RESEARCH ARTICLE

Influence of Different Therapy on Hematological Markers and Fertility Response in Anestrus Sahiwal Cows

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Abstract

The study was designed to evaluate influence of different therapy on hematological markers, resumption of ovarian cyclicity and conception rate in 24 anestrus Sahiwal cows, which were randomly and equally allocated to four groups as G1, G2, G3 and G4. Lugols iodine (2%, cervical painting with per rectal massage of genitalia thrice on alternate day) XXX herbal bolus (1 bid for 2 days, repeated on day 11 & 12 later in non-respondents) and modified ovsynch protocol (progesterone 100 mg s/c once a day for 6 days followed by GPG) was administered in G1, G2 and G3, respectively, while G4 kept as untreated control. All these animals were dewormed with bolus fenbendazole 3 g plus ivermectin 100 mg, once and supplemented with mineral mixture 30 g/day for 21 days before above therapies. Blood samples were collected thrice, *i.e.*, on day 0, day of induced estrus/AI and day 21 post-AI (day 0, 21 and 63 in non-respondents) for hematological analysis. In general, hemogram and leucogram did not vary significantly within and among different groups, except neutrophil and lymphocyte. Cent per cent estrus induction response was observed in treatment group G1, G2 and G3, however, none of the cows resumed cyclicity in untreated control group G4. The treatment to estrus induction interval was 9.17±2.34, 8.33±2.36 and 16±0.00 days; treatment to fertile estrus interval was 33.6±8.40, 42.0±8.57 and 29.4±5.14 days and overall conception rate was 83.33, 66.66 and 83.33 % in group G1, G2 and G3, respectively, while nil in untreated anestrus cows.

Keywords: Anestrus, Conception, Estrus induction, Sahiwal cows, Therapy.

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Introduction

Bovine anestrus syndrome is a most common single cause of infertility in dairy cattle that leads to huge economic losses in terms of prolonged calving interval, reduced calf crop and shorter productive life. Malnutrition is the most common cause of true anestrus featured by absence of estrus cyclicity with small smooth inactive ovaries. Bovine anestrus syndrome is a multifactorial disorder of the reproductive cycle which is characterized by absence of estrus signs manifested either due to lack of expression of estrus or failure of its detection. It can be normal physiological (pregnancy, lactation and in early postpartum period) or pathological (metritis, fetal resorption/PCL, pyometra, maceration and mummification). Many non-hormonal and hormonal drugs have been used to treat anestrus (Kumar *et al*., 2011; Dhami *et al*., 2015; Dhami *et al*., 2019; Kumar *et al*., 2020[a]). Among herbal therapeutics, various type of tropical plants are used traditionally to augment the reproductive performance. Some plants contain secondary metabolites which are effective in inducing estrus in postpartum anestrus cows (Pugashetti *et al*., 2009). Deficiency of minerals is also an important cause of anestrus in animals (Kumar *et al*., 2020[b]). This study was therefore designed to assess influence of different treatment protocols on hematological markers as well as on fertility response in true anestrus Sahiwal cows.

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Materials and Methods

Selection of Experimental Animals

The study was carried out under farm conditions during the year 2019-20 in the semi-urban areas of Ayodhya district in

UP. At preliminary stage, 34 cows were selected on the basis of history, breeding records and per-rectal examination of genitalia. The cows which had not exhibited apparent clinical sign of estrum, and had normal genital tract with atonic uterus and smooth, small inactive ovaries, even after 90 days postpartum were selected for further screening. Trans-rectal examinations of cows were performed twice 10 days apart and the cows with no palpable structures (follicle and/or corpus luteum) on ovaries were considered as true anestrus. The screened cows were subjected to deworming with broad spectrum anthelmimtic Fenbendazole 3 gm and Ivermectin 100 mg (Apptizole plus, Sri Govind Pharmaceuticals) once orally and further supplemented with mineral mixture 30 g/day (Mustmin Forte, Sri Govind Pharmaceuticals) for 21 days. Nine cows resumed cyclicity during mineral mixture supplementation were excluded from further treatment. Finally, 24 anestrus cows were taken up for detailed study and were randomly allocated to four equal groups as G1, G2, G3 and G4 (6 animals in each), and were managed as follows.

