using different treatments and to know its influence on conception rate and progesterone, calcium, and phosphorus profile.

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How to cite this article: Niaz, S., Naikoo, M., Islam, R., Lone, F.A., & Khan, A.A. (2022). Induction of Estrus in Postpartum Anestrus Crossbred Cattle. Ind J Vet Sci and Biotech. 18(4), 21-25.

Source of support: Nil

Conflict of interest: None.

Submitted: 29/03/2022 **Accepted:** 25/08/2022 **Published:** 10/09/2022

Materials and Methods

Treatment Protocols

The study was conducted on post-partum anestrus cows in 5-villages at the nearby locality of Faculty of Veterinary

Sciences and Animal Husbandry Shuhama, Srinagar (SKUAST-Kashmir) during the year 2015. A total of 120 animals were examined for reproductive status, and 30 animals were included in this study, including 24 anestrus and 6 normal cyclic animals.

The cows with a post-partum period of 90 days or more were only included. They were subjected to thorough clinico-gynecological examination twice at an interval of 10 days. The animals with normal appetite, properly dewormed and vaccinated against common diseases, without gross uterine pathology, and those with smooth ovaries were utilized for this study. The animals were randomly divided into five treatment groups as under.

Ovsynch Protocol (Group-I)

Six anestrus cows were treated with Ovsynch protocol which was initiated with the administration of 0.020 mg (5 mL) Buserelin acetate intramuscular (IM) on day 0, 500 µg (2 mL) cloprostenol sodium (IM) on day 7, the second shot of 0.010 mg of Buserelin acetate (IM) on day 9 and finally FTAI was performed on day 10.

TRIU-B Insert (Group-II)

The anestrus cows (n = 6) of this group were inserted with Triu-B (Virbac) - progesterone impregnated device containing 958 mg progesterone - intra-vaginally on day 0, an injection of 500 µg (2 mL) of cloprostenol sodium (IM) on day 7 with the removal of insert on the same day and FTAI was performed 55 hours after the last injection.

Mineral Mixture (Group-III)

Six anestrus cows were supplemented with a standard chelated mineral mixture (Agrimin forte, Glaxo) 40 g PO daily for 45 days or till the induction of estrus whichever was earlier. They were inseminated on observed estrus.

Normal Cyclic Control (Group-IV)

The normal cyclic cows (n=6) brought to the Veterinary Clinical Service Complex (SKUAST-Kashmir) as a routine for insemination were included, and no treatment was given to them before or after AI.

Anestrus Control (Group-V)

Six anestrus cows were kept as untreated control without any treatment and were observed for the occurrence of estrus and AI.

The pregnancy was confirmed on day 90 post-AI through examination per rectum in all groups.

Blood Sampling for Serum Profile

The blood samples were collected on days 0 (before treatment) and 21 (post-AI) without anticoagulant in sterile centrifuge tubes through jugular vein puncture. These were allowed to clot at room temperature in a slanting position. The clotted samples were centrifuged at 3000 rpm for 20 minutes, and serum harvested in sterile microfuge tubes was stored at -20°C. Using standard kits, it was later analyzed for the concentration of progesterone by ELISA and serum calcium and phosphorus by biochemical kits on an auto-analyzer.

Statistical Analysis

The plasma progesterone and mineral profile data were analyzed using one-way variance analysis and t-test to compare variation within and between groups. The conception rates in different groups were compared by the Chi-square test (Snedecor and Cochran, 1994).

Results and Discussion

Induction of Estrus and First Service Conception Rate (FSCR)

Estrus induction response was 100 % in Groups 1 (ovsynch) and 2 (TRIU-B insert) as all animals responded to treatment and exhibited estrus, whereas it was nil in anestrus control group 5. In group 3 (mineral mixture), 4 animals (66.66%) responded to mineral supplements and expressed estrus. In group 4 (cyclic control) all the animals were inseminated on observed estrus. The conception rate in group 1 was 33.33% (2/6). In group 2 it was 50.00% (3/6), and in group 3, 75.00% (3/4), and in group 4 (cyclic control), it was 83.33% (5/6). In group 5 (anestrus control), none of the animals expressed estrus or inseminated; hence, the conception rate was 0.00%.

