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Serum Trace Minerals Profile in Anestrus Buffaloes Treated With Different Estrus Synchronization Protocols

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ABSTRACT

The present study was conducted to analyse the serum trace minerals (copper, cobalt, iron, zinc, manganese and selenium) concentration in postpartum anestrus buffaloes. A total of 24 postpartum anestrus buffaloes were randomly selected after taking reproductive history and gynaeco-clinical examination 10 days apart. These 24 animals were divided into four groups; Group A (Ovsynch), Group B (Heatsynch) and Group C (CIDR plus Ovsynch) and Control group (no treatment) each containing six animals. Blood sample was collected on day 0, 7, 9 and 10 of treatment and serum was analysed for various trace minerals. The mean serum copper level (µg/ml) increased significantly ($p<0.01$) from day 0 to day 7, 9 and 10 in group A, B and C. The mean serum cobalt concentration increased significantly ($p<0.05$) from day 0 to day 7, 9 and 10 in group A, B and C. Serum iron concentration increased significantly ($p<0.05$) from day 0 to day 9 and 10 except on day 7 in group A, B and C and on day 9 in group A. The mean zinc level (µg/ml) increased significantly ($p<0.05$) in group B and C on day 9 and 10 except day 7. The serum manganese concentration on day 0 increased significantly ($p<0.01$) to day 9 and 10, the value on day 0 was also found significant from day 7 in group C. The mean serum selenium, the value on day 0 increased significantly ($p<0.01$) to day 7, 9 and 10 in group A, B and C except the value on day 7 in group B. Thus, it could be concluded that deficiency of copper, iron, zinc and selenium either alone or in combination could be responsible for anestrus condition. By improving the trace minerals status, fertility can be improved in buffaloes.

Key words: Postpartum, anestrus, buffaloes, trace minerals

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INTRODUCTION

India has emerged as the largest milk producer in the world. As per the 17th livestock census of India buffaloes constitute 36 per cent of total bovine population in India and account for 56 per cent of country's total milk production. To improve calf yield from a dairy animal and ultimately milk yield, the reproduction should receive greater empha-

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sis. One of the major factors of economic importance in buffalo reproduction is the delayed return to postpartum cyclicity (Honparkhe *et al.,* 2008). Postpartum anestrus can be defined as the lack of oestrus symptoms (despite of effective oestrus detection) within >90 days after calving, while normal buffalo in exactly the same conditions already have been seen in heat. Physiologically postpartum anestrus cannot be escaped because it is helpful in involution of uterus within 15–45 days after parturition.

Mineral deficiency exists widely in livestock and the severity of the deficiency depends upon the type of feed, physiological status of the animals and the agro-climatic conditions of the region. Deficiency of a single or multiple minerals or their imbalances may cause various reproductive failures, such as infertility, poor conception, anoestrous etc. (Hidiroglou, 1979).

There are certain biochemical parameters, which directly influence the reproductive performance of animals either through stimulating hormone synthesis, hormone action or response of the target tissue by acting as precursor for hormone synthesis. Nutritional factors needed for successful reproduction are the same as those needed for maintenance, growth and lactation, that include protein, energy, mineral and vitamins. Deficiency or excess of any of these components is serious enough to affect reproduction and also other physiological functions (Parmar *et al.,* 2015). Lack of minerals and trace elements such as copper, cobalt, manganese, zinc, etc., upset the proper functioning of the genital organs (Acharya, 1960). Trace elements may function as cofactors, as activators of enzymes or stabilizers of secondary molecular structure (Valee and Wacker, 1976). Ruminants frequently are subjected to severe dietary deficiencies of trace elements such as copper, cobalt, selenium, iodine, manganese, iron and zinc.Keeping this in view, the present study was designed to estimate the serum trace minerals (copper, cobalt, iron, zinc, manganese and selenium) concentration in postpartum anoestrous buffaloes in and around Rewa district of M.P.

