RESEARCH ARTICLE

Impact of Different Therapeutic Protocols on Blood Biochemical Markers and Fertility in Anestrus Sahiwal Cows

Safayat Husain[1], Rajesh Kumar[1]*, Bhoopendra Singh[1], Sushant Srivastava[1], Rabindra Kumar[1], Pramod Kumar[2], Nilufar Haque[3]

Abstract

Bovine anestrus syndrome is a multifactorial disorder of the reproductive system. The problem is severe in suburban and rural areas of the country. The present study was aimed to evaluate the effect of different therapy on biochemical parameters and fertility response in 24 anestrus Sahiwal cows. Anestrus cows were randomly divided into four equal groups of six animals each, and were subjected with Lugol's iodine 2%, cervical painting with per rectal massage of genitalia thrice on alternate day (G1, n=6), XXX herbal bolus 1 bid for 2 days, repeated on day 11 & 12 later in non-respondents (G2, n=6), and modified ovsynch protocol which consisted of 100 mg hydroxy progesterone caproate injected daily s/c for 6 days followed by inj. GPG (G3, n=6), while one group was kept as untreated control (G4, n=6). Blood samples were collected thrice from all cows, *i.e.,* pretreatment day-0 (S1); post-treatment at induced estrus/AI (S2) and day 21 post-AI (S3), for estimation of serum progesterone and biochemical constituents. In general, except progesterone, the mean values of serum glucose, total cholesterol, total protein, albumin, calcium and phosphorus were significantly ($p<0.05$) lower at d-0 than the corresponding values at induced estrus and day 21 post-AI in treatment groups G1, G2 and G3; whereas, the periodic values did not differ significantly in control group G4. The estrus induction response was 100% in all treated cows with comparable conception rates among different therapeutic protocols (83.33, 66.66 & 83.33 %) as against nil response in control group G4. The results suggest that bovine anestrus syndrome can be managed well with comparatively low-cost Lugol's iodine and herbal therapeutics.

Keywords: Anestrus, Biochemical parameters, Estrus induction, Sahiwal cows, Therapy

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Introduction

Bovine anestrus syndrome is a multifactorial disorder of the reproductive system, characterized by the absence of estrus either due to lack of estrus or failure of its detection. The problem is severe in suburban and rural areas of the country. Wide variation in the incidence of anestrus has been reported in cattle and buffalo across the country (Butani *et al*., 2008 and Ashoo *et al*., 2020). The clinical syndrome can be diagnosed using anamnesis, ovarian palpation, progesterone estimation, and ultrasonography; being multifactorial, it can be treated according to their cause; however, there is no single panacea to correct it. Many non-hormonal and hormonal drugs have been tried and tested to treat anestrus syndrome (Kumar et al., 2011; Dhami et al., 2015; Kumar *et al*., 2020; Kumar *et al*., 2021). Among herbal therapeutics, various types of tropical plants are used traditionally to augment reproductive performance. Some plants contain secondary metabolites which are effective in inducing estrus in post-partum anestrus cows. The hormonal drugs being costly cannot always be affordable under field conditions. Thus, the present study was aimed to assess comparative efficacy of Lugol's iodine, XXX bolus (herbal heat inducer) and modified ovsynch protocol on fertility response in post-partum anestrus cows and its effect on blood biochemical profile.

Materials and Methods

The study was carried out under farm conditions during the year 2019-20 in the semi-urban areas of Ayodhya district in UP, India. The experimental protocol was approved by Institutional Animal Ethics Committee of the College. At preliminary stage, 34 cows were selected on the basis of history, breeding records and per-rectal examination. The

[1]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science & Animal Husbandry, Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, UP-224229.

[2]Department of Veterinary Physiology & Biochemistry College of Veterinary Science & Animal Husbandry Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya, UP-224229.