The Ovsynch protocol appears to induce estrus and ovulation in a high percentage of anestrus dairy cows (Bhoraniya *et al.,* 2012; Hussain *et al.,* 2020). Ansari *et al.* (2008) also reported a comparable 100% estrus response with Ovsynch protocol in cows. On the contrary, a lower estrus induction rate was reported by Sathiamoorthy and Subramanian (2003). The conception rate of 33.33% (2/6) observed in group 1 was almost similar to the earlier reports of Sathiamoorthy and Subramanian (2003), Bhoraniya *et al.* (2012) and Lakher *et al.* (2019). On the contrary, some authors reported a lower pregnancy rate in cows ((Aali *et al.,* 2008), while others reported a higher pregnancy rate (Ansari *et al.,* 2008; Abubaker *et al.,* 2013). The association of GnRH and PGF2α influences the recruitment and growth of follicular waves and luteolytic activity. The ovulations are better synchronized, allowing the fixed time insemination. Velladurai *et al.* (2014) supplemented TANUVAS mineral mixture for 15 days with or without subsequent ovsynch protocol in crossbred cows and recorded 100.0 vs. 62.5% ovulatory estrus and 37.5 vs. 25.0% conception, respectively.

In group 2 (TRIU-B insert), all six cows responded to the treatment and exhibited estrus. On the contrary, the incidence of estrus in animals treated with CIDR was lower in dairy cows (Cevik *et al.,* 2010; Naikoo *et al.,* 2016). It might be because, unlike CIDR, TRIU-B insert comprises 4 wings, thus allowing its more appropriate intra-vaginal placement.

Further, TRIU-B is composed of synthetic progesterone wich attributed to its tight induction and synchronization of estrus. A total of three cows out of six (50%) conceived at first estrus in this group. A similar pregnancy rate was reported in dairy cows (Cevik *et al.,* 2010; Ammu *et al.,* 2012). On the contrary, a lower pregnancy rate was reported by Sathiamoorthy and Subramanian (2003), whereas others reported a higher pregnancy rate in anestrus cows (Bhoraniya *et al.,* 2012).

In group 3, 66.66% (4/6) of cows showed behavioral estrus during 45 days of mineral mixture supplementation. The conception rate was 75.00% (3/4). A higher estrus induction rate and almost similar conception rate were reported in cows by some workers (Hussain *et al.,* 2009; Ray and Banerjee, 2012). Dhami *et al.* (2019) nutritionally supplemented anestrus bovines (n = 265) either with chelated area-specific mineral mixture (ASMM, 50-60 g/d/h) or multi-minerals boli-one on alternate day, with and without bypass fat (75-80 g/d/h) for one month and recorded 34-56% estrus induction response and 33-50% conception rate, both being better with mineral plus bypass fat supplementation than minerals alone. Joshi *et al.* (2020) treated 12 anestrus buffaloes with chelated mineral mixture @ 50 g/animal/day for 90 days, keeping 12 anestrus animals as control with the farmer's feeding schedule, and recorded the early expression of estrus and higher conception rate with significantly improved macro-micro-mineral status in the supplemented group. The variation may be due to the effect of breed/species, feeding practices, geographical location and, more importantly, the composition of the standard mineral mixtures supplemented.

Plasma Progesterone (p_4) Profile

The mean serum concentrations of progesterone (ng/mL) in different groups of cows observed before treatment and on day 21 post-AI are shown in Table 1. It increased non-significantly from day 0 (before treatment) to day 21 post-AI among the cattle of all groups. The mean progesterone concentration of all the anestrus groups on day 0 varied from 1.36 to 3.11 ng/mL. Almost similar p_4 levels from 0.32 to 3.54 ng/mL on day zero were also reported by Garg *et al.* (2008). The plasma P_4 levels around day 90 post-partum and on the day of initiation of treatment in anestrus cows indicated that at least a few cows in each group showed elevated progesterone levels compared to the other anestrus animals under different groups (Ammu *et al.,* 2012; Joshi *et al.,* 2019).

