# PLASMA MINERAL PROFILE AND PREGNANCY STATUS IN BUFFALO SUBJECTED TO DOUBLESYNCH IN SUMMER AND WINTER SEASON

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**ABSTRACT**

The buffalo subjected to doublesynch protocol in summer (n=20) and winter (n= 25) season were investigated for plasma Zn, Cu, Ca and P at the start of protocol and at AI. The pregnancy rate following protocol was 40% in summer and 48% in winter. In summer, plasma P was low ($p<0.05$) in buffaloes, irrespective of pregnancy status, as compared to winter season. However, plasma Zn, Cu and Ca had no variation between seasons ($p>0.05$). Plasma Zn, Cu and P were invariably high, both in pregnant and non-pregnant buffalo, at time of AI ($p<0.05$). In brief, plasma mineral profile was not apparently related to pregnancy outcome following doublesynch protocol in summer and winter season.

**Keywords :** Buffalo, Doublesynch, Estrus, Minerals, Season

Minerals have a significant role in successful establishment of pregnancy. The deficiency of minerals leads to subfertility and infertility in dairy animals. The literatures in dairy cattle suggests that even marginal mineral deficiency is manifested as reduced fertility before other clinical symptoms are apparent (Hadiya *et al.,* 2010 and Panda *et al.,* 2015). Fixed time artifical insmeination (FTAI) is commonnly used as assisted reproductive technology to augment fertility in dairy buffalo (Warriach *et al*., 2015). However, the success of these protocols in different seasons might be effected by circulating mineral concentrations in dairy animals. The present study was designed to investigate the mineral profile in buffalo subjected to a FTAI protocol (Doublesynch) in summer and winter season.

Murrah buffalo (Parity, 1-5; b wt, 400-600 kg; body condition score, 3-4) free from any apparent pathological disorders of reproductive organs and cycling at regular intervals, as confirmed by history and corpus luteum on either of ovaries, in summer (n=20) and winter (n=25) season were synchronized for FTAI by doublesynch protocol. This protocol involved administration (i.m.) of a synthetic prostaglandin $F_{2á}$ ($PGF_{2á}$) analogue (500ìg, Cloprostenol sodium on day -2 and day 7, as well as two injections of a GnRH analogue (10ìg, Buserelin acetate) on day 0 and day 9 followed by AI with frozen thawed semen at 8 h and 24 h after day 9 GnRH (Cirit *et al*., 2010). Pregnancy diagnosis was carried out 45 days later by transrectal ultrasonography.

Jugular vein blood samples were collected on day -2 and on the day of AI (dAI) in heparinised vials. After centrifugation at 1500 X g for 10 minutes, plasma was harvested and stored at $-20^{0}$C until analysis. Plasma Calcium (Ca) and Phosphorus (P) were estimated by fully automated Random Access Clinical Chemistry Analyzer (EM 200TM Erba Mannheim, Germany) by using kits procured from Transasia Biomedical Limited, Germany (Arsenazo and UV Phosphomolybdate method, respectively). Plasma Zinc (Zn) and Copper (Cu) were estimated by atomic absorption flame photometer (Pinaacle 900T, Perkin Elmer) following wet digestion of plasma samples. Student's t test using SPPS version 16 was used for statistical difference of plasma mineral profile between summer and winter season as well as between pregnant and nonpregnant buffalo.

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**Table: Postpartum reproductive parameters following anti-oxidant therapy in Surti buffalo**

