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Surya Kumar Maurya
Department of Veterinary Obstetrics and Gynecology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Ram Sagar
Department of Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Padma Nibash Panigrahi
Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Atul Saxena
Department of Veterinary Obstetrics and Gynecology, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Corresponding Author:
Ram Sagar
Department of Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, Uttar Pradesh, India

Studies on blood mineral and hormonal profile in post-partum anestrous buffalo

Surya Kumar Maurya, Ram Sagar, Padma Nibash Panigrahi and Atul Saxena

Abstract
The present study was designed to determine alteration on blood mineral profile, hormonal profile and enzymatic activity in post-partum anestrous buffalo before and after supplementation of mineral mixture and antioxidants. Thirty Post-partum anestrous buffaloes were randomly selected from different blocks of Mathura, Uttar Pradesh, India and divided in to three groups 10 animals each. Group A (n=10) animals were acted as a control without having any treatment. Group B (n=10) animals were fed with mineral mixture @ 50 g daily for 25 days (composition: dicalcium phosphate- 40%, calcium carbonate-30%, sodium chloride- 22%, ferrous sulphate- 2.5%, copper sulphate-2.05%, potassium iodide-0.05%, zinc sulphate-0.75%, cobalt chloride-0.05%, manganese chloride-0.6%, magnesium sulphate-2%). Group C (n=10) animals were fed with antioxidant (vitamin E+selenium, in the proportion of 1500 IU (1.5gram) of vitamin E and 50 mg of sodium selenate equivalent to 10 ppm of selenium) for 25 days. Blood mineral profile, hormonal profile and serum enzymatic activity were determined at day 0 and 25 days of supplementation. There was a significant increase in calcium, copper, zinc, estradiol and insulin concentrations as well as total protein, glucose and ascorbic acid activities in both treatment groups. In group A, out of 10 anestrous buffalo only 1 animal exhibited estrous after 24 days. On contrary 5 animals in group B and 6 animals in group C exhibited estrous and conceived in the first service.

Keywords: buffalo; post-partum anestrous; macro-micro nutrients; hormone; antioxidants

Introduction
Buffalo is an integral part of agriculture in Asia having pivot role in the Indian livestock industry contributing towards the production of milk, meat and draft power. Reproductive problem is more in buffalo than cattle, hence buffalo is considered as difficult breeders primarily because of its inherent susceptibility of environmental stress which cause anestrous and sub estrous [1]. this conditions are responsible for a prolonged inter-calving period resulting a greet economic losses to the dairy industry. Subestros or silent heat contributed the largest factor responsible for poor reproductive efficiency in buffaloes. Some other factors are also reported to be responsible for lower reproductive efficiency in buffalo includes nutritional deficiency, environmental factors and extensive follicular atresia during the reproductive life of female [2].

It has been reported that nutritional deficiency is the important factor responsible for infertility in buffaloes [3]. As minerals and trace elements such as copper, cobalt, manganese, zinc, etc., play important role in the proper functioning of the genital organs and related activities [2]. Trace elements may function as cofactors, as activators of enzymes or stabilizers of secondary molecular structure [4]. Buffaloes are frequently subjected to severe dietary deficiencies of trace elements such as copper, cobalt, selenium, iodine, manganese, and zinc. Concomitant infertility in buffalo is believed to be associated with enzymatic dysfunctions resulting from these deficiencies [5]. Optimum protein level is necessary for the development of endocrine and sex organs. The ill effect of low protein on reproduction is through pituitary and sex glands. Protein deficiency retards the development of reproductive organs and was considered to be a factor responsible for failure or delay in the onset of postpartum estrus [6]. Cholesterol is synthesized from acetate with a series of intermediate substances. It is an essential precursor for steroid hormones of the testis, ovary and adrenal cortex [7]. Various minerals are the essential nutrients bearing a significant role in the reproductive performance of ruminants. Deficiency or excess of minerals like Co, Cu and Zn have been associated with subnormal fertility and anoestrous conditions.

Hence the study was designed to determine alteration on blood mineral profile, hormonal profile and enzymatic activity in post-partum anestrous buffalo.

