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EFFECT OF DIFFERENT HORMONAL TREATMENTS ON MICRO-MINERALS IN POST PARTUM BUFFALOES

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ABSTRACT

Forty-eight anestrus postpartum Murrah buffaloes were chosen randomly selected and divided into four groups with equal representation in each group. The buffaloes were treated with different progesterone implants and each buffalo received an intramuscular injection of 600 I.U. PMSG (Folligon) intramuscularly on the day when the device was removed. The concentration of copper increased non-significantly on day 3, 6 and 9 of treatment. However, the difference in the average concentration of serum levels Copper, Zinc and Iron within and were observed among the groups on the sampling days and found to be non-significant (P>0.05).

Keywords: *Bubalus bubalis*, buffaloes, postpartum, anestrus, copper, zinc and iron, Hormonal treatment

INTRODUCTION

Post-partum anestrus refers to the period following calving when dairy animals do not display estrus. This condition is widely recognised as a prevalent and challenging issue in dairy animals particularly frustrating for producers. Field studies on reproductive disorder have indicated that anestrus is primary cause of infertility in buffaloes with inactive or non-functional ovaries being the main contributing factor (EL-Wishy, 2007). The occurrence of anestrus is notably higher in buffaloes than in cattle with the issue intensifying during summer months (Singh *et al*., 2010). Compared to cows, buffaloes tend to have fewer pre antral and antral follicles and display a higher rate of follicular atresia (Danell, 1987). Kumar *et al*. (2013) reported an estimated loss of Rs. 372.90 per day in buffalo due to production losses. True anestrus is the condition where both ovaries are small, smooth, inactive with the absence of graffian follicle or corpus luteum

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and characterized by cessation of sexual cycle and psychic manifestation of estrus. In buffaloes smooth, inactive ovaries were one of the most common causes of anestrus (Newar *et al.,* 1999; Baruah *et al.,* 2000). Greater allocation of energy towards milk production can lead to anestrus by postponing the recommencement of follicular activity (Bauman and Currie, 1980). Failure of re-establishment of cyclical ovarian activity prolongs postpartum anestrus in dairy animals (Peter *et al.,* 2009).

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 or excess of mineral elements like Ca, P, Cu, Fe and Zn is associated with subnormal fertility and anestrus condition (Parmar, 2013).

MATERIALS AND METHODS

Anamnesis/Examination

Buffaloes were selected based on their history, with no signs of estrus for 90 days or more postpartum. The duration of the anestrus period ranged from 3 to 6 months. Each buffalo underwent two rectal examinations at a 10 days interval to confirm inactive, smooth ovaries, flaccid uterine horns and a pale vestibule. Any pathological causes of anestrus were excluded. The average body condition score of animals was 3.5 (1= thin and 5= obese).

Grouping of animals and treatment protocol

Based on the selection criteria, 48 post-partum anestrus buffaloes were included in the trial and randomly assigned to 4 groups with 12 animals in each group. The buffaloes in Group I were treated with CIDR (Controlled Intravaginal Drug Release, Pfizer Ltd.) for 9 days, in Group II were treated by Crestar ear implant for 9 days, in Group III were treated by Polyurethane sponge vaginal implant (containing 1.5 gm of Natural Micronized Progesterone) for 9 days and in Group IV were treated with Polyurethane sponge vaginal implant (containing 1.5 gm of Natural Micronized Progesterone along with 1% carboxymethyl cellulose) for 9 days. All four groups were also administered an intramuscular injection of 600 I.U. PMSG (Folligon) on the day of implant removal.

Blood sampling schedule

Blood samples were collected from postpartum anestrus buffalo for micro-mineral studies from jugular vein. Approximately 10 ml of blood were drawn om Day 0, 3rd, 6th and on the day of implant removal (9th day) through venipuncture of jugular vein under aseptic conditions into the dry vials. The blood was then centrifuged for 10 to 15 minutes 3000 rpm and serum was harvested , collected in sterilized vials and stored at -20^{0}C for mineral analysis.

Mineral Analysis

Estimation of copper, zinc and iron was done on Atomic Absorption Spectrophotometer (AAS Model No, Avanta Sigma of Scientific Equipment Pvt. Ltd, Australia).

