

Induction of Estrus in Postpartum Anestrus Murrah Buffaloes using Chelated Minerals and Ovsynch Protocol

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ABSTRACT

A research trial aimed to assess the effect of chelated mineral mixture supplementation and Ovsynch protocol on induction of estrus was conducted on 120 days postpartum true anestrus Murrah buffaloes. A total of 18 true postpartum acyclic buffaloes selected were divided into three groups each of 6 animals. Group T₁ was treated with chelated mineral mixture Agrimin Forte (50 g/day) and Garbhov bolus (1 bolus/day) for 15 days. In Group T₂, chelated mineral mixture as in T₁ plus Ovsynch protocol comprising intramuscular injections of Buserelin acetate (10 µg), Cloprostenol (500 µg), and Buserelin acetate (10 µg) on days 0, 7 and 9, respectively, were given, and treated buffaloes were inseminated after detected heat. The control group (C) buffaloes received no treatment. Blood glucose, and serum macro-micro minerals profile was compared pre- and post-treatment between the groups. Estrus was induced in 66.67% and 83.33% of postpartum anestrus buffaloes, respectively, in T₁ and T₂ groups with intermediate to intense estrus intensities, as compared to the untreated control group (33.33%). After supplementation of the mineral mixture (T₁), concentrations of blood glucose, calcium, magnesium, copper, iron, and zinc increased significantly ($p \leq 0.05$); however, inorganic phosphorus, cobalt, and selenium levels did not vary significantly post-treatment. In group T₂, a significant increase was observed in the mean concentrations of blood glucose, copper, and cobalt. From the present study, it can be concluded that supplementation of mineral mixture and application of hormonal synchronization protocol successfully induced estrus in postpartum acyclic Murrah buffaloes and can be adopted as a potential tool to alleviate the inherited problems of poorly exhibited estrus.

Key words: Buffalo, Chelated mineral mixture, Estrus induction, Mineral profile, Ovsynch, Postpartum anestrus.

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INTRODUCTION

The bubaline species is the prevailing livestock component of the Indian economy. Buffalo have unique physiological adaptations, particularly with regard to adaptations in hot environmental conditions (Orskov, 2007). Buffaloes, however, have a markedly lower fertility than cattle due to late attainment of puberty, silent estrus/ovulation and high incidence of anestrus. During the postpartum period, several endocrine, immune and metabolic pathways are functionally altered so that physiological adjustments can take place often resulting in negative energy balance (Pande *et al.*, 2016). Postpartum anestrus is widely recognized as a prevalent and challenging issue in dairy animals particularly frustrating for producers. Buffaloes tend to have fewer pre-antral and antral follicles and display a higher percentage of follicular atresia in contrast to cows. Under optimal conditions, buffalo resume cyclicity 30 to 90 days postpartum but factors such as poor nutrition and body condition, suckling, adverse climate and managerial practices can delay this considerably (Perera, 2011). Only about 45% of Indian buffaloes resume cyclicity within 90 days postpartum and the rest 55% remain in anestrus for about 150 days (El-Wishy, 2007). During hot-humid months, estrus expression in buffalo tends to decrease, posing challenges in estrus detection. Buffaloes face challenges in hot-humid weather due to their dark skin

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and sparse sweat glands, which absorb more heat (Marai and Haebe, 2010). There is an increasing awareness of the effects of ambient heat stress on animal productivity in recent times.

The profitable dairy farming is predominantly based on two major interrelated factors, viz., nutrition and reproduction. Apart from energy and protein, minerals are the main limiting factor for production (Judson and McFarlane, 1998). Both macro and micro minerals are vital in augmenting production

and reproduction in farm animals and their deficiency causes impairment in body functions. Minerals play an intermediate role in the action of hormones and enzymes at cellular level in an integrated fashion. Besides working as a co-factor or activator of enzyme systems the elements like calcium have been found to sensitize for the action of hormones. Deficiency of minerals is associated with subnormal fertility and anestrus condition. On the other hand, supplementation of these minerals improves reproductive efficiency, decreases service period and intercalving interval and also improves conception rate (Selvaraju *et al.*, 2009). In ruminants, number of factors like season and physiological variations such as those in pregnancy and lactation can affect serum chemistry and minerals levels (Yildiz *et al.*, 2005). Blood metabolic profile changes are important as they help in monitoring the severity of metabolic diseases and their treatment. Seasonal anestrus and postpartum anestrus in buffalo can be controlled using a range of therapeutic measures targeting at follicular wave and ovulation synchronization using hormonal procedures, non-hormonal therapies, nutrition and management (Singh *et al.*, 2009). The present study was attempted to evaluate the efficacy of mineral supplementation along with Ovsynch protocol in induction of estrus in postpartum anestrus Murrah buffaloes during hot humid season.