Materials and Methods

Thirty Post-partum an-estrous buffaloes were randomly selected from different blocks of Mathura, Uttar Pradesh, India and divided in to three groups 10 animals each. Buffalo was designated anestrous based on owner"s history, ultrasonographic examination (absence of corpus leuteum and relatively medium to large sized follicles) and progestrone concentrations. All the buffaloes were dewormed with albendazole @ 10 mg/kg body weight before the start of experiments. Group A (n=10) animals were acted as a control without having any treatment. Group B (n=10) animals were fed with mineral mixture @ 50 g daily for 25 days (composition: dicalcium phosphate- 40%, calcium carbonate-30%, sodium chloride- 22%, ferrous sulphate- 2.5%, copper sulphate-2.05%, potassium iodide-0.05%, zinc sulphate-0.75%, cobalt chloride-0.05%, manganese chloride-0.6%, magnesium sulphate-2%). Group C (n=10)animals were fed with antioxidant (vitamin E+selenium, in proportion of 1500 IU (1.5gram) of vitamin E and 50 mg of sodium selenate equivalent to 10 ppm of selenium) for 25 days.

Collection of blood

Approximately 10 ml blood samples were collected in the vaccutainers without anticoagulant from all the selected buffaloes on days 0 (prior to treatment) and day 25 (end of supplementation treatment) by jugular vein puncture. The serum was separated after clotting of blood by centrifugation at 3000 rpm for 15 min and stored at −20°C in a deep freezer until analyzed.

Estimation of macro and micro nutrients

Serum samples were digested by concentrated acids (3 ml perchloric + 2 ml nitric acid per ml serum) for 24 hours [8]. Then all samples were diluted, filtered and analyzed for micro-minerals (Zn and Cu) using a flame emission atomic absorption spectrophotometer. The flame conditions were these as recommended by the instrument manufacturer.

Estimation of hormonal profile

Progesterone, estradiol and insulin activity was estimated by ELISA kit as per manufacturers instruction.

Serum biochemistry

Serum calcium, phosphorus and glucose were estimated by Span diagnostic kits (Surat, India) by biochemical analyser according to manufacturer instruction.

Statistical analysis

The data were expressed as mean ± SEM. The standard error of mean and p-values was used to determine whether there is any significant difference among different treatment groups using one-way analysis of variance (ANOVA) following standard procedure [9].

Results

Estimation of blood mineral profile in post-partum anestrous buffalo

Table 1 reveled alteration on blood mineral profile in post-partum anestrous buffalo before and after treatment. There was a significant increase in calcium, copper and zinc concentrations in both the treatment group after the end of fed supplementation as compared to the healthy control group (table 1). Whereas the mean concentration of phosphorus, magnesium and cobalt varies non significantly as compared to the control group on day 25.

Serum biochemistry

There was significantly increased concentrations of total protein, glucose and ascorbic acid in both the treatment group on day 25 as compared to control group (table 2). Whereas mean cholesterol concentration was statistically similar in all three groups before and after feed supplementation.

Hormone profile

Table 3 depicted alteration on serum hormonal profile in post-partum anestrous buffalo before and after treatment. The progesterone activity was statistically similar in all three groups before and after feed supplementation. On the contrary mean activity of estradiol and insulin increased significantly in both the treatment group at the end of the experimental period (day 25).

Study of fertility response in post-partum anestrous buffalo

In group A, out of 10 anestrous buffalo only 1 animal exhibited estrous after 24 days. The responded animal had post-partum anestrous of 60 days while all non-responded animals had a post-partum anestrous of average 113.89 days. On contrary 5 animals in group B and 6 animals in group C exhibited estrous. All the animals were conceived in first service (table 4).

Discussion

Physiological condition as well as health status of animals can be revealed by the changes in blood constituents. There are certain hormonal and metabolic parameters, which influence directly the process of reproduction in animals [1, 5]. Anestrus can be defined as the failure or deficiency of the expression of estrus. A period of anestrus after parturition is considered to be a normal physiological event but becomes an abnormal condition if its duration exceeds its accepted average [2]. In livestock animals, one of the main factors that have a negative impact on their reproductive performance is the anestrus condition. In India, anestrus remains an important problem for reproduction in buffalo. The present study aims to compare the serum hormonal, metabolic and minerals profiles in postpartum anestrus Indian buffaloes before and after supplementing mineral mixture and antioxidants.

