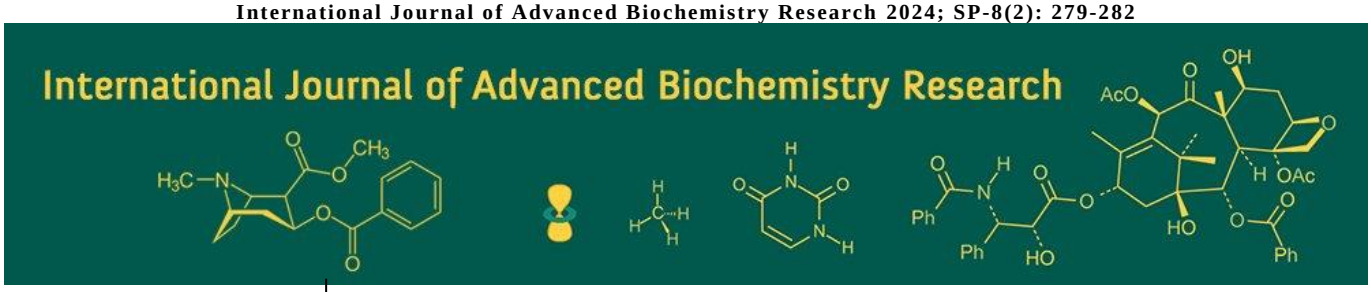

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Impact of mineral mixture, herbal remedies, and hormonal treatment on hematological parameters and ovarian activity resumption in post-partum anoestrus Murrah buffalo

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Abstract
This experiment was conducted in 24 postpartum anoestrus buffalo. Those are presented at Veterinary Clinical Complex, International Institute of Veterinary Education and Research, Bahu-Akbarpur, Rohtak. The experimental buffaloes were divided into four groups, each comprising 6 animals (n=06). Group I, which was untreated postpartum anoestrus, and Group II received the mineral mixture supplement. Group III received Prajana HS treatment, and Group IV received intramuscular administration of Buserelin acetate (20 µg) at day 0, followed by intramuscular administration of Cloprostenol sodium (500 µg) on day 7, and the same dose of second GnRH on day 9. The timed artificial insemination was performed at 12 and 24 hrs. Overall estrus induction and conception rate were recorded as 33.33% and 50% in mineral mixture (G2), 66.66% and 75% in Prajana HS (G_3), 83.33% and 80% in GnRH-PG-GnRH regimen group. On the basis of Overall estrus induction, conception rate and hematological parameters concluded that Prajana HS, and GnRH-PG-GnRH regimen group an effective roll in the treatment of anoestrus buffalo heifers.

Keywords: Postpartum anoestrus buffalo, ovarian activity, estrus induction, conception rate

Introduction
Buffalo are important animals for draught as well as meat production in addition to yielding highest amounts of milk with high fat and Solid Non Fat (SNF) content. There are 108.7 million buffalo in India (About 21.2% of the world's total). Anoestrus in buffaloes has been reported in India between 9% and 85.5%, with economic losses per animal of about Rs. 372.90 per day (Chohan, 1998) [4]. Anoestrus is the most common reproductive problem that affects cattle and buffalo in India. Its effects are particularly severe in the country's rural and suburban areas and have a detrimental impact on livestock economics and productivity. This reproductive cycle dysfunction is characterized by the lack of overt estrous signals, which could be due to nonexpression or inability to recognize it. Anestrus has been observed in adult animals, postpubertal heifers, nursing mothers, and pregnant individuals during the first postpartum phase. The syndrome may be associated with pyometra, fetal resorption, mommification, and other uterine problems (Kumar *et al*., 2012) [10].
Anoestrus is a major factor in buffaloes' poor reproductive results, which prolongs the intercalving time (Devkota *et al*., 2012) [6]. An insufficient and unbalanced diet reduces GnRH secretions, claim Mwaanga and Janowski (2000) [15]. High yielders create more prolactin hormones, which suppresses GnRH. According to Youngquist and Thelfall (1986) [26], nursing is yet another important etiological factor that contributes to the development of anoestrous behavior in cows. Published research have shown that a wide range of factors, including species, breed, parity, season, degree of nutrition, mental health conditions, and geographic location, have been linked to the occurrence of anestrus in buffaloes. According to reports, it varies from 9.09–82.50% in various regions of the nation (Prajapati *et al.*, 2005) [18] to 12.29.12% in Madhya Pradesh and Jabalpur (Kumar *et al.*, 2012) [10].

