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Herbal, Homeopathic and Hormonal Therapy Improves Macromineral Profile and Fertility Outcomes in Postpartum Anestrus Buffaloes

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ABSTRACT

The study was aimed to assess the effect of *Aegle marmelos* (Indian Bael), Oophorinum and Progesterone on serum calcium, phosphorus and fertility outcomes. A total of 24 postpartum anestrous buffaloes were randomly allocated in 4 groups as T0, T1, T2 and T3; each group comprised of six animals. All the animals were dewormed with fenbendazole (10mg/kg) before commencement of experiment. The T0 (untreated control) buffaloes were supplemented with mineral mixture (50 gm daily for 10 days). In addition to mineral mixture, the T1, T2 and T3 buffaloes were treated with *Aegle marmelos*, Oophorinum and progesterone hormone, respectively. The serum calcium and phosphorus values was significantly ($P<0.05$) increased after therapy in Oophorinum treated buffaloes as compared to other groups. The estrus induction response was 50, 100 and 33.33% in *Aegle marmelos,* Oophorinum and Progesterone treated groups, respectively. Among responded animals, the overall conception rate after three inseminations was 0, 66.66, 83.33 and 100%, respectively for T0, T1, T2 and T3 groups. In conclusion, herbal and homoeopathic therapeutics can be used effectively to manage clinical syndrome of post-partum anestrus in Buffaloes.

Key words: Aegle marmelos, Anestrus, Buffaloes, Herbal therapy, Homeopathy, Oophorinum.

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INTRODUCTION

Fertility is one of the key limiting factors in lifetime production of bovines (Butani *et al*., 2011). Livestock is an integral part of the rural society of the country. Furthermore, buffalo considered as "Black gold of India", with 109.85 million populations (DAHDF, 2019), plays a pivotal role to significantly improve economic status of rural people by contribution of milk, meat, hides etc. Buffalo has recently gained a lot of attention by virtue of its high milk yield with high fat percentage, tolerance to hot and humid climate, lean meat, draught ability and a reasonable growth rate on roughage feeding (Mondal *et al*., 2007). Unfortunately, buffalo is still considered as problem breeder due to several reproductive puzzles viz. delayed puberty, silent estrus, summer anoestrus, post-partum anoestrus and repeat breeding syndrome (Butani *et al*., 2008; Ashoo *et al*., 2020). Battery of therapeutic agents have been tried and tested for the management of clinical syndrome of anoestrus with varying success rate (Warriach *et al*., 2008; Kumar *et al*., 2010; Kumar *et al*., 2011; Butani *et al*., 2013a; Butani *et al*., 2013b; Kumar *et al*., 2018).

In current sphere, numerous medicinal plants and homeopathic drugs have been tried to manage problem of the anoestrus in bovines (Gupta *et al*., 2011; Kumar *et al*., 2020a). India has one of the ancient, richest and the most diverse cultural tradition called folk tradition associated with the use of medicinal herbs for the management of various disorder in human and animals. Medicinal herbs like *Saracaasoca* (Ashoka), *Trigonellafoenum-graecum* (Methi), *Bambusa aruninacea*, *Carica papaya*, *Garlic*, Neem (Kumar *et al*., 2007) and *M. Koenijii* and *A. marmelos* (Dutt *et al*., 2011) have been used to manage infertility in past few years. Many previous workers have reported use of herbal preparation/indigenous medicine to induce estrus in acyclic cattle (Husain *et al*., 2020; Kumar *et al*., 2020a) and buffaloes with varying success.

Many therapeutic agents has been tried and tested to manage anestrus in bovines across the world however, homeopathic therapy gaining popularity by virtue of its low cost and devoid of untoward side effects need to be validated in field conditions (Kumar *et al*., 2020a). Oophorinum a nosode prepared from ovarian extract can be used in low potency for treatment of ovarian functional disorders in human beings. Furthermore, Mineral has an important bearing in reproduction of domestic animals. Deficiency as well as excess of the minerals results in compromised reproductive function. In order to maximize reproductive efficiency and to get optimum economic return, the ration of animal should contain adequate quantity of minerals (Kumar *et al*., 2020b). Therefore, the study was planned with the objective to study the fertility response as well as changes in macromineral profile in anoestrus buffaloes with herbal, homeopathic and hormonal therapeutic regimens.

MATERIALS AND METHODS

Selection of experimental animals

The study was carried out under field conditions during year 2019-20 in the semi-urban areas of Ayodhya district, Uttar Pradesh, India. The experiment was conducted on the postpartum anestrus Murrah buffaloes after prior permission from the Institutional Animal Ethics Committee (Reference no. IAEC/CVSc/2/P-31/2020/25) and written consent of the owner. The animals were selected on the basis of history, breeding records and per rectal examination of genitalia. The Murrah buffaloes which had not exhibited apparent clinical sign of estrum and had normal genital tract with atonic uterus and smooth, small inactive ovaries, even after day 60 post-partum were included in this study.

The selected anestrus buffaloes (n=24) were divided into four treatment groups namely T0, T1, T2 and T3, with each group comprised of six animals. The screened buffaloes were subjected with broad spectrum anthelmintic Fenbendazole 3g once orally. Buffaloes of all groups fed mineral mixture 25g daily for 10 days. In addition, T1 buffaloes were fed *Aegle marmelos* poweder (dose was calculated by using dose equivalent system as described by Miert (1986); T2 buffaloes were subjected with Oophorinum (30c) 10 drop, orally, daily for 10 days and in T3 buffaloes 100mg hydroxy progesterone caproate was injected daily subcutaneously for 10days.