MATERIALS AND METHODS

The present investigation was undertaken at Veterinary Gynaecology and Obstetrics Department of College of Veterinary Science and Animal Husbandry, Anjora, DSVCKV, Durg (Chhattisgarh, India) during April to November 2023 following approval of IAEC of the college. Murrah buffalo stationed in private dairy farms of Durg (Chhattisgarh) constituted experimental animals. The pluriparous buffaloes, aged 4 to 8 years in good body condition weighing 450 to 500 kg, and beyond 120 days postpartum with history of normal calving were gynaecologically examined twice at 11 days interval. The buffaloes (n=18) having normal reproductive tract, ovaries without palpable corpora lutea (CL), and not observed in estrus within 120 days after parturition were designated as "true anestrus" and selected for the study. They were housed in asbestos roof shed with concrete flooring in tail-to-tail system and maintained under uniform feeding and managerial conditions, milked twice daily, and were dewormed with Ivermectin (Hitek[®] bolus) 200 µg/kg body weight, orally before the experiment initiated.

Experimental Design

The selected 18 postpartum acyclic Murrah buffaloes were randomly assigned to three treatment groups each comprising of 6 animals. The buffaloes of treatment group T₁ (mineral mixture supplementation) received chelated mineral mixture (CMM) Agrimin Forte[®] (Virbac Animal Health) @ 50 g/animal/day and Garbhov[®] bolus (Sushima Pharmaceuticals) @ 1 bolus/animal/day mixed with feed for 15 days. Similarly,

acyclic buffaloes of treatment group T₂ (mineral mixture plus Ovsynch protocol) were treated with CMM for 15 days and then administered with intramuscular injection of Receptal[®] (GnRH analogue, Buserelin acetate 0.0042 mg/mL, 2.5 mL) on day 0, Inj. Vetmate[®] (PGF₂α analogue, Cloprostenol 250 µg/mL, 2 mL) on day 7, and second injection of Receptal[®] on day 9. The buffaloes of control group (C) received routine diet without any treatment. Per-rectal palpation was done in all three groups to observe changes in ovarian follicular structure to note the response to treatments.

Estrus and Fertility Response

The estrus response was defined as the percentage of treated animals that exhibited estrus. Mean interval from completion of treatment to onset of estrus, duration of estrus and intensity of estrus was also noted. The intensity of estrus was measured based on the observations of symptoms and score card (15 point scale) stipulated by Rao and Rao (1981) and graded as weak (≤5); intermediate (6 to 10) and intense (11 to 15) estrus. The buffaloes found in estrus were inseminated 12 h after the exhibition of the estrus symptoms. The animals not returning to estrus were examined per rectally for confirmation of pregnancy at 60 days post-breeding and fertility response was noted. Conception rate was calculated as percentage of buffaloes conceived of the total buffaloes inseminated at observed (induced) estrus.

Blood Collection and Biochemical Estimations

Blood samples (10 mL) were aseptically collected from jugular vein immediately before start of treatment and also at the end of treatment. Serum was separated by centrifugation at 500 x g for 10 to 15 min and stored in sterile vials in a deep freezer at -20°C till biochemical estimation.

Blood glucose concentrations in freshly collected whole blood were quantified using a glucometer and blood glucose strips. Serum concentrations of major minerals like Ca, Pi and Mg were estimated by semi auto-analyzer (Microlab 300) using serum diagnostic kit as per manufacturers' specifications. Trace elements, viz., Co, Cu, Fe, Zn and Se concentrations in serum were estimated using Atomic Absorption Spectrophotometer (AAS4141; Electronics Corporation of India Limited, Hyderabad) employing flame atomization techniques after acid digestion of samples.

Statistical Analysis

The data obtained on various parameters before and after treatment were analysed statistically for the significance of differences within and between groups by t-test and one way analysis of variance and Duncan's multiple range test as per standard statistical method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Estrus and Fertility

The results obtained on estrus induction response, mean interval from completion of treatment to onset of estrus, duration and intensity of induced estrus and conception rates in anestrus buffaloes under different treatment groups and in control group are summarized in Table 1. Higher estrus induction response (66.67%) with conception rate of 50% was reported in in group T₁ over control group (33.33% & 50.0%). In agreement with this, Singh *et al.* (2016) and Maurya *et al.* (2021) reported favourable estrus and fertility response, but of varied degree among buffaloes treated with mineral supplementation. The wide variations in response to mineral treatment have been attributed to type of mineral mixture supplemented (chelated or ionic), duration of treatment, dose and rate of supplementation, age and parity as well as production performance of treated buffaloes etc. which are not widely reported till now. Although reports in augmentation of fertility with mineral mixture supplementation are scanty pertaining to anestrus buffaloes during hot-humid season, however Mujawar *et al.* (2019) indicated favourable estrus and fertility response with mineral treatments.

