RESEARCH ARTICLE

Effect of Herbal, Homeopathic and Hormonal Drug on Hematology, Ovarian Cyclicity and Conception Rate in Postpartum Anoestrus Cows

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Abstract

The study was designed to assess the effect of herbal, homeopathic, and hormonal drugs namely Janova, Sepia and GnRH-PG-GnRH on hematological parameters, resumption of ovarian cyclicity and conception rate in 40 postpartum anoestrus and 8 normal cyclic cows. The anoestrus cows were randomly divided into five groups as G0, GI, GII, GIII, GIV; 8 cows in each group. Group G0 (untreated anoestrus) and G_V (normal cyclic cows) served as positive and negative control, respectively. All cows in GI, GII, GIII and GIV were subjected to dewormer and mineral mixture supplementation for 10 days, while group GII, GIII, and GIV were additionally treated with Janova (herbal heat inducer), Sepia (a homeopathic drug) and GnRH-PG-GnRH (Ovsynch) protocol, respectively. Different therapeutic protocols revealed variable and significant effects on most of the hematological parameters, except differential leucocyte count, before and after treatment. The values were also significantly higher in normal cyclic than anoestrus groups. The oestrus induction response in G0, GI, GII, GIII, GIV, and GV was 0.00, 50.00, 62.50, 75.00, 87.50, and 100.00 %, and corresponding overall conception rates of 3 cycles were 0.00, 75.00, 80.00, 66.66, 57.13 and 75.00 %, respectively. In conclusion, aforesaid therapeutic regimens have definite bearings on oestrus induction as well as on conception and can be used to manage postpartum anoestrus in cows.

Keywords: Anoestrus, Conception, Oestrus, Hematology, Herbal, Homeopathy, Hormone.

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Introduction

Postpartum anoestrus is one of the most prevalent reproductive problems in dairy cows despite technological advances in animal husbandry (Kumar *et al*., 2013). The anoestrus causes significant economic loss due to prolonged calving interval, reduced calf crop, and shorter productive life. In the majority of cases, the causes of true anoestrus are a low plane of nutrition, chronic or debilitating diseases, senility, seasonal changes, heavy milk yield and management errors. To resume cyclicity postpartum, the threshold LH concentration is necessary. Prolonged period of ovarian inactivity and failure of ovulation is mainly due to suboptimal plasma LH level. Negative energy balance, malnutrition, stressors, deficient endogenous opioid peptides, suckling and lowered insulin concentration, are risk factors for the subthreshold LH pulse (Patil *et al*., 1992). A battery of therapeutic strategies has been tried and tested to manage postpartum anoestrus in cows (Kumar *et al*., 2011; Dhami *et al*., 2015; Dhami *et al*., 2019; Mangrole *et al*., 2019) however, homeopathic therapy gaining popularity by its low cost and devoid of untoward side effects, has not been tested in the veterinary field. Evaluation of hematological parameters is of diagnostic value to determine the health or disease status of animals. This study was aimed to assess the effect of different therapeutic regimen on haematological parameters, resumption of ovarian cyclicity and conception rate in anoestrus cows.

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Materials and Methods

Selection of Experimental Animals

The study was conducted at the Department of Veterinary Gynaecology and Obstetrics of the College, ANDUAT, Ayodhya, with the aim to develop an economic and effective therapeutic regimen for postpartum anoestrus cows. The postpartum anoestrus cases presented at government veterinary hospital Khandasa block and villages in adjoining areas of the university were also taken under study. The

postpartum anoestrus cows were screened on the basis of history, breeding records, and trans-rectal palpation of genitalia. The cows which had not exhibited apparent clinical sign of oestrus, and had normal genital tract with an atonic uterus and smooth, small inactive ovaries for at least 3 months postpartum were selected as anoestrus cows. Trans-rectal palpation of ovaries were performed twice at ten days apart to rule out cyclic cases and to confirm the cases of anoestrus.