[3]Directorate of Research Extension & Farm, WBUAFS, Kolkata, West Bengal, India

Corresponding Author: Rajesh Kumar, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, ANDUAT, Ayodhya, U.P., India, e-mail: drrajesh25@gmail.com

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cows, which did not exhibit apparent clinical sign of estrum, and had normal genital tract with atonic uterus and smooth, small inactive ovaries, even after 90 days post-partum were selected for further screening. Trans-rectal examinations of cows were performed twice 10 days apart and the cows with no gross or palpable structures (follicle and/or corpus luteum) on ovaries were considered as true anestrus. The screened cows were subjected to deworming with Fenbendazole 3 gm and Ivermectin 100 mg (Apptizole plus, Sri Govind Pharmaceuticals) once orally and simultaneously supplemented with mineral mixture 30 g/day (Mustmin Forte, Sri Govind Pharmaceuticals) for 21 days. Nine cows resumed cyclicity during mineral mixture supplementation and were excluded from the study. Out of 25 non-responsive cows, 24 anestrus cows were taken up for study and were randomly allocated to four equal groups as G1, G2, G3 and G4 (6 animals in each group). In G1 cows, Lugol's iodine (2%) was painted on external os of cervix along with gentle massage of genitalia on alternate days for 3 occasions; In G2 cows, herbal heat inducer (XXX bolus, SG Pharma) was administered @ 1 bolus twice a day for 2 days, and the same regimen was repeated on 11th and 12th day in non-respondent cows; In G3 cows, 100 mg hydroxy progesterone caproate was injected daily subcutaneously for 6 days followed by Buserelin acetate (10 µg, i/m) on 7th day, Cloprostenol sodium (500 µg, i/m) on 14th day, and Buserelin acetate (10 µg, i/m) again on 16th day (*i.e.*, Modified Ovsynch protocol), and G4 control cows received no treatment.

Estrus detection was carried out by visual observation thrice in a day, morning, afternoon and evening. Animal if found in estrus was further confirmed by transrectal palpation of genitalia. Cows in estrus were inseminated twice 12 hours apart with good quality frozen thawed semen. Pregnancy was confirmed by rectal examination 60 days post-AI in non-returned cases.

Blood samples were collected from all treated cows (G1, G2 and G3) thrice, *i.e.*, pretreatment on day-0 (S1); post-treatment at induced estrus/ AI (S2) and on day 21 post-AI (S3). The positive control /untreated anestrus cows (G4) were also sampled thrice at 21 days apart (day-0; day 21st and 42nd). Approximately 10 ml blood was collected aseptically from jugular vein puncture using 16G needle and kept in vaccutainer containing rapid clot activator in slanting position for 1-2 h and then serum was separated by centrifugation at 3000 rpm for 5 minutes and stored at -20°C in deep freezer until analysis. Glucose, total cholesterol, total protein, albumin, calcium and phosphorus were estimated using diagnostic kits provided by Span Diagnostic Ltd, and progesterone estimation was done using RIA technique at a commercial laboratory.

Data were presented as mean ± SE and analyzed using a completely randomized design and Duncan's MRT for the effect of groups and periods at a significance of $p<0.05$ (Snedecor and Cochran, 1994).

Results and Discussion

Comparative Blood Biochemical Profile

The mean (± SE) values of serum progesterone and biochemical constituents observed on different days in treatment groups, and the control group are depicted in Table 1. The mean serum progesterone values were at nadir at the time of induced estrus in treatment groups, whereas in control cows, the values did not differ significantly among three periodic samples. At pretreatment and induced estrus samplings, the progesterone levels did not differ significantly between groups, but on day 21 post-AI the progesterone levels were significantly higher in all three treatment groups compared to values of the control group (Table 1). In the present study, comparatively higher P4 values at the initial or anestrus stage (> 1 ng/ml) might be due to missing diagnosis of CL in few cows taken under study, which is in agreement with Dawson (1975), who reported 20% false result by trans-rectal ovarian palpation. Likewise, Kumar *et al*. (2009) recorded variations in progesterone level in anestrus, subestrus, repeat breeding, and ordinary cyclic cows (1.65 ± 0.17, 3.99 ± 0.58, 0.77 ± 0.07, and 0.76 ± 0.10 ng/ml, respectively), and Dutta *et al*. (2019) observed lower P4 concentration in anestrus cows due to absence of active corpus luteum on the ovary.