Statistical Analysis

To find out the statistical significance the data were analyzed using Analysis of Variance (ANOVA) and General Linear Models of SPSS 16.0 software. Significance was assessed at the 5% level ($P<0.05$).

RESULTS AND DISCUSSIONS

Analysis of the data revealed that the mean concentration of copper in all groups ranged from 29.29±2.85 to 53.65±2.23 µg/dl from day 0 to day 9. The mean concentration of zinc varied between 123.66±20.46 to 193.33±13.71 µg/dl from day 0 to day 9. The mean concentration of Iron ranged from 323.16±66.03 to 533.25±30.79 µg/dl from day 0 to day 9th. However, the differences, in the mean concentration of serum copper, zinc and iron within and between the groups on sampling days were statistically non-significant (P>0.05).

The mean level of copper did not differ significantly during treatment period (from hormonal implant insertion to withdrawal of the implant) within any of the groups or between the groups (Table 1). Similar findings were reported by Kumar *et al.* (2015d) in buffaloes, that there was non-significant change in concentration of copper under Ovsynch, ovsynch + CIDR and Heat synch protocol and Dhamsaniya *et al.* (2016) also made similar observation in buffalo's heifer under Gn-RH protocol. Chandolia and Verma (1987) and Sarvaiya and Pathak (1991) reported non-significant variation in the values of copper in normal cycling than anestrus buffaloes. However, Newar *et al.* (1999) and Jain *et al.* (2003) reported significant increase in the values of copper in normal cycling than anestrus buffaloes.

The reference value of serum copper in buffaloes has been reported as 58 to 136 µg/dl (Abid Ellah *et al.*, 2014). In present study, the mean copper concentration in all groups ranged from 29.29±2.85 to 53.65±2.23 µg/dl, which is lower than the reference value. This lower copper concentration may be a contributing factor to post-partum anestrus observed in our study as it causes impaired pulsatile release of LH (Phillipo *et al.*, 1987). Higher level of copper (70.59±2.59 µg/dl) in cyclic buffaloes was reported by Akhtar *et al.* (2009). Deshpande and Deopurkar (1981) reported that copper plays a major role in the fertility of animals. It was also reported that animal with postpartum interval (>75 days), animal having follicular cysts, those with smooth and inactive ovaries have copper levels of 78, 52 and 65 µg/100 ml serum, respectively. Following copper supplementation in these animals, the postpartum interval was shortened and inter calving periods were reduced (Mahendran and Zubiary, 1969). Copper is essential for haemoglobin formation, growth, hairs pigmentation and lactation. Besides, copper is also required for cellular respiration, bone formation, proper cardiac function, connective tissue development, keratinization and tissue pigmentation. Also, copper is an essential component of several physiologically important matallo-enzymes including cytochome oxidase, lysyl oxidase, Superoxidase, dismutase, dopamineβ-hyroxylase and tyrosine (Mc Dowell, 1992).

The mean level of copper did not differ significantly during treatment period (Hormonal implant insertion to withdrawal of implant) within or between the groups (Table 1). Similar findings were reported by Kumar *et al.* (2015d) in buffaloes, that there was non-significant change in concentration of copper under Ovsynch, ovsynch + CIDR and Heat synch protocol and Dhamsaniya *et al.* (2016) also made similar observation in buffalo's heifer under Gn-RH protocol. Chandolia and Verma (1987) and Sarvaiya and Pathak (1991) reported non-significant variation in the values of copper in normal cycling than anestrus buffaloes. However, Newar *et al.* (1999) and Jain *et al.* (2003) reported significant increase in the values of copper in normal cycling than the buffaloes which were anestrus.

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The mean level of zinc did not significantly differ during the treatment period (from hormonal implant insertion to withdrawal of implant) within any of the groups or between the groups (Table 1). Similar findings were reported by Kumar *et al.* (2015b) in buffaloes, of a non-significant change in concentration of zinc under Ovsynch, Ovsynch + CIDR and Heat synch protocol and Dhamsaniya *et al.* (2016) also made similar observation in buffalo's heifer under Gn-RH protocol. Ullah *et al.* (1983); Chandolia and Verma (1987) reported a non-significant increase in the values of zinc. However, Newar *et al.* (1999) in buffaloes and Sharma *et al.* (1999) in cows reported significant increase in the values of zinc in cyclic animals than acyclic animals.