MATERIALS AND METHODS

The present study was carried out on 24 postpartum anestrus buffaloes from different villages in and around Rewa district (M.P.) which had not exhibited signs of estrus for ≥90 dayspostpartum. Buffaloes were divided into treatment and control groups. Eighteen anestrus buffaloes in the treatment group were further divided into 3 treatment groups A, B and C with 6 buffaloes in each group. The remaining 6 anestrus buffaloes were grouped as control (Group D) in which no treatment was given. In group A, animals were subjected to treatment with Ovsynch protocol and were administered GnRH analogue (Buserelin acetate 20μg, i.m) on day 0, followed by $PGF_{2\alpha}$ (Cloprostenol sodium 500μg, i.m) on day 7 and again GnRH analogue (Buserelin acetate 20μg, i.m) on day 9. In group B, animals were subjected to treatment with Heat synch protocol and were administered GnRH analogue (Buserelin acetate 20μg, i.m) on day 0, followed by $PGF_{2\alpha}$ (Cloprostenol sodium 500μg, i.m) on day 7 and then estradiol benzoate 1 mg on day 8. In group C, animals were subjected to treatment with CIDR plus Heat synch protocol and animals were administered CIDR (controlled internal drug release device) implant (for 7 days) along with estradiol benzoate (1mg, i.m) on day 0 and the implant was removed on day 7, followed by $PGF_{2\alpha}$ (Cloprostenol sodium 500μg, i.m) on day 7 and then estradiol benzoate 1 mg on day 8.

Blood collection for serum trace mineral estimation

Five millilitre blood was collected aseptically from jugular vein in vaccutainers (containing clot activating factor) from all the animals on day of treatment (day 0) and day 7, day 9 and day 10 after initiation of treatment. Serum was separated from the collected blood by centrifugation at 1500 rpm for 30 minutes and stored in labelled vials in a deep freezer at -20°C till trace mineral estimation by Inductively coupled plasma optical emission spectrometry (ICP-OES).The effect of different hormonal protocols on the level of serum trace minerals (μg/ml) was studied before start of treatment (day 0) and after start of treatment (day 7, 9 and 10) under Ovsynch, Heatsynch, CIDR plus Heatsynch and control groups.

Statistical analysis

The data were analyzed using SYSTAT software, Version 12, San-Jose California USA. Data from different experiments were presented as Mean ±SE. The pair-wise comparison of means was carried out using Fisher's multiple comparison test as per standard statistical method described by Snedecor and Cochran (1994). The difference at $p \leq 0.05$ was considered to be satisfactory significances.The results of incidence of anestrus, estrus induction and conception rate were expressed in percentage.

RESULTS AND DISCUSSION

The effect of different hormonal protocols on the level of serum trace minerals (μg/ml) *i.e.*, Cu, Co, Fe, Zn, Mn and Se were estimated before start of treatment (day 0) and after start of treatment (day 7, 9 and 10) under group A (Ovsynch), group B (Heatsynch), group C (CIDR plus Heatsynch) and group D (control group) in postpartum anestrus buffaloes and their mean values are presented in Tables 1-6.

Table 1: Serum copper concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$0.67^{ap}\pm0.01$	$0.72^{bp}\pm0.01$	$0.76^{cp}\pm0.01$	$0.78^{cpr}\pm0.01$
Group B (n=06)	$0.68^{ap}\pm0.01$	$0.71^{bp}\pm0.01$	$0.75^{bpr}\pm0.01$	$0.80^{cp}\pm0.01$
Group C (n=06)	$0.68^{ap}\pm0.01$	$0.70^{bpq}\pm0.01$	$0.72^{bqr}\pm0.01$	$0.75^{cr}\pm0.01$
Group D (n=06)	$0.67^{ap}\pm0.01$	$0.68^{aq}\pm0.01$	$0.68^{aq}\pm0.01$	$0.68^{aq}\pm0.01$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05)

Table 2: Serum cobalt concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$0.58^{ap}\pm0.01$	$0.61^{bpq}\pm0.01$	$0.61^{bp}\pm0.01$	$0.62^{bp}\pm0.02$
Group B (n=06)	$0.59^{ap}\pm0.01$	$0.61^{ap}\pm0.01$	$0.63^{bpr}\pm0.01$	$0.64^{bq}\pm0.01$
Group C (n=06)	$0.59^{ap}\pm0.01$	$0.62^{bp}\pm0.01$	$0.65^{bqr}\pm0.01$	$0.65^{bq}\pm0.01$
Group D (n=06)	$0.58^{ap}\pm0.01$	$0.59^{aq}\pm0.01$	$0.59^{aq}\pm0.01$	$0.58^{ar}\pm0.01$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05)

Table 3: Serum iron concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$3.17^{ap}\pm0.01$	$3.19^{ap}\pm0.02$	$3.21^{abp}\pm0.01$	$3.24^{bp}\pm0.01$
Group B (n=06)	$3.19^{ap}\pm0.01$	$3.22^{apr}\pm0.01$	$3.32^{bq}\pm0.01$	$3.34^{bq}\pm0.01$
Group C (n=06)	$3.20^{ap}\pm0.01$	$3.24^{acqr}\pm0.01$	$3.28^{bcq}\pm0.01$	$3.30^{br}\pm0.01$
Group D (n=06)	$3.17^{ap}\pm0.01$	$3.19^{ap}\pm0.01$	$3.19^{ap}\pm0.02$	$3.19^{as}\pm0.01$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05).