In the present study 83.33% estrus induction with 60.0% conception rate was observed with CMM plus Ovsynch protocol in group T₂, which was quite high than in T₁ or control groups. Although, no studies have yet been reported combined response of mineral mixture plus hormones for induction and synchronization of estrus in buffaloes, however, numerous reports are available for Ovsynch and their modifications in augmenting fertility in buffaloes. Similar reports of estrus induction response (81 to 87%) and estrus behaviours have been reported by Ravikumar *et al.* (2007) in postpartum anestrus buffaloes, by Karena and Darwish (2010) in cyclic Egyptian buffaloes during summer months and by Thorat *et al.* (2012) and Mujawar *et al.* (2019) in Marathawadi buffaloes. Also, a higher response of 100% was reported by Kumar *et al.* (2015) and Virmani *et al.* (2018) with application of G-P-G protocol, while lower estrus/

ovulation response of 40-60% was documented by Nakrani *et al.* (2014) among buffaloes treated with Ovsynch based hormonal protocols.

Blood Glucose

In the present study, post-treatment blood glucose levels increased significantly ($p < 0.05$) in group T₁ and T₂ as compared to their pre-treatment levels (Table 2), which corroborated with reports of Joshi *et al.* (2020) and Maurya *et al.* (2021). Richards *et al.* (1989) advocated that increased glucose level was correlated with increased energy status of the reproductive phase and also elevated the progesterone production directly by increasing LH production. Low blood glucose in buffaloes decreased the hypothalamic-hypophyseal-ovarian axis signal communication leading to anestrus (Kumar *et al.*, 2015). Higher values of blood glucose after supplementation of minerals and application of hormonal protocol in present study indicated that minerals might have role in improved energy metabolism.

Serum Major Minerals

Overall mean serum concentrations of major minerals, viz., Ca, Pi and Mg measured in different treatment groups of postpartum anestrus buffaloes are presented in Table 2. A significant increase in serum concentrations of Ca and Mg was observed as a result of CMM supplementation in group T₁. Effect of CMM supplementation plus hormonal induction was evident with noticeable change in mineral status of treated acyclic buffaloes in group T₂. Mean Pi concentration did not vary post-treatment; however it was well within physiological range in pre-treatment group. Similar increase in levels of minerals as a result of supplementation was also reported by Lall *et al.* (2004), Biswas *et al.* (2005) and Savalia *et al.* (2013). Minerals like Ca, Pi and Mg also influence the ability of animals to utilize other trace minerals. The traditional inorganic forms of trace minerals rapidly dissociate in the rumen and are free to interact with antagonists, resulting in the loss of trace minerals prior to absorption by the animal. Bailey *et al.* (2001) reported that chelated organic minerals are bound to organic ligands and thus improve the bioavailability of the mineral.

Table 1: Estrus induction, interval of onset of estrus, estrus intensity, duration of estrus and conception rate in different treatment regimens

Reproductive parameters	Treatment Groups		
	T ₁ (n=6)	T ₂ (n=6)	Control (n=6)
No. of animals exhibited estrus (%)	4 (66.67%)	5 (83.33%)	2 (33.33%)
Interval from last treatment to onset of estrus (range)	99.00±10.25h (72-120h)	46.00±6.87h (36-72h)	-
Estrus: Intense intensity	25% (1/4)	20% (1/5)	-
Estrus: Intermediate intensity	75% (3/4)	60% (3/5)	100% (2/2)
Estrus: Weak intensity	-	20% (1/5)	-
Duration of estrus (range)	16.50±1.28h (12-22h)	17.80±1.77h (12-30h)	15.00±1.22h (12-18h)
Conception rate (no. conceived)	50.0% (2/4)	60.0% (3/5)	50.0% (1/2)



Table 2: Serum concentrations of blood glucose and minerals (Mean \pm SE) in treatment groups of postpartum anestrus buffaloes