| Season | Mineral | Pregnancy status | Day of doublesynch | |
|---|---|---|---|---|
| | | | Day -2 | Day of AI |
| **Zn**, ppm | **Summer** (NP=12, P=8) | **NP** | 1.3±0.1$^{A}$ | 1.8±0.1$^{B,a}$ |
| | | **P** | 1.4±0.1$^{A}$ | 1.7±0.1$^{B}$ |
| | | **Overall** | 1.4±0.1$^{A}$ | 1.7±0.1$^{B}$ |
| | **Winter** (NP=13, P=12) | **NP** | 1.3±0.1$^{A}$ | 1.5±0.1$^{B,b}$ |
| | | **P** | 1.2±0.1$^{A}$ | 1.6±0.1$^{B}$ |
| | | **Overall** | 1.3±0.1$^{A}$ | 1.5±0.1$^{B}$ |
| **Cu**, ppm | **Summer** | **NP** | 1.3±0.2$^{A}$ | 2.0±0.2$^{B}$ |
| | | **P** | 1.2±0.1$^{A}$ | 1.7±0.1$^{B}$ |
| | | **Overall** | 1.3±0.1$^{A}$ | 1.9±0.1$^{B}$ |
| | **Winter** | **NP** | 1.3±0.2 | 1.7±0.1 |
| | | **P** | 1.6±0.2 | 1.9±0.1 |
| | | **Overall** | 1.4±0.1 | 1.8±0.1 |
| **Ca**, mg/dl | **Summer** | **NP** | 8.6±0.2 | 8.8±0.3 |
| | | **P** | 8.4±0.4 | 9.1±0.2 |
| | | **Overall** | 8.5±0.2 | 9.0±0.2 |
| | **Winter** | **NP** | 9.0±0.4 | 9.2±0.4 |
| | | **P** | 9.2±0.5 | 9.5±0.3 |
| | | **Overall** | 9.1±0.3 | 9.3±0.2 |
| **P**, mg/dl | **Summer** | **NP** | 4.2±0.3$^{a}$ | 4.5±0.3$^{a}$ |
| | | **P** | 3.6±0.3$^{c,A}$ | 4.8±0.2$^{c,B}$ |
| | | **Overall** | 4.0±0.2$^{A}$ | 4.6±0.2$^{B}$ |
| | **Winter** | **NP** | 5.2±0.2$^{b}$ | 5.5±0.3$^{b}$ |
| | | **P** | 5.2±0.4$^{d}$ | 6.0±0.3$^{d}$ |
| | | **Overall** | 5.3±0.2$^{\#}$ | 5.7±0.2$^{\#}$ |

$^{A\ vs\ B}$p<0.05, within a row; p<0.05, between summer and winter within a column for a parameter ($^{a\ vs\ b,\ c\ vs\ d}$) or for overall value of parameter ($^{\#}$); AI Artificial insemination, NP Non pregnant, P Pregnant

In present study, the pregnancy rate following doublesynch protocol in buffaloes was 40% in summer and 48% in winter season. In buffalo subjected to doublesynch protocol, plasma Zn was high in non-pregnant as well as pregnant counterparts at the time of AI as compared to values at the start of protocol (p<0.05, Table). This might be due to role of Zn in secretion of GnRH from hypothalamus (Kaswan and Bedwal, 1995). Moreover, during follicular phase of estrous cycle, ovarian tissues require more Zn (Brem *et al*., 2003). Plasma Cu and Ca values were similar at the start of protocol and at AI (p>0.05, Table), except for higher plasma Cu at AI in summer season (p<0.05, Table). Copper has role in steroid hormone synthesis as well as prevents ovaries from free radical damage (Ahmed *et al*., 2009). In fact, plasma Cu increases under the influence of estrogen hormone (Sato and Henkin, 1973). In a study, higher plasma copper was observed during follicular phase as compared to luteal phase following synchronized estrus using $PGF_{2á}$ in ewes (Hassanein *et al*., 1999). Plasma P was high at AI during summer season as compared to values at start of protocol (p<0.05, Table). Moreover, plasma P was high in buffaloes in winter season as compared to summer season (p<0.05, Table). In present study, there was no seasonal variation in plasma Zn, Cu and Ca in buffalo either pregnant or non-pregnant (p>0.05, Table), contrary a previous study exhibiting high blood mineral profile in winter in buffalo (Chhabra *et al*., 2015). In brief, plasma P was low in summer season both in pregnant and non-pregnant buffalo, whereas other minerals were not related to pregnancy outcome in either of the seasons.

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