The reference value of serum zinc of buffaloes has been reported as 53.82 to 149.86 µg/dl (Abid Ellah, *et al*., 2014). In our study, the mean concentration of zinc concentration in all groups varied between 123.66±29.30 to 193.33±13.71 µg/dl, which is towards higher side compared to reference value of zinc.

Zinc is the main component of many enzymes system. It is largely involved in nucleic acid metabolism, carbohydrates metabolism and protein synthesis. Synthesis of DNA, RNA and protein are greatly reduced in zinc deficiency; hence its deficiency impairs cellular division, growth and repair. Zinc plays a role in the production, storage and secretion of individual hormone as well as in the effectiveness of receptor sites and end organ responsiveness. All phases of reproductive process in the female can be adversely affected by zinc deficiency (Mc Dowell, 1992 and Hidiroglou, 1979). Mc Dowell (1992) also opinioned that serum zinc level below 0.6-0.84 µg/ml may cause clinical signs of deficiency. Cunnane *et al*. (1983) suggested that zinc have dose association with prostaglandin synthesis.

The mean level of iron did not differ significantly during the treatment period (from hormonal implant insertion to withdrawal of implant) within any of the groups or between the groups (Table 1). Similar findings were

Table 1. Effect of various hormonal treatments on Cu, Zn and Fe (µg/dl) level in anestrus buffaloes.

Groups		Day 0	Day 3	Day 6	Day 9
Group I	Cu	30.90±6.36	45.17±5.07	29.35±8.61	49.40±4.53
	Zn	147.42±18.90	152.90±17.54	135.80±25.90	139.73±21.17
	Fe	533.25±30.79	430.26±47.50	416.86±61.14	421.69±47.52
Group I	Cu	37.78±7.88	35.41±9.57	43.65±9.04	44.05±8.83
	Zn	159.54±19.87	140.21±15.06	116.12±20.46	158.92±33.86
	Fe	453.76±29.26	528.26±37.41	492.36±72.57	458.23±52.10
Group I	Cu	36.60±5.25	29.29±2.85	32.66±6.50	32.41±5.34
	Zn	180.67±18.81	142.48±14.01	193.33±13.71	145.10±16.37
	Fe	462.35±16.08	451.84±32.53	474.97±33.91	482.73±51.49
Group I	Cu	35.35±4.58	37.78±5.50	30.41±5.59	53.65±2.23
	Zn	149.55±18.96	150.44±20.02	147.51±27.56	123.66±29.30
	Fe	466.26±39.04	472.29±35.98	340.16±26.44	323.06±66.03

reported by Kumar *et al.* (2015b) in buffaloes, of a non-significant change in concentration of zinc under Ovsynch, ovsynch + CIDR and Heat synch protocol and Dhamsaniya *et al.* (2016) also made similar observation in buffalo's heifer under Gn-RH protocol. Chandolia and Verma (1987) reported a non-significant increase in the values of iron. However, Khattab *et al.* (1995); Sharma *et al.* (1999) reported significant increase in the values of iron in cyclic than acyclic buffaloes.

The reference value for serum iron in buffaloes has been reported as 61.01 to 152.66 (Abid Ellah *et al*., 2014). In our study, the mean concentration of iron in all groups ranged from 323.06±66.03 to 533.25±30.79, which is 4-5 times on higher side compare to reference value of iron, but in range with reports of Akhtar *et al.* (2009); Kumar *et al*. (2016).

Approximately 60% body iron is present as haemoglobin, a complex of protoporphyrin synthesis and globin. Role of iron in haemoglobin synthesis is well known and its deficiency causes malnutrition stress resulting in normochomic anaemia in animals which in turn affects the response of ovarian receptors to estrogen hormone (Mgongo *et al*., 1953).

CONCLUSION

It is concluded from above study that hormonal treatment does not affect the serum concentration of Copper, Zinc and iron. The mean level of serum copper, zinc and iron did not differ significantly during the treatment period (from hormonal implant insertion to withdrawal of implant within any of the group or between the groups.

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