Experimental Design

The selected anoestrus cows (n = 40) were randomly divided into five groups as G0 GI, GII, III, and GIV; with eight cows in each group, and eight normal cyclic cows were assigned to GV. The group G0 (untreated anoestrus cows) and GV (normal cyclic cows) served as a positive and negative control, respectively, while cows in groups GI, GII, GIV were administered with fenbendazole @ 7.5 mg per kg body weight orally once to reduce worm load and chelated mineral mixture @ 50 g orally once in a day for 10 days to ameliorate nutritional deficiency. The cows in group GII, GIII, and GIV were additionally treated with oral capsule Janova (Ayurvet Ltd.), Sepia (SBL Pvt Ltd), and injection GnRH-PG-GnRH (Ovsynch) regimen, respectively. The cows of all groups were observed for oestrus detection in morning and evening, and if found in standing oestrus were inseminated with frozen-thawed semen. The pregnancy was confirmed by trans-rectal palpation 60 days post-AI in non-returned cases.

Experimental Design

Group	Treatment
G0	Positive control (untreated anoestrus cows)
GI	Deworming (day 0) + Mineral mixture supplementation @ 50 g orally daily for 10 days
GII	Deworming (day 0) + Mineral mixture supplementation @ 50 g orally daily for 10 days + Janova capsule 2 daily for first 2 days followed by 3 capsules on 11^{th} and 12^{th} day
GIII	Deworming (day 0) + Mineral mixture supplementation @ 50 g orally daily for 10 days + Sepia 30, ten drops orally daily for 10 days
GIV	Deworming (day 0) + Mineral mixture supplementation @ 50 g/h daily for 10 days followed by GnRH-PG-GnRH (Ovsynch) regimen
GV	Negative control (untreated normal cyclic cows)

Blood Sampling

Approximately 10 mL of blood was collected aseptically in a clean sterile EDTA vial by jugular vein puncture using 16 G sterilized needle from each animal before and after treatment for hematological examination.

Resumption of Cyclicity and Conception Rate

After treatment, all the animals were visually observed for the expression of oestrus in the morning, afternoon, and evening at least for 30 minutes each and confirmed by trans-rectal examination. Cows were inseminated two times at an interval of 12 hours with good quality frozen-thawed semen if found in oestrus. Pregnancy was confirmed 60 days post-insemination in non-returned cases.

Statistical Analysis

Data were represented as Mean ± SE and analyzed by using statistical methods for significance at $p < 0.05$ (Snedecor and Cochran, 1989).

Results and Discussion

Effect on Hematological Parameters

The mean values of hemoglobin (Hb) concentration, packed cell volume (PCV) and erythrocyte sedimentation rate (ESR) were found to be significantly ($p < 0.05$) lower and total leucocyte count (TLC) higher in all groups of anoestrus cows (G0 to GIV) as compared to normal cyclic control group (GV) before start of treatment. After 20 days of deworming alone in G1 and deworming plus mineral mixture supplementation together with the herbal, homeopathic and allopathic course of treatment (GII to GIV), there was a significant improvement in values of all these hematological traits as compared to pre-treatment values in concerned groups. The impact of mineral mixture plus herbal, homeopathic and allopathic treatment was however insignificantly more than the deworming alone as seen from the data of various hematological indices post-treatment in groups GI To GIV (Table 1).

The present findings were following Ruginosu *et al.* (2010), who reported an improved mean value of hemoglobin (10.6 ± 0.20 vs. 9.20 ± 0.30 g/dL) after treatment in anoestrus cows. They also recorded a slightly higher value of TLC in cows with chronic genital infection compared to healthy cows (8.20 ± 2.20 vs. 7.26±1.04 $x10^3$/µL). Ahmed *et al.* (2003) observed a non-significant difference in TLC of cyclic and acyclic cows. The present findings were also in agreement with Pariza *et al.* (2013), who found significantly lower mean hemoglobin concentration and higher TLC and ESR in anoestrus than the control group of cows. Kumar *et al.* (1991) reported lower values of PCV in anestrous cows and heifers and postulated that the cyclic oestrus cows have a higher metabolic rate, which causes an increase in the production of RBC and this lead to increased values of other hematological parameters. The lower hemoglobin in anoestrus animals might be due to anemia caused by gastrointestinal parasites or nutritional deficiency of protein and micro minerals. Though the importance of hemoglobin levels has not been directly implicated in reproductive disorders, a low level of hemoglobin influences tissue oxygenation of the reproductive tract, which in turn could affect the cyclicity. The degree of leukocytosis depends upon several factors including nature of causative agent, severity of infection, resistance of animal, and localization of inflammatory response.