In the current study, significant increase in calcium concentration in poat partum anestrous buffalo was in agreement with Chaurasia *et al* [10]. Ca is essential in the field of reproduction for contraction of the uterine muscle, LH secretion from the pituitary gland and fertilization [2]. Ca is also known to affect the animal's ability to utilize other trace elements; it may disrupt reproductive functions through its influence on certain enzyme systems [5]. Serum phosphorus (P) level of buffaloes in the anestrus state showed significantly lower level compared to regular cyclic buffaloes, this concurs with Chaurasia *et al* [10], Kumar *et al* [3] and Mourad [11]. Whereas, Khasatiya *et al* [12] between cyclic and anestrus buffaloes did not record any variation in P level. The participation of P in phospholipids and cAMP formation may be an essential factor for its effect on reproductive performance. Marginal P insufficiency may prompt anestrus

condition because of the close connection between P and reproductive hormones [10]. A phosphorus deficiency causes impaired ovarian activity, irregular estrus, anestrus, reduced conception rate, high incidence of cystic follicles, and generally depressed fertility [2,13]. Administration of P has been found to alleviate the anestrus conditions in buffalo heifers [14]. The results of this study for a significant decrease in Zn agrees with the findings of Yadav *et al* [15], Akhtar *et al* [16] and Mourad [11], whereas Jayachandran *et al* [17] did not find any variation in Zn level between cyclic and anestrus buffaloes. It was reported that Zn level is related to the steroid reproductive hormone concentrations which indicated that there was a close relationship between blood Zn level and progesterone-estrogen levels for optimum reproductive performance [13]. This element plays an important role in the repair of the endometrium following parturition, speeding the return to normal reproductive function and ovarian activity [2]. Zn deficiency has been shown to reduce the synthesis and secretion of FSH and LH, disruption of the estrus cycle in buffalos and impair ovarian development [18]. Supplementation with Zn and other elements has been found to improve anestrus conditiond in buffalo heifers [14]. The significant decrease in serum Cu levels of the present study is in agreement with Yadav *et al* [15] and Akhtar *et al* [16]. The importance of Cu for growth, production and reproductive functions has been well established; ceruloplasmin and superoxide dismutase are several Cu-containing proteins that are essential for the physiological functions of Cu [13]. It was reported that Cu deficiency may have adverse effects on female reproduction via the hypothalamus-pituitary-gonadal axis on LH secretion leading to a decrease in the ovarian oestradiol secretion and absence of the expression of estrus in animals [19].

Serum biochemistry

In the current study, there were significant increased concentrations of total protein, glucose and ascorbic acid in both the treatment group which was in agreement with the results of Khasatiya *et al* [12]. Depending upon the feed intake of the animal the serum protein levels change with different phases of reproduction. Protein lack retarded the improvement of reproductive organ and was regarded as a factor responsible for the postponement in beginning or failure of postpartum estrus [20]. The mean cholesterol concentration was statistically similar in all three groups before and after feed supplementation which was in agreement with Kumar *et al* [21]. Cholesterol acts as a precursor for the formation of steroid hormones in thica and luteal cells in the ovarian [5]. The mechanism by which estrogens affect the interrelationships of pituitary-thyroid-adrenal functions is by affecting the carbohydrate metabolism that in turn increase the production of cholesterol in endocrine gland tissue from acetate, and that explains the increase in serum cholesterol during estrus [15].

Hormone profile

The significantly low serum estradiol levels in postpartum anestrus animals in the present study agree with the findings of Ahmed *et al* [18], Akhtar *et al* [22] and Kalasariya *et al* [12], in buffaloes and Saleh *et al* [23] in cattle. It was reported that the recent diagnostic tools of ovarian inactivity not depend only on rectal palpation but also can use blood P_4 and E_2 profile, as serum P_4 and E_2 level reduce in anestrus animals due to the absence of LH surge and impaired follicular growth accompanied by ovarian inactivity. In anestrus animals, a broad variety has been recorded in serum P_4 and E_2 concentration demonstrating continual follicular growth and atresia [18]. It is a fact that the chief function of estrogen is the manifestation of estrus by regulating the functionality of the tubular genital tract; it also coordinates sexual behavior and receptivity to the male animals by sensitizing the central nervous system [5].