The mean blood glucose concentration was significantly lower ($p < 0.05$) at pretreatment (anestrus) than at induced estrus and 21 days of post-AI in all treatment groups, but no such differences were observed between days 0, 21, and 42 in the untreated control group. Similarly, the significantly lower blood glucose level in anestrus cows has been reported by many workers (Ray *et al*., 2016; Dhami *et al*., 2019; Kumar *et al*., 2021). Blood glucose is an important factor, which modulates reproduction and lowers glucose level is postulated as the cause for acyclicity and decreased fertility rate. The loss of ovarian activity in hypoglycemic animals might be due to releasing gonodotrophins from the hypophysis. Furthermore, Kumar and Saxena (2010) postulated that lower glucose and insulin concentrations in blood were associated with nutritional anestrus. Indeed, glucose appears to have a pivotal role in the release of LH, which in turn reflects its role in modulating GnRH release.

Serum cholesterol levels did not differ significantly during periodic sampling in the control group, while in all three treatment groups, the levels were significantly lower at pretreatment sampling than induced estrus and 21 days post-AI. Kumar *et al*. (2009), Ray *et al*. (2016), Soni *et al*. (2018), and Kumar *et al*. (2021) also recorded lower cholesterol levels in anestrus cows. Conversely, Virmani *et al*. (2011) opined that cholesterol levels significantly decreased as cows approached cyclicity. Furthermore, Dhami *et al*. (2019) reported non-significantly higher total cholesterol levels in anestrus and subestrus cows compared to repeat breeding cows. In the current study, cholesterol level was

Table 1: Effect of different therapeutic protocols on serum progesterone and biochemical parameters of anestrus Sahiwal cows (Mean ± SE)

Blood parameters	*Period*	*Lugol's iodine (G1)*	*XXX bolus (G2)*	*Modified ovsynch (G3)*	**Untreated control (G4)**
Progesterone (ng/ml)	S1	1.98 ± 0.31^{a}	2.08 ± 0.28^{ab}	2.00 ± 0.32^{a}	1.48 ± 0.14^{a}
	S2	0.56 ± 0.03^{a}	0.58 ± 0.08^{a}	0.57 ± 0.02^{a}	1.43 ± 0.14^{a}
	S3	5.19 ± 0.99^{Ab}	4.35 ± 0.92^{Ab}	4.97 ± 0.81^{Ab}	1.43 ± 0.14^{Ba}
Glucose (mg/dl)	S1	47.67 ± 6.21^{a}	56.33 ± 7.89^{a}	37.33 ± 3.45^{a}	41.33 ± 3.69^{a}
	S2	87.50 ± 4.20^{Ab}	94.17 ± 11.55^{Ab}	75 ± 3.71^{ABb}	47.33 ± 4.43^{Ba}
	S3	65.17 ± 7.75^{Aab}	74.83 ± 3.85^{Aab}	68.50 ± 2.90^{Ab}	49.33 ± 4.53^{Aa}
Total cholesterol (mg/dl)	S1	84.1 ± 2.48^{a}	81.81 ± 3.75^{a}	82.38 ± 3.70^{a}	79.95 ± 3.52^{a}
	S2	150.8 ± 13.2^{Ab}	121.1 ± 6.51^{Ab}	148.0 ± 6.85^{Ab}	79.30 ± 3.48^{Ba}
	S3	156.9 ± 13.17^{Ab}	124.1 ± 6.31^{Ab}	144.2 ± 8.88^{Ab}	79.3 ± 3.48^{Ba}
Total protein(g/dl)	S1	6.55 ± 0.17^{a}	5.36 ± 0.70^{a}	5.73 ± 0.27^{a}	5.42 ± 0.30^{a}
	S2	8.56 ± 0.15^{Ab}	8.55 ± 0.15^{Ab}	9.46 ± 0.18^{Ab}	5.43 ± 0.29^{Ba}
	S3	8.88 ± 0.09^{Ab}	8.71 ± 0.08^{Ab}	9.28 ± 0.16^{Ab}	5.42 ± 0.25^{Ba}
Albumin (g/dl)	S1	2.58 ± 0.10^{a}	2.54 ± 0.08^{a}	2.56 ± 0.11^{a}	2.45 ± 0.08^{a}
	S2	3.94 ± 0.28^{Ab}	3.66 ± 0.10^{Ab}	3.68 ± 0.13^{Ab}	2.45 ± 0.09^{Ba}
	S3	4.02 ± 0.16^{Ab}	3.73 ± 0.10^{Ab}	3.70 ± 0.12^{Ab}	2.44 ± 0.09^{Ba}
Calcium (mg/dl)	S1	7.97 ± 0.18^{a}	8.46 ± 0.09^{a}	8.14 ± 0.29^{a}	8.19 ± 0.09^{a}
	S2	9.88 ± 0.36^{Ab}	9.41 ± 0.49^{ABa}	9.48 ± 0.27^{ABab}	8.17 ± 0.11^{Ba}
	S3	10.15 ± 0.40^{Ab}	9.44 ± 0.61^{ABa}	9.70 ± 0.19^{Ab}	8.17 ± 0.11^{Ba}
phosphorus (mg/dl)	S1	3.37 ± 0.33^{a}	4.29 ± 0.23^{a}	3.96 ± 0.17^{a}	3.02 ± 0.22^{a}
	S2	5.98 ± 0.28^{Ab}	7.25 ± 0.75^{ABb}	8.44 ± 0.62^{Bb}	3.07 ± 0.18^{Ca}
	S3	5.80 ± 0.26^{Ab}	7.41 ± 0.77^{ABb}	8.40 ± 0.74^{Bb}	3.07 ± 0.17^{Ca}