Experimental Design

Group	*Treatment*
G1 (n = 6)	Painting with 2% Lugol's iodine solution on external os of cervix along with gentle massage of genitalia on alternate days for 3 occasions.
G2 (n = 6)	Herbal heat inducer (XXX bolus, SG Pharma) was administered @ 1 bolus twice a day for 2 days; if cow did not respond within 10 days then the same regimen was repeated on 11th and 12th day.
G3 (n = 6)	Hydroxy progesterone caproate 100 mg, once in a day s/c was administered for first 6 days followed by GPG, *i.e.,* 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).
G4 (n = 6)	Untreated anestrus cows (without any treatment) as positive control

Estrus detection was done 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 hr apart with good quality frozen thawed semen. Pregnancy was confirmed by rectal examination 60 days post-AI in non-returned cases.

Blood Sampling

Approximately 10 ml blood was collected aseptically in clean sterile EDTA vial by jugular vein puncture from each animal at the commencement of treatment, at induced estrus (or 21st day after 1st sample), and on day 21st post-AI (or 42nd day after second sample in non-respondents) for hematological examination.

Statistical Analysis

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

Results and Discussion

Resumption of Ovarian Cyclicity and Conception

The estrus induction response (EIR) observed was 100 % in group G1, G2 and G3, however, none of the cows resumed cyclicity in untreated control group G4. The treatment to estrus induction interval (TEI) (mean± SE in days) was 9.17±2.34, 8.33±2.36 and 16±0.00 days; treatment to fertile estrus interval (TFEI) was 33.6±8.40, 42.0±8.57 and 29.4±5.14 days and overall conception rate was 83.33, 66.66 and 83.33 % in group G1, G2 and G3, respectively, while zero in untreated anestrus group G4 (Table 1).

Mechanism by which Lugol's iodine acts on the reproductive system is not well understood (Mwaanga *et al*., 2004). The findings in G1 suggests possible role of iodine for induction of estrus and increased conception rate in anestrus cows, which concurred with the reports of (Sarkar, 2006) who also observed high conception rate (81%) with use of Lugol's iodine and ovarian massage. Different route of administration of Lugol's iodine have been tried with variable estrus induction response (45-91%) among cattle and buffalo (Sarkar 2006; Gupta *et al.,* 2011; Kumar *et al.,* 2011). However, Pandey *et al.* (2011) observed longer mean estrus induction interval (14.12±0.4 days) but conception rate at par with intrauterine infusion of 20 ml Lugol's iodine (0.25%) on day 5th and on day 17th of the estrous cycle. Kumar *et al.* (2011) reported lower estrus induction response (77.92%) with treatment to estrus induction interval of 26.70±4.52 days with conception rate 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, 62.5 and 70.0% estrus induction response and 50.0 and 85.7 % conception rates have been reported in true anestrus cows (El-Shahat and Badr, 2011) and buffalo (Ahlawat *et al.,* 2016) with the use of Lugol's iodine. Lugol's iodine

Table 1: Effect of different therapeutic regimen on estrus induction response and conception rate

			TEI (days)	*TFEI (days)*	*Conception rate (CR)*			
Group	*n*	*EIR*	*(Mean ± SE)*	*(Mean ± SE)*	*Induced Cycle$_1$*	*Cycle$_2$*	*Cycle$_3$*	*Overall*
G1	6	6/6 100%)	9.17±2.34	33.6±8.40	50.00% (3/6)	33.33% (1/3)	50.00% (1/2)	83.33% (5/6)
G2	6	6/6 (100%)	8.33±2.36	42.0±8.57	16.66% (1/6)	40.00% (2/5)	33.33% (1/3)	66.66% (4/6)
G3	6	6/6 (100%)	16.00±0.00	29.4±5.14	50.00% (3/6)	66.66% (2/3)	0	83.33% (5/6)
G4	6	0/6	NA	NA	NA	NA	NA	NA

solution has been used traditionally as intrauterine douche or cervical paint for induction of estrus, which causes local irritation and inflammation leading to enhanced blood flow and reflex stimulation of anterior pituitary for secretion of gonadotrophin and consequently animal resumes cyclicity. Furthermore, it may also improve thyroid activity which in turn increases body metabolism, one of triggering factors of ovarian factors, since one of the main causes of ovarian afunction or dysfunction is energy utilization imbalance (Short *et al.,* 1990).