Materials and Methods

The postpartum anoestrus buffalo were presented at the Veterinary clinical complex, International Institute of Veterinary Education and Research, Bahu-Akbarpur, Rohtak. The anoestrus animal was confirmed by history and two successive rectal examinations at 10 days interval. The animal having smooth ovaries with no palpable any follicular structure and having no clinically detectable abnormalities in their genital tract was categorized as anoestrus animal. The selected (n=24) postpartum anoestrus buffalo were divided into four groups and each group having six animals (n=6). Deworming with albendazol @ 10 mg/kg body weight was done in all animals before the start of treatment to prevent them from the stress of parasitism. Group-1 was kept as control and no treatment was given. Group-II was treated with Mineral mixture supplementation for 20 days (1-20 days) @ 50 gram orally once a day and Group-III Mineral mixture supplementation for 20 days (1-20 days) @ 50 gram orally once a day and Prajana HS @ 3 capsule daily for 2 days followed by 3 capsules P.O. for two days on 11th day of study. Group-IV was administered Buserelin acetate (20 µg) intramuscularly at day 0, followed by Cloprostenol (500 µg) intramuscularly on day 7 and same dose of second GnRH on day 9. The timed artificial insemination was performed at 12 and 24 hrs. Sahli's hemoglobinometer was used to determine the hemoglobin concentration of the blood (Samuel, 1986) [23]. Erythrocyte sedimentation rate (ESR) was assessed using the Wintrobe method, and the packed cell volume (PCV) was calculated using a microhaematocrit tube. The hemocytometer method developed by Samuel (1986) [23] was used to count leucocytes. Based on morphological and staining features, the Differential Leucocytes Count (DLC) was carried out in accordance with Coles, (1986) [5].

Following therapy, the animals were carefully watched to see if they were displaying estrus. Careful visual observation of animals in the morning, afternoon, and evening for at least half an hour allowed for the detection of the induction of estrus. An additional confirmation was obtained by a rectal examination. Animals were inseminated twice, separated by 12 hours, once estrus was detected. Per rectal examination was carried out to confirm the pregnancy 45 days after the second artificial insemination.

Treatment Protocol

The selected twenty four (n=24) postpartum anestrous buffalo will be divided into four groups, each group having six (n=6) animals. The treatment will be given as per following schedule and various parameters were assessed.

Table 1: Treatment Protocol

Group	Treatment
G-I	Anoestrus without treatment (Control)
G -II	Deworming (day 0) + Min. Mix. Supplementation for 20 days (1-20 days),
G -III	Deworming (day 0) + Min. Mix. Supplementation for 20 days (1-20 days) + Pragana capsule daily for 2 days followed by 3 capsule orally for two days on 11th day
G -IV	On day 0, buserelin acetate (20 µg) was injected intramuscularly. On day 7, cloprostenol (500 µg) was injected intramuscularly. On day 9, the same dosage of second GnRH was injected.

Results and Discussion

All the induced estrus buffalo were confirmed by palpation of uterine horn and ovary. Be found that G-II treated with mineral mixture showed the symptoms of estrus on an average of 33.33% with an interval of 35 days from initiation of treatment. Out of two estrus buffalo, one buffalo were conceived after the first insemination and another buffalo was not conceived after second and third insemination and total conception rate was recorded as 50%. In Prajana HS treated G-III, 66% buffalo heifers were responded to commercial herbal preparation i.e. Prajana HS treatment for the onset of estrus with an interval of 25 days from initiation of treatment and out of six buffalo, four buffalo exhibit sing of estrus and two were conceived at first insemination (50%) and one buffalo conceived at second insemination (25%) and total conception rate in experimental buffalo heifers were reported as 75%. In G-IV five buffalo were responded to GnRH-PG-GnRH regimen. Five buffalo show sing of estrus out of them three buffalo were conceived at first insemination (60%) and one buffalo conceived at second insemination (20%) total conception rate in experimental buffalo were reported as 80%