Means bearing common superscripts do not differ significantly ($p>0.05$) within a row (A, B, C) and column (a, b). Period; S1 day 0, S2 day of induced estrus, S3 day 21st post-AI (or day 0, 21st and 42nd in control group).

improved as animals resumed cyclicity. Indeed, cholesterol is an essential precursor for testis, ovary, and adrenal cortex steroid hormone synthesis. Lower cholesterol concentration might cause anestrus in post-partum cows (Kumar *et al*., 2009).

The pretreatment serum total protein and albumin values (S1) were significantly ($p<0.05$) lower as compared to post-treatment values (S2 and S3) in all treatment groups (G1, G2, and G3); however, the periodic values (S1, S2, and S3) did not differ significantly in the untreated control group. Like current findings, significantly lower total protein and albumin levels were recorded in anestrus crossbred (Kumar *et al*., 2009) and Sahiwal cows (Virmani *et al*., 2011), but significantly increased by 7th day post-treatment as animals reached towards normalcy. Furthermore, Mondal *et al*. (2019) and Kumar *et al*. (2021) reported significantly ($p < 0.05$) lower serum total protein and albumin in anestrus than cyclic crossbred cows. Indeed, lack of protein or insufficient protein intake was considered a factor responsible for failure or delay in post-partum estrus. In the present study, significantly increased total protein and albumin levels at induced estrus indicate that lower protein concentration might cause post-partum anestrus condition in dairy cows as values were improved as cows attained cyclicity. The improved protein level in treated cows suggests positive effects of the drug on total protein and albumin levels.

The pretreatment serum calcium and phosphorus concentrations (S1) were significantly ($p<0.05$) lower in all treatment groups (G1, G2, and G3) as compared to post-treatment values (S2 and S3); however,, the period effect was not significant in the control group. Likewise, Kumar *et al*. (2009) recorded lower serum calcium level in anestrus against subestrus, repeat breeding, and normal cyclic cows. Furthermore, lower calcium levels in anestrus cows was observed by Ray *et al*. (2016) against estrus cows; Soni *et al*. (2018) against normal cyclic cows and Mangrole *et al*. (2019) against induced estrus cows. Moreover, Dhami *et al*. (2019) reported non-significantly higher plasma calcium levels in repeat breeding cows than anestrus and subestrus cows. The present findings are indicative of the positive impact of the drug on serum calcium levels. Calcium appears to have indirect action on reproduction in cattle (Dhoble and Gupta, 1986) by sensitizing the tubular genital tract for the action of hormones (Kumar *et al*., 2020). Similar to our findings, many previous reports suggest lower phosphorus levels cause bovine anestrus (Virmani *et al*., 2011; Ray *et al*., 2016; Soni *et al*., 2018; Kumar *et al*., 2021). The present finding suggests the importance of phosphorus level in cyclicity and indicates the positive

effect of the drug on phosphorus concentration in treated cows.