The findings in group G2 indicate the possible role of nutritional supplement as well as herbal heat inducer in estrus induction and increased conception rate, as all cows were premedicated with dewormer and mineral mixture supplement. This therapy was found to be significantly beneficial as compared to untreated contemporaries. Variable estrus induction response (50-75%) and conception rates (66-83%) have also been reported with use of herbal heat inducers like Prajana HS, Janova in postpartum anestrus cattle and buffalo (Hussain *et al.,* 2009; Chaudhiry *et al.,* 2018; Kumar *et al.,* 2020[c]), which might be due to variation in age, breed, parity and nutritional status of animals in question. Sahatpure *et al.* (2016) reported lower estrus induction response and conception rate with Prajana capsule (66.67% and 75.00%) and CoFeCu (50.00% and 66.67%) in postpartum anestrus crossbred cows.

In accordance with the present observations in group G3 with modified ovsynch protocol, Dhami *et al.* (2015) and Kumar *et al.* (2020[c]) reported comparable estrus induction response (100 and 87.5 %), but lower conception rate (50% each) with use of conventional ovsynch protocol in postpartum anestrus cows. Variable EIR and CR with different therapy might be due to difference in age, breed, parity, nutritional and health status of animals as well as differential

Table 2: Effect of different therapy on hemogram and leucogram of anestrus Sahiwal cows

Blood parameter	*Sampling period*	*Treatment groups (n=6 cows each)*			
		G1 (Lugol's iodine)	*G2 (XXX herbal bolus)*	*G3 (Modified ovsynch)*	*G4 (Untreated control)*
Hemoglobin (g/dl)	S1	9.72 ± 0.30	9.05 ± 0.91	9.47 ± 0.44	8.97 ± 0.74
	S2	10.23 ± 0.41	9.07 ± 0.58	11.12 ± 0.32	8.93 ± 0.65
	S3	9.33 ± 0.48	9.07 ± 0.58	10.63 ± 0.37	9.40 ± 0.32
Packed cell volume (%)	S1	37.83 ± 1.92	41.67 ± 3.58	38.50 ± 2.31	36.50 ± 1.91
	S2	40.33 ± 3.53	41.83 ± 3.40	43.83 ± 1.20	34.50 ± 2.46
	S3	35.83 ± 2.40	38.33 ± 2.35	43.00 ± 1.65	33.00 ± 1.63
Total erythrocyte count ($x10^6$/cmm)	S1	6.88 ± 0.24	6.81 ± 0.65	6.39 ± 0.32	5.97 ± 0.69
	S2	7.58 ± 0.31	7.13 ± 0.84	6.67 ± 0.35	6.03 ± 0.68
	S3	6.73 ± 0.35	6.90 ± 0.68	6.28 ± 0.37	6.17 ± 0.52
Total leuco-cyte count ($x10^3$/cmm)	S1	19.3 ± 6.76	21.48 ± 4.02	9.63 ± 1.06	10.44 ± 2.95
	S2	15.73 ± .65	12.42 ± 1.83	9.20 ± 1.02	9.51 ± 2.14
	S3	13.22 ± 1.13	11.25 ± 1.75	9.22 ± 1.10	9.47 ± 2.20
Neutrophils (%)	S1	42.5 ± 1.5^{a}	41.5 ± 0.9^{a}	41.6 ± 0.7^{a}	44.5 ± 1.3^{a}
	S2	30.3 ± 1.3^{ABb}	27.9 ± 0.9^{Ab}	34.7 ± 0.8^{Bb}	42.3 ± 1.1^{Ca}
	S3	27.8 ± 1.4^{Ab}	25.8 ± 0.5^{Ab}	34.1 ± 0.8^{Bb}	42.3 ± 0.60^{Ca}
Lymphocytes (%)	S1	40.92 ± 0.25^{Aa}	48.23 ± 3.80^{Ba}	49.47 ± 0.08^{BCa}	45.63 ± 1.04^{AB}
	S2	56.72 ± 1.19^{Ab}	61.00 ± 0.95^{Ab}	57.26 ± 0.57^{Ab}	48.24 ± 0.57^{B}
	S3	66.32 ± 0.09^{Ac}	66.24 ± 1.94^{Abc}	56.55 ± 0.58^{Bbc}	47.80 ± 0.57^{C}
Monocytes (%)	S1	7.69 ± 2.46^{Aa}	4.70 ± 0.95^{ABa}	2.09 ± 0.99^{Ba}	4.42 ± 0.57^{ABa}
	S2	3.90 ± 1.90^{Aab}	5.33 ± 1.56^{Aa}	2.52 ± 0.66^{Aa}	4.25 ± 0.51^{Aa}
	S3	1.77 ± 1.18^{Ab}	2.15 ± 0.96^{Aa}	3.00 ± 0.65^{Aa}	4.53 ± 0.49^{Aa}
Eosinophils (%)	S1	8.48 ± 3.49	5.18 ± 2.71	6.43 ± 0.82	4.99 ± 0.86
	S2	8.70 ± 3.22	5.36 ± 3.27	5.12 ± 1.34	4.74 ± 1.05
	S3	3.70 ± 1.10	5.42 ± 1.10	5.95 ± 1.09	4.92 ± 0.98
Basophils (%)	S1	0.41 ± 0.02	0.39 ± 0.01	0.41 ± 0.03	0.46 ± 0.03
	S2	0.39 ± 0.02	0.41 ± 0.01	0.40 ± 0.02	0.47 ± 0.03
	S3	0.41 ± 0.02	0.39 ± 0.02	0.40 ± 0.02	0.45 ± 0.03