Table 2: Effect of mineral mixture, commercial herbal drug (Prajana HS) and GnRH-PG-GnRH regimen on postprtum anoestrus buffalo percent estrus induction and conception rate

Treatment Group	No. of anoestrus Buffalo	No of Animals Exhibited Estrus signs	Pregnancy after artificial insemination 1st 2nd 3rd	Total Animal Conceive
G-1	6	0	- - -	-
G-2	6	2 (33.33%)	1 (50%) 0 0	1 (50%)
G-3	6	4 (66.66%)	2 (50%) 1 (25%) 0	2 (75%)
G-4	6	5 (83.33%)	3 (60%) 1 (20%) 0	5 (80%)

The mean value of the hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), total leucocytes count (TLC) and differential leucocytes count (DLC) in anoestrus buffalo heifers before treatment and after treatment are summarized (Table.3).

The results of Dhoble and Gupta (1981) [7], Kumar *et al.* (1991) [12], and Rameez Ali *et al.* (2012) [20] are comparable to the hemoglobin concentrations found here. Since the study's animals were raised in various field conditions, the higher hemoglobin value in normally cycling buffaloes may indicate their physical and nutritional health, while the lower hemoglobin value in anoestrus buffaloes may indicate poor nutrition and poor health. Anemia brought on by a gastrointestinal parasite or a nutritional shortage of microminerals could be the reason of the reduced hemoglobin levels (McDowell, 1992; Srivastava and

Kharche, 1986) [28, 24] Even while hemoglobin levels have not been directly linked to reproductive diseases, a decline in hemoglobin value is suggestive of several systemic conditions that may have an indirect impact on the reproductive organs' ability to function. A low hemoglobin level affects the reproductive tract's tissue oxygenation, which may have an impact on cyclicity (Ramakrishna, 1997) [19].

The packed cell volume was lower during early stage of life and it increased with advanced age. Kumar *et al*., (1990) [11] observed that hematocrit value showed a marked decrease in one month old calves as compared to day old and other ages. Similar results were found by Patil *et al.* (1992) [17], who came to the conclusion that growing buffalo heifers had better PCV values than adult buffaloes due to their higher erythrocyte values. This could be because of a high basal metabolic rate, which accelerated erythropoiesis and produced more erythrocytes overall. According to Rizvi (1973) [21], the wintertime has a higher value of erythrocyte sedimentation rate than other seasons. The erythrocyte sedimentation rate value in heifers was also influenced by the age of the animals. After puberty was reached, a rise in ESR values was noted. According to Patil *et al.* (1992) [17], the erythrocyte sedimentation rate was low in neonatal buffaloes but increased with age. These findings are consistent with the reports from Horadagoda *et al.* (2002) [9], Patil *et al.* (1992) [17], and Rameez Ali *et al.* (2012) [20]. Numerous physiological characteristics and environmental factors have an impact on the erythrocyte parameter and differential leucocyte counts (Zadnik, 1991) [27].