Among the differential leucocyte counts, the mean percentages of neutrophils, monocytes, and basophils were

Table 1: Effect of mineral mixture, herbal heat inducer (Janova), homeopathic medicine (Sepia) and GnRH-PG-GnRH protocol on haematological profile in postpartum anoestrus cows (Mean ± SE)

		Treatment					
Parameter	*Status*	G_0	G_I	G_{II}	G_{III}	G_{IV}	G_V
Hemoglobin	Before	8.90 ± 0.05^{Y}	9.03 ± 0.08^{Y}	9.13 ± 0.07^{Y}	9.09 ± 0.06^{Y}	9.11 ± 0.07^{Y}	11.36 ± 0.36^{X}
	After	$8.91\pm0.06^{a\,Y}$	$9.99\pm0.11^{B,ab}$	$11.15\pm0.23^{B,bc}$	$11.38\pm0.29^{B,bcd}$	$11.64\pm0.29^{B,cde}$	11.43 ± 0.23^{X}
TLC	Before	9.36 ± 0.04^{Y}	9.34 ± 0.05^{Y}	9.38 ± 0.06^{Y}	9.36 ± 0.06^{Y}	9.40 ± 0.57^{Y}	7.36 ± 0.09^{X}
	After	$9.35\pm0.05^{a\,Y}$	$7.5\pm0.07^{B,ab}$	$7.29\pm0.10^{B,bc}$	$7.30\pm0.05^{B,bcd}$	$7.18\pm0.05^{B,bcde}$	7.33 ± 0.05^{X}
PCV	Before	27.35 ± 0.49^{Y}	28.77 ± 0.24^{Y}	27.11 ± 0.46^{Y}	27.87 ± 0.33^{Y}	28.62 ± 0.32^{Y}	31.26 ± 0.34^{X}
	After	27.38 ± 0.40^{a}	$29.50\pm0.35^{B,ab}$	$32.27\pm0.31^{B,bc}$	$32.76\pm0.50^{B,cd}$	$33.84\pm0.55^{B,cde}$	31.56 ± 0.41^{X}
ESR	Before	7.05 ± 0.10^{Y}	7.10 ± 0.09^{Y}	7.21 ± 0.01^{Y}	7.18 ± 0.07^{Y}	7.10 ± 0.08^{Y}	7.81 ± 0.03^{X}
	After	$7.03\pm0.04^{a\,Y}$	$7.52\pm0.07^{B,ab}$	$7.96\pm0.08^{B,bc}$	$7.93\pm0.08^{B,bcd}$	$8.16\pm0.10^{B,cde}$	7.82 ± 0.06^{X}

Means bearing different superscripts in a column (A, B) and in a row (X,Y) & (a, b, c, d, and e) differed significantly for each attribute.

Table 2: Effect of mineral mixture, commercial herbal drug (Janova), Homeopathic medicine (Sepia-30) and GnRH-PG-GnRH Protocol on differential leukocyte count of postpartum anoestrus cows (Mean ± SE)