S1 day 0, S2 day of induced estrus, S3 day 21st post-AI (or day 0, 21st and 63rd in non-respondents). Means of a parameter bearing different superscript within a row (A, B) for groups and within a column (a, b, c) for periods differ significantly ($p < 0.05$).

efficacy of drug towards fertility response.

Effect of Different Therapy on Hemogram and Leucogram

Numerically higher Hb, PCV and TEC were observed at induced estrus in treated cows (G1, G2 & G3) as compared to untreated anestrus cows (Table 2), though the differences were statistically non-significant. This might be due to small sample size as well as premedication of cows with dewormer and mineral mixture before commencement of treatment, which minimize worm load and improve feed intake, which in turn may improve nutritional status and hemogram. The current findings were to some extent in agreement with Pariza *et al*. (2013), Sahithi *et al*. (2019), Mangrole *et al*. (2019) and Kumar *et al*. (2020[c]) who reported significantly lower Hb and PCV level in anestrus cows than those of estrus or cyclic contemporaries. Similarly, Das *et al*. (2005) and Pariza *et al*. (2013) recorded lower TEC values in anestrus cows as compared to estrus or cyclic cows.

The TLC, eosinophil and basophil count did not differ significantly within and among the groups (G1, G2 and G3) as compared to corresponding values in untreated anestrus cows (Table 2). Pariza *et al*. (2013) postulated that bacterial infection might be a cause of anestrus in zebu cattle. Anestrus condition might be associated with bacterial infection or bacterial endotoxin (Debus *et al*., 2002; Mondal *et al*., 2019). Our findings corroborate with Mangrole *et al*. (2019) who recorded non -significant difference in eosinophil count between anestrus and estrus cows. Conversely, Mondal *et al*. (2019) observed significantly lower eosinophil count in anestrus cows than those of cyclic contemporaries. Furthermore, Kumar *et al*. (2020[c]) recorded significantly higher eosinophil count in anestrus cows as compared to values at induced estrus, but basophil count did not vary significantly within and among the groups. Significantly lower neutrophil count observed at induced estrus in treated cows (G1, G2 & G3) as compared to untreated anestrus cows (Table 2) was in agreement with Mondal *et al*. (2019) and Mongrole *et al*. (2019) who observed significantly higher neutrophil count in anestrus cows as compared to cyclic or estrus cows, however no significant variation was reported by Kumar *et al*. (2020[c]).

Significantly higher lymphocyte count was observed at induced estrus in treated cows as compared to untreated anestrus cows (Table 2). Higher lymphocyte count was recorded by Mondal *et al*. (2019) in cyclic cows and Mongrole *et al*. (2019) in induced estrus cows as compared to anestrus contemporaries. Conversely, Kumar *et al*. (2020[c]) reported higher lymphocyte count in anestrus cows than those of estrus or cyclic cows. In general, periodic values of monocyte count did not differ significantly in all groups, except G1, which shows significant reduction at induced estrus (Table 2). In contrast, on-significantly and significantly lower monocyte count was reported by Mondal *et al*. (2019) and Kumar *et al*. (2020[c]) in anestrus cows as compared to cyclic and estrus cows, respectively.

In conclusion, aforesaid therapeutic protocols, particularly Lugol's iodine and modified ovsynch, can be used to manage clinical syndrome of true anestrus in Sahiwal cows.

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