Table 4: Serum zinc concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$1.61^{ap}\pm0.02$	$1.62^{ap}\pm0.01$	$1.63^{apq}\pm0.01$	$1.64^{ap}\pm0.01$
Group B (n=06)	$1.62^{ap}\pm0.01$	$1.64^{abp}\pm0.01$	$1.66^{bcp}\pm0.01$	$1.68^{cq}\pm0.01$
Group C (n=06)	$1.63^{ap}\pm0.01$	$1.65^{acp}\pm0.01$	$1.67^{bcp}\pm0.01$	$1.68^{bq}\pm0.01$
Group D (n=06)	$1.61^{ap}\pm0.02$	$1.62^{ap}\pm0.01$	$1.62^{aq}\pm0.01$	$1.61^{ar}\pm0.02$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05).

Table 5: Serum manganese concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$0.13^{ap}\pm0.01$	$0.14^{abp}\pm0.01$	$0.15^{bp}\pm0.01$	$0.15^{bp}\pm0.01$
Group B (n=06)	$0.14^{ap}\pm0.01$	$0.15^{abpq}\pm0.01$	$0.16^{bcp}\pm0.01$	$0.17^{cq}\pm0.01$
Group C (n=06)	$0.14^{ap}\pm0.01$	$0.16^{bq}\pm0.01$	$0.16^{bp}\pm0.01$	$0.17^{bq}\pm0.01$
Group D (n=06)	$0.13^{ap}\pm0.01$	$0.14^{ap}\pm0.01$	$0.13^{aq}\pm0.01$	$0.14^{ap}\pm0.01$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05).

Table 6: Serum selenium concentration (μg/ml) in control and different treatment groups

Days Groups	Day 0	Day 7	Day 9	Day 10
Group A (n=06)	$0.06^{ap}\pm0.01$	$0.07^{bp}\pm0.01$	$0.08^{bcp}\pm0.01$	$0.09^{cp}\pm0.01$
Group B (n=06)	$0.07^{ap}\pm0.01$	$0.07^{abp}\pm0.01$	$0.08^{bp}\pm0.01$	$0.08^{bp}\pm0.01$
Group C (n=06)	$0.07^{ap}\pm0.01$	$0.08^{bp}\pm0.01$	$0.09^{cp}\pm0.01$	$0.09^{cp}\pm0.01$
Group D (n=06)	$0.06^{ap}\pm0.01$	$0.07^{ap}\pm0.01$	$0.07^{ap}\pm0.01$	$0.06^{aq}\pm0.01$

Mean ±SE with different superscript (a, b, c, d) in row and (p, q, r, s) in column differ significantly (p<0.05)

Serum copper concentration

The effects of different hormonal protocols on the level of serum copper level (μg/ml) are presented in Table 1. The mean serum copper concentration increased significantly (p<0.01) from day 0 to day 7, 9 and 10 in group A, B and C.

The significant difference was recorded between treatment groups and control groups. Higher serum copper level was recorded on day 10 in all treatment groups. The present study corroborated with the reports of Dabas *et al.* (1987) and Sharma *et al.* (1999). They observed significant ($p<0.05$) difference in plasma copper profile in anestrus buffalo-heifers. In contrary to present study non-significant differences were reported by Jain and Madan (1984). McDowell (1992) suggested that the critical level of copper to be 0.65 µg/ml below which the clinical signs of deficiency may occur. Sato and Henkin (1973) reported that estrogen hormone increases copper level in blood.

Serum cobalt concentration

The effects of different hormonal protocols on the level of serum cobalt level (µg/ml) are presented in Table 2. The mean serum cobalt concentration increased significantly ($p<0.05$) from day 0 to day 7, 9 and 10 in group A, B and C. Significantly higher levels were recorded in treatment groups as compared to control group.Sharma e*t al.* (2005) reported higher level of serum cobalt in GnRH treated buffaloes. Wagner (1962) reported that cobalt has been found to be required for synthesis of vitamin B_{12} and its deficiency has been associated with non-functional ovaries.Similar findings of increase in serum cobalt levels have been reported by Dhamsaniya*et al.* (2016) in GnRH treated buffaloes as compared to untreated control animal group. Khasatiya *et al.* (2005) also reported that the level of cobalt was significantly higher in treatment than the control group of anestrus Surti buffaloes.