The mean concentration of progesterone (ng/mL) in normal cyclic cows on day 0 was 2.39±1.04, and on day 21 post-AI, it was 4.57±1.38. The increased progesterone levels noted from day 0 to day 21 post-AI in Groups 1, 2, 3, and 4 could be attributed to ovulatory estrus and the establishment of pregnancy in some cows. Similarly, it was reported that higher plasma P_4 in pregnant than non-pregnant cows indicate luteal insufficiency or anovulation in non-pregnant animals. The pregnancy rate in cows was positively associated with higher progesterone concentration in the luteal phase of the cycle preceding AI (Bhoraniya *et al.,* 2012; Dhami *et al.,* 2019). In a similar trend, the previous studies on induction of estrus using ovsynch protocol reported an increase in P_4 level subsequent to 1st and 2nd GnRH inj. in cattle (Willard *et al.,* 2003; Hammam *et al.,* 2013; Naik *et al.,* 2013).

Plasma Calcium and Phosphorus Profile

The mean serum concentrations of Ca and P (mg/dL) recorded before treatment and on day 21 post-AI in different groups are shown in Table 1. The mean calcium concentration on day 0 was significantly higher in the normal cyclic control group as compared to the anestrus cows under all other groups. It was non-significantly higher on day 21 post-AI in all groups as compared to day 0. In general, there was an increase in the level of calcium from day 0 to day 21 in all groups. The mean serum concentration of P (mg/dL) was insignificantly higher in the animals under all groups on day 0 as compared to day 21. The values were in the normal physiological range. There were as such, no significant differences in the levels of phosphorus between and within the groups. Ammu *et al.* (2012) and Naikoo *et al.*(2016) recorded a similar insignificant effect of various synchronization protocols on the macro-minerals status of anestrus cattle. However in contrast to present findings, several workers have recorded significant improvement in serum mineral profile following chelated mineral mixture supplementation (Dhami *et al.,* 2019; Joshi *et al.,* 2020)

Calcium appears to affect reproduction in animals indirectly. It influences the animal's ability to use other trace elements, thus disrupting reproductive efficiency. This study

Table 1: Serum progesterone, calcium, and phosphorus concentration (mean ± SEM) in anestrus cattle under different groups

	Progesterone (ng/mL)		*Calcium (mg/dL)*		*Phosphorus (mg/dL)*	
Groups	*Day 0 (before treatment)*	*Day 21 post-AI*	*Day 0 (before treatment)*	*Day 21 post-AI*	*Day 0 (before treatment)*	*Day 21 post-AI*
Ovsynch (G1)	1.36 ± 0.58	4.48 ± 1.98	6.02 ± 0.54^{a}	8.06 ± 1.14^{a}	4.99 ± 0.69	4.74 ± 0.58
TRIU-B (G2)	2.81 ± 0.43	4.00 ± 1.76	7.05 ± 0.65^{a}	8.78 ± 0.78^{a}	5.31 ± 0.45	4.98 ± 0.57
Chelated mineral mixture (G3)	3.11 ± 0.85	11.31 ± 4.46	5.67 ± 0.56^{a}	7.53 ± 0.83^{a}	5.17 ± 0.29	5.11 ± 1.29
Normal cyclic control (G4)	2.39 ± 1.04	4.57 ± 1.38	11.54 ± 0.8^{b}	13.49 ± 0.57^{b}	4.43 ± 0.64	3.97 ± 0.50
Anestrus control (G5)	2.01 ± 0.41	-	5.01 ± 0.52^{a}	-	5.37 ± 0.59	-

Means bearing different superscripts (a, b) within the columns differ significantly ($p < 0.05$)

showed a significant difference in serum calcium levels between cyclic and acyclic animals, which is in accordance with other studies (Muneer *et al.,* 2013). The mean calcium concentration on day 0 was higher in normal cyclic control as compared to the anestrus cows under all other groups (Table1). The low calcium level in acyclic/ anestrus animals under the study might be due to failure to maintain normal calcium levels due to some subclinical level of metabolic disturbances or increased calcium excretion. The observations in cows studied also support the viewpoint that calcium plays a major role in maintaining the normal reproductive efficiency in cows. Because the lower plasma calcium level could be contributing factor to the anestrus condition as the release of GnRH and LH is a calcium-dependent mechanism.