Parameters		Treatment Groups		
		T ₁ (n=6)	T ₂ (n=6)	Control (n=6)
Blood glucose (mg/dL)	Before	67.83 \pm 2.81 ^a	65.50 \pm 2.68 ^a	59.83 \pm 2.87
	After	78.00 \pm 3.46 ^{Bb}	72.17 \pm 2.09 ^{Bb}	60.83 \pm 1.54 ^A
Calcium (mg/dL)	Before	7.09 \pm 0.70 ^a	7.20 \pm 1.10	6.62 \pm 0.30
	After	8.76 \pm 0.55 ^b	8.36 \pm 1.10	7.06 \pm 0.52
Phosphorus (mg/dL)	Before	5.38 \pm 0.66	5.37 \pm 0.56	6.57 \pm 0.52
	After	6.24 \pm 0.82	6.77 \pm 0.57	6.28 \pm 0.63
Magnesium (mg/dL)	Before	2.08 \pm 0.28 ^a	1.87 \pm 0.29	1.85 \pm 0.49
	After	2.50 \pm 0.33 ^b	2.07 \pm 0.38	1.74 \pm 0.40
Copper (ppm)	Before	0.73 \pm 0.20 ^{Aa}	1.34 \pm 0.16 ^{Ba}	1.08 \pm 0.10 ^{AB}
	After	1.38 \pm 0.16 ^{Bb}	2.12 \pm 0.17 ^{Cb}	0.90 \pm 0.11 ^A
Cobalt (ppm)	Before	1.76 \pm 0.64	0.68 \pm 0.22 ^a	0.68 \pm 0.15
	After	1.67 \pm 0.50	1.37 \pm 0.47 ^b	1.08 \pm 0.20
Iron (ppm)	Before	1.89 \pm 0.10 ^a	1.86 \pm 0.13	1.82 \pm 0.11
	After	2.13 \pm 0.06 ^b	2.05 \pm 0.28	1.65 \pm 0.12
Zinc (ppm)	Before	3.56 \pm 0.54 ^a	3.65 \pm 0.70	3.63 \pm 0.31
	After	4.91 \pm 0.39 ^b	3.85 \pm 0.46	4.15 \pm 0.73
Selenium (ppm)	Before	0.689 \pm 0.033	0.692 \pm 0.058	0.632 \pm 0.030
	After	0.772 \pm 0.043	0.768 \pm 0.052	0.699 \pm 0.019

Means bearing different superscripts A,B,C differ significantly between groups and a, b between period or stage ($p < 0.05$).

Overall, the status of treated buffaloes improved in terms of serum mineral concentrations and thus it can be concluded that supplementation of CMM had significant impact on absorption and retention of minerals, that reflected in serum values of these minerals.

Serum Trace Elements

Overall mean serum concentrations of trace elements Cu, Co, Fe, Zn and Se measured in ppm between different treatment groups and presented in Table 2 revealed that post-treatment Cu concentrations in group T₁ and T₂ increased significantly ($p < 0.05$) over pre-treatment values, and it also differed significantly ($p \leq 0.05$) between groups T₁, T₂ and control at both pre- and post-treatment stages. A significant ($p \leq 0.05$) increase in mean concentrations of Fe and Zn was recorded as result of CMM supplementation in group T₁. Similarly, a significant ($p < 0.05$) increase in Co level was also noted in CMM and hormonal treatment group T₂. Although the estimated values of minerals in present study were in physiological range, the effect of CMM supplementation and hormonal induction with Ovsynch protocol was clearly evident by improvement in serum concentrations of Cu, Co, Fe and Zn in both the treatments. However, none of treatment could alter the Se concentration of treated buffaloes. These findings of increase in serum trace elements in treatment groups were in close agreement with observations

of Lall *et al.* (2004) in anestrus buffalo heifers and Biswas *et al.* (2005) in postpartum Murrah buffaloes.

Cu and Zn are known to have a significant correlation with reproductive hormones progesterone and estradiol as they are specific activators of enzyme systems that assist in maintaining the activity of hormones in blood (Georgievskii, 1982). Setia *et al.* (1994) studied the distribution of trace elements during lactation stage in buffaloes and the concentration of Zn and Cu reflected the increasing trends with advancement of lactation in buffaloes as was also noted in present study. Mineral supplemented in chelated form increased the body development and the early pregnancy rates in buffaloes.

CONCLUSION

From the present study it may be inferred that estrus may be successfully induced in anestrus or sub-estrus buffaloes with supplementation of minerals, alone or along with hormonal treatment. These can be adopted as a potential tool to alleviate the infertility problems in buffaloes that are attributed to its 'shy' nature manifested in silent- or sub-estrus and poor estrus expression.

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