Serum iron concentration

The effects of different hormonal protocols on the level of serum iron level (µg/ml) are presented in Table 3. The mean serum iron concentration increased non-significantly from day 0 to day 7 in all the groups (treatment and control), while significant rise was seen in group B and C on day 9 and 10, whereas, in group A the value of day 0 were found significant ($p<0.05$) on day 10. Mean serum iron concentration in untreated control group (group D) were found to be non- significant ($p>0.05$) on day 0, 7, 9 and 10.

The present findings are in agreement with findings of Dutta *et al.* (2001) and Sharma *et al.* (2005) and Sharma *et al.* (2010) wheresignificant rise in mean serum Iron concentration from pre-treatment day to different days post-treatment was recorded. Dhamasaniya *et al.* (2016) reported non-significant ($p>0.05$) rise in mean serum Iron concentration within different treatment groups at all time intervals. Although, Shah (1999) found significantly higher overall pooled mean iron level in anestrus treated than anestrus control Surti buffaloes. Kumar and Sharma (1993) reported that lower level of Iron causes normochromic anemia in animals, which in turn affects the response of ovarian receptors to estrogen, resulting in anestrus condition in animals. Parmar *et al.* (2015) reported that a low level of iron could possibly result in improper oxygenation of uterus resulting in impaired uterine nutrition in the uterus for the conceptus causing death of embryo.

Serum zinc concentration

The effects of different hormonal protocols on the level of serum zinc level (µg/ml) are presented in Table 4. The mean serum zinc concentration in group A increased non-significantly at all the time interval from day 0 to day 7 while in group B and C this rise was significant on day 9 and 10.Hence, there was an overall increase in serum zinc concentration in all the treatment groups. Mean serum zinc concentration in untreated control group showed non-significant increase at all point of time. The findings of present study are in agreement with Akhtar *et al.* (2009), Soni (2014) and Parmar *et al.* (2015) reported non-significant ($p>0.05$) rise in mean serum zinc concentration of anestrus Surti Buffaloes between sampling days and groups. Dutta *et al.* (2001) reported low zinc levels in anestrus heifers. Fall in zinc levels are associated with fall in steroid hormone concentration which indicated that there was some correlation between plasma zinc levels and progesterone-estrogen levels for proper reproductive process. Carlson *et al.* (1982) and Apagar (1985) reported that lower zinc levels might interfere with prostaglandin receptor-mediated phase and consequently the luteolytic process which in turn causes some of the reproductive pathologies and optimum zinc levels are essential to maintain the activity of FSH and LH.

Serum manganese concentration

The effects of different hormonal protocols on the level of serum manganese level (µg/ml) are presented in Table 5. The mean serum Manganese concentration increased significantly ($p<0.05$) on day 7 in treatment group C while this rise was significant on day 9 and 10 post treatment in treatment group A, B and C. Mean serum manganese concentration in untreated control group showed non-significant ($p>0.05$) increase at all point of time.Wilson (1966) reported manganese deficiency in dairy cattle results in anestrus or irregular return to oestrus, sometimes with extended periods of anestrus.

Serum selenium concentration

The effects of different hormonal protocols on the level of serum selenium level (µg/ml) are presented in Table 6. The mean serum concentration increased significantly ($p<0.05$) on day 9 and 10 post treatment in group A and on day 7, 9 and in group B and C while serum selenium levels remain non-significant ($p>0.05$) in untreated control buffaloes. Akhtar *et al*. (2009) reported higher selenium levels in cyclic buffaloes and in anestrus buffaloes it dropped significantly. Hindiroglou (1979) reported that selenium deficiency in cattle is associated with reduced fertility while Harrison *et al*. (1984) reported that high selenium concentration reduces the incidence of anestrus.

CONCLUSIONS

It was concluded that deficiency of Copper, Iron, Zinc and Selenium either alone or in combination, could be responsible for anestrus condition. By improving the trace minerals status, fertility can be improved in buffaloes. Mineral mixture should be supplied daily in the animals ration to suffice the requirement of the trace minerals. There was no consistent trend of trace minerals among different groups.

CONFLICT OF INTEREST

Authors have no conflict of interest.

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