A non-significant difference in TLC value was noted by Ahmad *et al.* (2003) [1] between cyclic and non-cyclic cows. The fact that the animals in the anoestrus group had neutrophilia, a sign of infection, suggests that the infection was causing the bone marrow to release neutrophils through the plasma's leucocytosis-inducing factor. Stress and a dietary deficit that compromises immunity may be the root cause of subclinical illness. There were no notable variations seen in the current TLC, eosinophils, lymphocyte, monocyte, and basophil counts, nor in the percentages of eosinophils, monocytes, or basophils. In anoestrus and normal cyclic buffaloes, the mean counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were not statistically significant. Brar *et al.* (2002) [3] also observed similar findings. Due to varying environmental conditions and feeding habits, the current finding about the impact of mineral supplementation on anoestrus animals was less than that of Lall *et al.* (2000) [13] and Pander *et al.* (2003) [16]. After receiving a mineral mixture supplement for 12.9 days, 72.13% of buffaloes came into heat, and 59.02% of them conceived overall in the field, according to Pander *et al.* (2003) [16], while Lall *et al.* (2000) [13] reported that 70% of buffaloes showed estrus and conceived within a period of 2-4 weeks. The results on the impact of Prajana HS on anoestrus animals were in similar with the findings of Samsad and Hasan (1984) [22], who noted that 73.3% of Bangladeshi buffaloes conceived and 75% of them displayed estrus. Whereas Tanwar *et al.* (1986) [25] carried out an estrus induction study in a semi-arid area of India and found that 20% of buffaloes conceived following treatment with Prajana HS and 36.67% of buffaloes reached estrus after 18 days. High levels of trieonic fatty acids are included in the herbal product Prajana HS, which is non-hormonal. In order to optimize ovarian function and subsequently induce timely ovulation, estrus, and conception, these fatty acids serve as precursors for the manufacture of prostaglandin (PG), follicular stimulating hormone (FSH), luteinizing hormone (LH) and other hormones.

Table 3: Effect of mineral mixture, commercial herbal drug (Prajana HS) and GnRH-PG-GnRH regimen on hematological constituent (mean ±se) in postpartum anoestrus buffalo Mean bearing different superscript in the column (A, B) and in a row (a, b, c, d, e, and f) significantly differed repeatedly for each attributes

Parameter	Status	Treatments			
		G-1	G-2	G-3	G-4
Heoglobin	Before	9.49±.20^{Y}	9.56±0.18	9.39±.0.15^{Y}	9.64±0.16^{X}
	After	9.51±.0.17^{a,Y}	10.96±0.21^{A,ab}	12.42±.0.12^{A,bcd}	13.08±0.16^{A,cde}
Total Leucocytes	Before	8.12±0.16^{Y}	8.24±0.07^{Y}	8.31±0.13^{Y}	8.28±0.18
	After	8.11±0.09^{Y}	7.42±12^{ab}	7.46±0.13^{A,bc}	7.15±0.07^{A,bcd}
Packed cell volume	Before	31.40±0.38^{Y}	28.56±0.12^{Y}	26.89±0.45^{Y}	28.98±0.64^{Y}
	After	31.32±0.14^{Y}	31.02±0.28^{A,ab}	32.74±0.12^{A,bc}	35.76±0.31^{A,cd}
Erythocyte Sedimentation rate	Before	7.12±0.08^{Y}	6.92±0.16^{Y}	7.13±0.12	7.15±0.32^{X}
	After	7.11±0.05^{a}	7.56±0.19^{A,ab}	8.28±0.08^{A,bc}	8.76±0.61^{A,cd}
Neutrophil	Before	26.50±0.42^{Y}	26.75±0.36	25.87±0.40^{a}	27.13±0.32^{X}
	After	26.63±0.47	27.34±0.42^{ab}	27.16±0.35^{A,bc}	28.31±0.41^{A,ab}
Lymphocyte	Before	68.63±0.23	65.62±0.43	67.32±0.52	67.63±0.46
	After	68.13±0.24	66.21±0.15^{A,}	65.12±0.40^{A,bc}	65.50±0.33^{A,bcd}
Monocyte	Before	2.43±0.16	2.51±0.17	2.62±0.31Y	2.68±0.19X
	After	2.47±0.11a	2.68±0.38A,bc	2.56±0.16A,bc	4.23±0.05A,cd
Eosinophil	Before	3.15±0.26	3.27±0.30	3.47±0.25	3.01±0.23
	After	3.12±0.21a	2.98±0.16ab	2.98±0.17A,bc	2.75±0.25A,bc
Basophil	Before	0.51±0.12	0.52±0.14	0.51±0.18	0.50±0.12
	After	0.51±0.07a	0.52±0.17	0.50±0.06abc	0.50±0.16bc

Conclusion

Postpartum anoestrus in buffalo is complicated problem and oestrus can be effectively induced with mineral mixture, Pragana HS and GnRH-PG-GnRH regimen. It helps to induce the behavioral oestrus by means of enhancing ovarian stimulation.

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