After initial treatment, the behavioural estrus in both groups of animals was detected by careful visual observation at morning, afternoon and evening at least for 30 minutes every day. After detection of normal behavioural signs, the estrus was further confirmed by per rectal examination. The animals in estrus were inseminated twice at 12h intervals, if returned to estrus, they were inseminated again at second and third subsequent estrus. Pregnancy diagnosis was made 45 days post-insemination.

Approximately 6 ml of blood was collected before treatment and at subsequent estrus after treatment aseptically in clean sterile vials via jugular vein puncture. Samples were centrifuged at 1200g for 15 min at 4 °C. The plasma was separated and preserved at -20 °C until analysis. The plasma was analyzed for estimation of serum calcium and

phosphorus using diagnostic kits as per manufacturer instructions.

Statistical analysis

The 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

Response to estrus

The estrus induction response was 50, 100 and 33.33% in *Aegle marmelos,* Oophorinum and Progesterone treated groups, respectively. The highest estrus induction response was achieved with homeopathic remedy (Oophorinum) followed by herbal (*Aegle marmelos*) and hormonal treatment (Progesterone). It seems that homoeopathic drug Oophorinum work well as far as estrus induction response was concerned.

Pregnancy outcome

The conception rate in *Aegle marmelos* treated buffaloes were 33.33%, 33.33 % and nil at induced estrus and two subsequent estrus with overall conception rate of 66.66 per cent in three consecutive cycle. Likewise, the conception rate in Oophorinum treated buffaloes were 50, 16.66 and 16.66 per cent at induced estrus and two subsequent estrus, respectively; with 83.33% over all conception rate in three cycles. Similarly, in progesterone treatment group, the conception rate was 50%, 50% and nil in at induced estrus and two subsequent estrus respectively; with 100% over all conception rate in three cycles. Present findings are comparable well with observations of Chandel *et al.* (2009), Das *et al*. (2016) and Kumar *et al*. (2020a).

Effect of different therapies on serum calcium and phosphorus profile

Serum Calcium (mg/dL)

The mean value of calcium concentration before and after treatment has been depicted in table 1. The estrus animals had a significantly (P<0.05) higher serum calcium than anoestrus buffaloes. Similar findings had been reported by Chaurasia *et al*. (2010) and Kumar *et al.* (2019). The mean values of serum calcium in both groups were higher than the values reported by Chaurasia *et al*. (2010), Kumar *et al*. (2010), Yotov *et al*. (2013) and lower than that of Doijad *et al*. (2018). Calcium plays an important role in maintaining homeostasis of vertebrate animals including hormone secretion. The calcium absorption and its circulating level in blood depend upon parathyroid hormone.

Phosphorus (mg/dL)

The mean value of phosphorus before and after treatment has been depicted in table 1. Significantly (P<0.05) higher serum phosphorus level was noticed in estrus than anoestrus animals, whereas Hafez (2019) did not found significant variation between estrus and anoestrus buffaloes. Similar findings of higher values of serum phosphorus in cyclic buffaloes as compared to non cyclic buffaloes had been reported by Chaurasia *et al.* (2010), Kumar *et al.* (2010), Ali and Shukla (2012) and Yotov *et al.* (2013). The mean values of serum phosphorus in both groups were higher than the values by Chaurasia *et al*. (2010), Kumar *et al.* (2010), Ali and Shukla (2012),Yotov *et al.* (2013) and Doijad *et al.* (2018).

The absorption of phosphorus is a correlated phenomenon with calcium which depends upon the level of phosphorus and calcium giving closer ratio of 1:2. The involvement of phosphorus, phospholipids and cyclic AMP synthesis may be key factor of its effect on reproduction (Yadav *et al*., 2006; Yotov *et al*., 2013). A close correlation between the reproductive hormones and inorganic phosphorus exists. The marginal phosphorus deficiency may lead to anoestrus conditions. The low levels of inorganic phosphorus in non cyclic buffaloes might be due to intake of phosphorus deficient feed. It might also be due to phosphorus deficient rations in non cyclic buffalo.

CONCLUSIONS

In conclusion, the herbal and homoeopathic therapies can be used to manage postpartum anestrus syndrome in buffaloes under field conditions. Moreover, homoeopathic drug Oophorinum is cost effective as well as easy to administer. As mineral deficiency is one of the causes of clinical syndrome of anestrus, area specific mineral mixture supplementation might be one of the strategies to ameliorate the bovine anestrus syndrome.

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Table 1: Effect of different therapeutic regimen on Serum Calcium and Phosphorus level in Anestrus Buffaloes

	Calcium (mg/dL)		Phosphorus (mg/dL)		Fertility response	
					ER%	CR%
Group	**Pre-Treatment**	**Post- Treatment**	**Pre- Treatment**	**Post- Treatment**		
T0 (Positive Control)	8.67 ± 0.13^{A}	$9.00\pm0.17^{B,a}$	4.67 ± 0.27	4.37 ± 0.20^{a}	Nil	Nil
T1 (*Aegle marmelos*)	8.45 ± 0.11^{A}	$8.82\pm0.09^{B,a}$	4.43 ± 0.17	4.32 ± 0.14^{a}	50	66.66
T2 (Oophorinum)	8.65 ± 0.12^{A}	$10.48\pm0.16^{B,b}$	4.65 ± 0.21^{A}	$5.28\pm0.14^{B,b}$	100	83.33
T3 (Progesterone)	8.73 ± 0.16^{A}	$9.20\pm0.07^{B,a}$	4.70 ± 0.24	4.77 ± 0.14^{a}	33.33	100.0

Mean bearing different superscript in the column (a, b, c and d) and in a row (A, B) differed significantly ($P<0.05$) for each attributes. ER (Estrus Response), CR (Conception Rate in three cycles among responded buffaloes).

CONFLICT OF INTEREST

The authors declare that they have no competing interest with this manuscript.

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