Fertility Response with Different Treatment Protocol

In G1, G2, and G3 all cows (100 %) resumed cyclicity. The treatment to estrus induction interval (EII) was 9.17 ± 2.34, 8.33 ± 2.36 and 16 ± 0.00 days; treatment to fertile estrus interval (FEI) was 33.6 ± 8.40, 42.0 ± 8.57 and 29.4 ± 5.14 days and overall conception rate (CR) was 83.33, 66.66, and 83.33 % in three groups, respectively; however, none of the cows resumed cyclicity in the control group (G4).

The findings in G1 suggest a possible role of iodine for induction of estrus and increased conception rate in anestrus cows. Similarly, Sarkar (2005) observed a high conception rate (81%) using Lugol's iodine and ovarian massage. Conversely, Pandey *et al.* (2011) observed a higher mean estrus induction interval (14.12 ± 0.4 days), but the conception rate was at par with an intrauterine infusion of 20 mL Lugol's iodine (0.25%) on day 5th and day 17th of the estrous cycle. Furthermore, Kumar *et al.*(2011) reported lower estrus induction response (EIR) (77.92%) with treatment to EII of 26.70 ± 4.52 days and CR of 40, 20, and 10 % in three consecutive cycles in true anestrus crossbred cows treated with parenteral phosphorus, vitamin A and 10 mL Lugol's iodine solution (0.25%) on os cervix. Conversely, lower EIR and CR were recorded in actual anestrus cows (62.5% and 50%) (El-Shahat and Badr, 2011) and true anestrus buffaloes (70% and 85.71%) (Ahlawat *et al.*, 2016) using Lugol's iodine. The ovarian inactivity might be due to energy utilization imbalance. Iodine might enhance absorption of thyroid hormone, thus, increases body metabolism, which might be one of the triggering factors for resumption of ovarian function; however, the mechanism by which Lugol's iodine acts on the reproductive system is not clearly understood (Mwaanga *et al.*, 2004). Lugol's iodine applied as cervical paint causes local irritation, inflammation, enhanced uterine blood flow, and probably causes reflex stimulation of anterior pituitary for secretion of gonadotrophin, which causes resumption cyclicity.

Observations in G2 suggest the possible role of herbal heat inducer for induction of estrus and improved conception rate. The current therapy was found to be significantly beneficial as compared to untreated contemporaries. Unlike the current study, lower EIR and CR was noted with Prajana capsule (66.67% and 75.00%) and Janova (62.5% and 80.0%) by Sahatpure *et al.*, 2016) and Kumar *et al.* (2020) respectively in post-partum anestrus crossbred cows, and buffaloes (75% and 83.33%) (Chaudhary *et al.*, 2018). Like current observations in G3, Dhami *et al.* (2015) recorded 100 and 50% EIR and CR, respectively, in anestrus crossbred cows. Conversely, lower EIR (87.5%) and CR (50%) were observed in post-partum anestrus cows with the use of the ovsynch protocol by Kumar *et al.* (2020). Furthermore, variable EIR and CR were observed in anestrus cattle, and buffalo (Chaudhiry *et al.*, 2018 and Kumar *et al.*, 2020) with different therapeutic protocols might be due to differences in age, breed, parity, nutritional and health status of animals, depth of anestrus and differential efficacy of the drug.

Conclusion

The study showed a significantly varied blood biochemical profiles in anestrus and induced estrus cows, thus identifying post-partum anestrus cows. All therapeutic protocols were equally effective for induction of estrus in post-partum cows with variable conception rates. Lugol's iodine and XXX bolus (herbal heat inducer) can be utilized as an alternative to comparatively costly modified ovsynch protocol. Incidence of post-partum anestrus can be reduced with mass deworming and area-specific mineral mixture supplementation. Furthermore, pharmacokinetics and pharmacodynamics of herbal medicaments are yet to be explored better to understand the mechanism of action of such drugs.

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