		Treatment					
Parameter	*Status*	G_0	G_I	G_{II}	G_{III}	G_{IV}	G_V
Neutrophil	Before	25.88±0.35	26.38±0.46	26.50±0.42	26.63±0.46	26.38±0.38	27.88±0.35
	After	25.50 ± 0.19^{a}	28.50 ± 0.42^{b}	28.63 ± 0.42^{bc}	28.75 ± 0.45^{bcd}	28.38 ± 0.38^{bcde}	27.38±0.38
Lymphocyte	Before	67.00±0.50	66.38±0.42	66.63 ± 0.60^{A}	65.88 ± 0.61^{A}	65.88 ± 0.44^{A}	65.50±0.19
	After	67.13 ± 0.44^{a}	66.75 ± 0.59^{ab}	$64.63\pm0.60^{B,abc}$	$63.88\pm0.61^{B,bcd}$	$63.88\pm0.44^{B,cde}$	65.25±0.25
Monocyte	Before	3.25±0.25	3.25±0.37	3.25±0.37	3.63 ± 0.26^{A}	3.63 ± 0.18^{A}	3.70±0.25
	After	3.38 ± 0.26^{a}	4.13 ± 0.30^{ab}	4.13 ± 0.30^{abc}	$4.50\pm0.19^{B,abde}$	$4.63\pm0.18^{B,bcde}$	4.13±0.23
Basophile	Before	0.38±0.18	0.50±0.19	0.38±0.18	0.50±0.19	0.38±0.18	0.50±0.19
	After	0.50±0.18	0.63±0.18	0.38±0.18	0.50±0.19	0.75±0.16	0.63±0.18
Eosinophil	Before	3.38±0.18	3.50±0.19	3.50±0.19	3.50±0.19	3.38 ± 0.18^{A}	3.00±0.28
	After	3.38 ± 0.18^{a}	$2.25\pm0.16^{B.b}$	$2.50\pm0.19^{B,abc}$	$2.38\pm0.18^{B,abcd}$	$1.88\pm0.13^{B,bced}$	2.63±0.26

Means bearing different superscripts in a column (A, B) and in a row (a, b, c, d, and e) differed significantly for each attribute.

insignificantly lower and leucocytes and eosinophils higher in anoestrus positive control than the normal cycle negative control, and the values of all other treatment groups (GIto GIV) were in-between, with occasional significant differences in post-treatment values (Table 2). These findings on DLC concurred well with Ahmed *et al.* (2003), who also reported a non-significant difference in the DLC counts in the cyclic and acyclic animals. Leukocytosis induced as a result of infection promotes the release of neutrophils from the bone marrow through increased plasma concentration of leukocytosis-inducing factor by bacterial products. Lymphocytes differentiate in primary lymphoid organs where they commit a lymphocytic lineage, express B or T cell receptors, which are essential for cell survival or further maturation as well as function. Monocytes are a subset of circulating white blood cells that can further differentiate into a range of tissue macrophages of dendritic cells and are implicated in many inflammatory diseases.

Resumption of Cyclicity and Conception

The oestrus induction response in cows of group GI, GII, GIII, and GIV was 50.00, 62.50, 75.00 and 87.50 %, respectively, with corresponding oestrus induction interval of 32, 16, 15 and 11 days. The highest oestrus induction response was observed in G_{IV} followed by GIII, GII, and GI respectively. However, none of the cows in group G_0 (untreated anoestrus cows) showed a resumption of oestrus cyclicity during the course of study (Table 3). The results show the importance of different therapeutic regimens in the induction of oestrus in anoestrus cows.

The establishment of cyclicity in G_I might be due to supplementation of a mineral mixture containing iron, manganese, selenium, zinc, cobalt, and iodine and various essential amino acids which are known to normalize the reproduction function. The findings of an oestrus induction response in GI and GII also corroborated with Chaudhiry *et al*. (2018) who reported similar oestrus induction rate in anoestrus buffalo heifers, but Kumar *et al*. (2011) reported higher oestrus induction response (76.92 %) with a mean interval of 26.70±4.52 days using Lugol's iodine 10 ml on os cervix and supplementation of injectible phosphorus, vitamin A and cyclomin-7 bolus. Mathur *et al*. (2005) observed 80 % and 66.66 % oestrus induction response with induction interval of 10.25 days and 21.00 days in Frieswal