Table 1: Mineral profile in post-partum anestrous buffalo before and after treatment

Parameters	Day	Groups		
		Group A	Group B	Group C
Calcium (mg/dl)	Day-0	7.13 ± 0.40^{aA}	6.99 ± 0.53^{aA}	7.20 ± 0.41^{aA}
	Day-25	7.32 ± 0.41^{aB}	8.42 ± 0.37^{abB}	9.16 ± 0.38^{bB}
Phosphorus (mg/dl)	Day-0	4.98 ± 0.23^{aA}	5.05 ± 0.33^{aA}	5.04 ± 0.20^{aA}
	Day-25	5.12 ± 0.18^{aA}	5.14 ± 0.33^{aA}	5.54 ± 0.10^{aA}
Magnesium (mg/dl)	Day-0	3.15 ± 0.28^{aA}	3.06 ± 0.21^{aA}	3.01 ± 0.47^{aA}
	Day-25	3.09 ± 0.29^{aA}	3.12 ± 0.35^{aA}	4.42 ± 0.76^{aA}
Copper (µg/ml)	Day-0	0.23 ± 0.03^{aA}	0.33 ± 0.06^{aA}	0.29 ± 0.05^{aA}
	Day-25	0.30 ± 0.03^{aB}	0.40 ± 0.08^{bB}	0.56 ± 0.08^{bB}
Zinc (µg/ml)	Day-0	1.55 ± 0.08^{aA}	1.41 ± 0.07^{aA}	1.39 ± 0.11^{aA}
	Day-25	1.57 ± 0.07^{aB}	1.51 ± 0.11^{aB}	1.97 ± 0.12^{bB}
Cobalt (µg/ml)	Day-0	0.52 ± 0.08^{aA}	0.52 ± 0.06^{aA}	0.52 ± 0.05^{aA}
	Day-25	0.52 ± 0.08^{aA}	0.53 ± 0.08^{aA}	0.55 ± 0.06^{aA}

Means with small letters superscripts denotes comparison within rows (between groups) and capital letters within column (between days)

Table 2: Hormone profile in post-partum anestrous buffalo before and after treatment

Parameters	Day	Groups		
		Group A	Group B	Group C
Progesterone (ng/ml)	Day-0	0.75 ± 0.04^{aA}	0.73 ± 0.03^{aA}	0.64 ± 0.04^{aA}
	Day-25	0.74 ± 0.04^{aA}	0.75 ± 0.03^{aA}	0.73 ± 0.04^{aA}
Estradiol (pg/ml)	Day-0	9.41 ± 0.74^{aA}	9.62 ± 0.84^{aA}	7.75 ± 0.55^{aA}
	Day-25	9.21 ± 0.54^{aB}	14.28 ± 0.90^{bB}	13.70 ± 1.13^{bB}
Insulin (µIU/ml)	Day-0	7.51 ± 0.43^{aA}	7.29 ± 0.64^{aA}	7.47 ± 0.68^{aA}
	Day-25	7.58 ± 0.39^{bA}	7.32 ± 0.65^{bA}	5.48 ± 0.46^{aA}

Means with small letters superscripts denotes comparison within rows (between groups) and capital letters within column (between days)

Table 3: Alteration in serum biochemistry in post-partum anestrous buffalo before and after treatment

Parameters	Day	Groups		
		Group A	Group B	Group C
Total protein (g/dl)	Day-0	6.23±0.15^{aA}	6.69±0.40^{aA}	6.75±0.22^{aA}
	Day-25	6.58±0.31^{aB}	7.73±0.26^{bB}	7.37±0.19^{bB}
Glucose (mg/dl)	Day-0	56.97 ± 4.74^{aA}	60.85±5.34^{aA}	62.44±5.48^{aA}
	Day-25	64.12±3.94^{aB}	70.55±5.60^{abB}	84.39±4.69^{bB}
Cholesterol (mg/dl)	Day-0	66.32±5.88^{aA}	66.33±6.29^{aA}	70.06±5.00^{aA}
	Day-25	66.86±5.60^{aA}	65.71±6.73^{aA}	75.40±4.06^{aA}
Ascorbic acid (µg/ml)	Day-0	3.90±0.49^{aA}	3.89±0.44^{aA}	3.63±0.44^{aA}
	Day-25	4.03±0.47^{abA}	3.65±0.35^{aA}	5.08±0.51^{bA}

Means with small letters superscripts denotes comparison within rows (between groups) and capital letters within column (between days)

Table 4: Study of fertility response in post-partum anestrous buffalo

Group	No of Animal in heat after treatment	Interval between treatment and response (days)	Post-partum anestrous period (days)		No of animals conceived	Service per conception	Percentage of rate of success of pregnancy
			Responder	Non-responder			
Group A	1	24	60	113.89±37.14	1	1	10
Group B	5	33.865.89	313.64±84.56	194.17±45.28	5	1	50
Group C	6	48.447.99	242.5±84.35	113.13±37.24	6	1	60

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