The present findings on phosphorus levels between groups and within the group agreed with the findings of other workers (Bhoraniya *et al.,* 2012). However, increased levels of phosphorus in cyclic animals in comparison to post-partum anestrus cows were reported in cows by Muneer *et al.* (2013). Interestingly, it was observed that the overall Ca: P ratio was disturbed (Ca being on the lower side) in the animals under study, which might be attributed for the anestrus condition of the animals (Yasothai, 2014).

Conclusions

From the results, it can be inferred that the application of both ovsynch protocol and intra-vaginal TRIU-B insert are good tools for induction of estrus and timed ovulation and enhancement of pregnancy rate in post-partum anestrus crossbred cows. The chelated mineral mixture supplementation to the anestrus animals resulted in very prominent estrus signs as well as a good fertility booster. The increasing linear trend of plasma P4 observed from days 0 to 21 post-AI was in association with early resumption of ovarian activity, ovulation, and ultimately conception. The overall disturbed Ca:P ratio might be attributed to the anestrus condition of the animals, and the scientific interventions definitely benefit the farmers in minimizing the calving interval in anestrus animals.

Acknowledgments

The authors are highly thankful to Virbac India Ltd. for providing us with some medicines/ medicaments free of cost for the study.

References

Aali, M., Pretheeban, T., Giritharan, G., & Rajamahendran, R. (2008). Pregnancy rates and peripheral progesterone levels following Ovsynch or CIDR ovulation synchronization/ timed artificial insemination protocols in post-partum dairy cows. *Canadian Journal of Animal Science, 88,* 457-461.

Abubaker, P.S.S., Kurien, M.O., Ghosh, K.N.A., Simon, S., & Anil, K.S. (2013). Ovulation synchronization for improving fertility in post-partum dairy cows. *Journal of Veterinary and Animal Science, 44*, 42 - 45.

Ammu Ramakrishnan, Dhami, A.J., Naikoo, M., Parmar, B.C., & Divekar, B.S. (2012). Estrus induction and fertility response in post-partum anestrus Gir cows. *Indian Journal of Animal Reproduction, 33*(1), 37-42.

Ansari, S.M.A., Rao, K.S., & Raju, K.G.S. (2008). Studies on post-partum anoestrus with special emphasis on induction of oestrus in crossbred cows. In: *Proceedings of the 24th annual convention of ISSAR and national symposium,* KVAFSU Bangalore, India, pp. 11-13.

Bhoraniya, H.L., Dhami, A.J., & Killedar, A. (2012). Influence of estrus synchronization protocols on fertility, plasma progesterone and biochemical constituents in Kankrej cows. *Indian Journal of Animal Reproduction*, *33*(2), 14-18.

Cevik, M., Selcuk, M., & Dogan, S. (2010). Comparison of pregnancy rates after timed Al in ovsynch, heatsynch and CIDR-based synchronization protocol in dairy cows. *Kafkas University Veterinary Fak Derg, 16*, 85-89.

Dhami, A.J., Patel,, J.A. Hadiya, K.K. Parmar, S.C., & Chaudhari, D.V. (2019). Nutritional infertility and ameliorative measures in dairy animals of middle Gujarat. *The Indian Journal of Veterinary Science and Biotechnology, 14*(3), 5-9.

Garg, M.R., Bhanderi, B.M., & Gupta, S.K. (2008). Effect of supplementing chelated minerals and vitamins to overcome infertility in field animals. *Indian Journal of Dairy Science, 61*, 181-184.

Hammam, A.M.M., Abdel-Gawad, E.M.M., Ali, A.H., & Amer, H.A. (2013). Effect of CIDR-Ovsynch regimen as a pharmacological agent on fertility, blood and, hormonal patterns of crossbred cows. *Global Journal of Pharmacology, 7*(4), 383-389.