Table 3: Efficacy of Janova, Sepia-30 and GnRH-PG-GnRH regimen on oestrus induction response and conception rate in postpartum anoestrus cows

Treatment Group	*No. of cows*	*Oestrus and ovarian response*	*Oestrus induction interval (days)*	*Conception after AI*			
				Induced oestrus	*IInd cycle*	*IIIrd cycle*	*Overall*
G0	8	None	None	None	None	None	None
GI	8	4/8 (50.00%)	32	2 (50%)	1 (25%)	-	3 (75.00%)
GII	8	5/8 (62.50%)	16	3 (60%)	1 (20%)	-	4 (80.00%)
GIII	8	6/8 (75.00%)	15	2 (33.33%)	1 (16.66%)	1 (16.66%)	4 (66.66%)
GIV	8	7/8 (87.50%)	11	3 (48.85%)	1 (14.28%)	-	4 (57.13%)
GV	8	8/8 (100.0%)	-	5 (62.5%)	1 (12.5%)	none	6 (75%)

and Sahiwal heifers, respectively, by using inj. vitamin A and Tonophosphan.

The oestrus induction response in GII with Janova was in agreement with the findings of Pugashetti *et al.* (2009). Available reports suggested that Janova exerts gonadotropin like action and synchronizes the release of FSH, LH and estradiol for inducing ovulatory oestrus. Feeding of Janova probably stimulates the hypothalamus-hypophyseal ovarian axis, which results in synchronizing the hormonal release and inducing oestrus in the early postpartum anoestrus period with comparatively higher conception rate (Singal, 1995). On the other hand, Sahapure *et al.* (2016) reported lower oestrus induction response with the use of herbal heat inducer Prajana HS and CoFeCu tablet and Estroform powder (7.5 g/day, 2 days) and tablet Mintrus (1 tab/day, 20 days). Similarly, Rajkumar *et al.* (2006) reported a lower oestrus induction response using the homeopathic remedy in anoestrus cows. Contrary to the present findings of Ovsynch in GIV, higher resumption of cyclicity was reported by De Jarnette *et al.* (2001) and Dhami *et al.* (2015). The differential resumption of cyclicity and conception rate might be due variation in the breed, climate, method and frequency of oestrus detection, presence of large follicle at the time of treatment, body condition and milk yield of the animal.

The conception rates at induced oestrus in group GI, GII, GIII, and GIV were 25.00, 20.00, 16.66, and 14.28 %, respectively, with corresponding pooled conception rates of three cycles as 75.00, 80.00, 66.66 and 57.13 % (Table 3). In GV (negative control), the pooled conception rate was 75.00%, and none of the cow in positive control resumed the estrous cycle and conceived. Conception rate in GI and GII was in accordance with Sahatpure *et al.* (2016), who reported a conception rate of 75% by using estroform powder and mintrus tablet and 66.67% with use of capsule Prajana HS and tablet CoFeCu. Like current findings in GIII with the use of Sepia (62.5% oestrus induction and 80% conception rate). Rajkumar *et al.* (2006) reported a 54.5% overall conception rate using homeopathic drug 15 pills, twice in day for 10 days in anoestrus cows. Chandel *et al.* (2009) also reported significantly higher oestrus induction response (71.42% vs 20%) and conception rate (78.95% vs. 0.00) using homeopathic combination "Hit-O-Gen" (Goel Vet Pharma) two tablets twice a day in anoestrus buffaloes. Williamson *et al.* (1995) observed the beneficial effect of Sepia 200c for the prevention of postpartum anoestrus in dairy cows and lowering calving to conception interval. Furhermore, Bhoraniya *et al.* (2012) and Dhami *et al.* (2015) reported comparable conception rates with GnRH-PG-GnRH protocol in anoestrus cows.

In conclusion, the postpartum anoestrus in cows is a multifactorial problem, and oestrus can be effectively induced with good conception rate using herbal Janova, homeopathic Sepia, and allopathic GnRH-PG-GnRH protocol together with a mineral supplement.

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