Hussain, S., Kumar, R., Singh, B., Srivastava, S., Kumar, R., Kumar, P., & Srivastava, A. (2020). Influence of different therapy on hematological markers and fertility response in anestrus Sahiwal cows. T*he Indian Journal of Veterinary Science and Biotechnology*, 16(2,3,&4), 76-80.

Hussain, Z., Bhat, A.S., Shaheen, M., & Islam, R. (2009). Comparative response of vitamin-mineral Vs herbal therapy in alleviating post-partum true anestrum in dairy cows. *Indian Journal of Animal Research, 3 ??*, 222-223.

Joshi, P.M., Patel, D.C., Patel, P.D., & Sarvaiya, N.P. (2020). Effect of chelated mineral mixture on blood profile and fertility in anoestrus buffaloes of tribal areas of Dahod district in Gujarat, India. *The Indian Journal of Veterinary Science and Biotechnology, 15*(3), 61-65.

Kumar, P.R., Singh, S.K., Kharche, S.D., Govindarajau, C.S., & Behera, BK (2014). Anestrus in cattle and buffalo, Indian perspective: A review. *Advances in Animal and Veterinary Sciences; 2*(3), 124-138.

Lakher, J.P., Awasthi, M.K., Khan, J.R., Poyam, M.R. (2019). Efficacy of ovsynch and ovsynch plus protocol for improvement of fertility in post-partum Sahiwal cows. *The Indian Journal of Veterinary Science and Biotechnology, 14*(4), 5-8.

Muneer, S., Rao, K.S., & Raju, K.J.S. (2013). Serum biochemical profiles and body condition score in crossbred cows affected with post-partum anestrum. *Theriogenology Insight, 3*(1), 21-24.

Naik, B.R., Sivakumar, A.V.N., Bramhaiah, K.V., Ravi, A,, & Chakravarthi, V.P. (2013). Estrogen and progesterone hormone level in Punganur cattle. Journal *of Agriculture and Veterinary Sciences, 2*(5), 50-53.

Naikoo, M., Dhami, A.J., & Ammu Ramakrishnan (2016). Effect of estrus synchronization on plasma progesterone profile and fertility response in post-partum suckled anestrous Kankrej cows. *Indian Journal of Animal Research*, 50(4), 460-465.

Naikoo, M., Dhami, A.J., Ramakrishnan, A., & Divekar, B.S. (2021). Plasma trace minerals profile and fertility without and with estrus synchronization therapy at day 90 post-partum in suckled Kankrej cows. *The Indian Journal of Veterinary Science and Biotechnology, 17*(2), 35-41.

Ray, K., & Banerjee, U. (2012). Fertility parameters and blood biochemical profile of Holstein Friesian x Jersey crossbred cows treated with herbal heat inducer and mineral mixture combination for induction of post-partum oestrus. *Animal Science Reporter, 6*(3), 109-115.

Sathiamoorthy, T., & Subramanian, A. (2003). Effect of GnRH and PGF2α combination on fixed time breeding and fertility in dairy cows. *Indian Veterinary Journal, 80,* 543546.

Snedecor, G.W., & Cochran, W.G. (1994). *Statistical Methods*. 8th edn, The Iowa State University Press, Ames, Iowa, USA.

Velladurai, C., Ezakial Napolean, R., Selvaraju, M., & Doraisamy, K.A. (2014). Pattern of induced oestrus and conception rate following ovsynch programme in post-partum dairy cows. *The Indian Journal of Veterinary Science and Biotechnology, 10*(2), 23-25.

Willard, S., Gandy, S., Bowers, S., Graves, K., & Elias, A. (2003). The effects of GnRH administration post-insemination on serum concentrations of progesterone and pregnancy rates in dairy cattle exposed to mild summer heat stress. *Journal of Animal Science, 59*, 1799-1810.

Yasothai, R. (2014). Importance of minerals on reproduction in Dairy cattle: A review. *International Journal of Science, Environment and Technology, 3*(6), 2051-2057.