



## Comparative study on plasma mineral profiles of normal cyclic and pregnant cows with infertile cows of North-Western Himalayan region of India

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### ABSTRACT

The present study was undertaken to study the effect of different minerals on infertility in cows (n=885) belonging to different Northern Western Himalayan regions of the state. Cows suffering from different types of infertility such as endometritis, anestrus, silent estrus and cows showing metestrual bleeding were evaluated and compared with normal cyclic and pregnant cows. Minerals evaluated were calcium, phosphorous, magnesium, copper, zinc, sodium, and potassium. The levels of calcium were statistically low in anestrus and silent estrus cows when compared with normal cyclic and pregnant animals. The levels of phosphorus were significantly low in cows suffering from endometritis and in cows showing abnormal metestrual bleeding in comparison to normal cyclic cows. Similarly, magnesium levels were high in pregnant cows than other categories. Copper was statistically high in normal cyclic and pregnant cows. Similarly, sodium and potassium were statistically high in normal cyclic cows than infertile and repeat breeder cows.

**Keywords:** Anestrus, Endometritis, Minerals, Normal cyclic cows, Silent estrus

Infertility in cattle is considered to be one of the major problems of reproduction and the various reasons of infertility still remain conundrum in many animals. It is also responsible for decreased reproductive performance of affected animals (Dutta *et al.* 2001). Under field conditions, when managemental practices are below standard, the deficiency of various nutrients can cause conception failure and early embryonic mortality in such animals (Pandey *et al.* 2009). Repeat breeding is thought to be one of the important factors involved in infertility which may put burden on the pockets of dairy farmers due to increased inter-calving interval, increased number of insemination up to next calving and reduced milk production (Parkinson 2009).

Among various nutrients, the mineral profile of animals can be used to check the health and nutritional status of cows. Normal levels of mineral parameters are essential for normal functioning of different body systems including reproductive system. So any deviation from normal levels of these minerals may cause reproductive failure. Therefore, the estimations of different minerals are helpful in characterization of such reproductive problems. The present study was conducted to determine the mineral profiles of normal cyclic and pregnant cows compared

with infertile cows suffering with endometritis, anestrus, silent estrus and animals showing metestrual bleeding in various parts of the Himachal Pradesh which are under the geographical region of North western region of the country.

### MATERIALS AND METHODS

Blood samples were collected from cows of different areas of different districts suffering from reproductive abnormalities. Overall, 885 blood samples were collected from different cows suffering from different reproductive disorders. Blood (10 ml) was collected in heparinised vial through jugular venipuncture in centrifuge tubes for separation of plasma. These blood samples were centrifuged at 3000 rpm for 10 min in portable centrifuge and the plasma was harvested at the spot. The plasma samples were stored at -20°C for pending analysis. The macro minerals like calcium (Ca), magnesium (Mg) and micro minerals viz. copper (Cu), zinc (Zn) were estimated from plasma using atomic absorption spectrophotometer (Perkin Elmer Analyst 400). The wavelengths used for estimation of Ca, Mg, Cu and Zn were 422.7, 285.2, 324.8 and 213.9 nm, respectively. Plasma sodium (Na) and potassium (K) were estimated by Flame photometer (Model T-129, Systronics India Ltd.). Similarly, phosphorus was estimated by using fully automatic biochemistry analyzer Mispal nano (Agappe Diagnostics Ltd., India) with the help of standard kits (Agappe Diagnostic Ltd., India) at 340 nm wavelength.

*Statistical analysis:* The obtained data was statistically

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analyzed by using student's t-test with the help of Instat graphical pad software.

## RESULTS AND DISCUSSION

The level of calcium was statistically low ( $p < 0.01$ ) in anestrus and silent estrus cows when compared with pregnant and normal cyclic cows (Table 1). Similarly, a significant ( $p < 0.05$ ) difference was also found in between anestrus and silent estrus. The normal required levels of calcium for optimum production and reproduction are 8.0-11.4 mg/dl (Merck Veterinary Manual 2005). The levels of calcium were marginally low in anestrus ( $7.96 \pm 0.079$  mg/dl) and low in cows suffering with silent estrus ( $7.45 \pm 0.15$  mg/dl). The serum calcium is required for regulation of function of various hormones during reproductive cycle of normal cyclic cows. Deficiency of this mineral can cause delayed uterine involution, retained placenta, prolonged calving and reduction in neutrophil function due to decrease in uterine muscle tone (Martinez *et al.* 2014). The low levels were found in anestrus whereas, excess of this mineral may affect the reproduction of animal by impairing absorption of phosphorous, copper, manganese, zinc and other elements from gastrointestinal tract (Yasothai 2014).

Ca deficiency is always associated with P deficiency and is responsible for reproductive disorders. Calcium:phosphorous (Ca:P) ratio plays an important role in bovine reproduction. The required normal ratio of Ca:P is between 2.5:1 and 1.5:1 for lactating healthy cows (Yasothai 2014). In our study, the ratios of Ca:P recorded were lying within the normal range. The mean ratio of Ca and P minerals is required for reproductive and ovarian cyclicity of cows. Any deviation in normal ratio may cause decrease in absorption of other minerals (Ugyen *et al.* 2016). Lower serum Ca and P ratio is thought to be the main reason for anestrus conditions in dairy cattle (Kumar *et al.* 2022).

Similarly, phosphorus (P) deficiency is considered when its levels in the blood are consistently below 4.5 mg/dl, however, phosphorous of bones is considered more sensitive measure of its status in animals body (Pradhan and Nakagoshi 2008). Deficiency of this mineral may result in reduced feed intake, reduced milk production, decreased fertility rate, impaired ovarian activity, irregular estrous cycles, increased occurrence of cystic ovaries,

delayed sexual maturity and decreased conception rates (Cromwell 1997). In present study, the levels of phosphorous were non-significantly lower in silent estrus ( $5.14 \pm 0.147$  mg/dl) and anestrus ( $5.11 \pm 0.058$  mg/dl) animals in comparison to normal cyclic ( $5.66 \pm 0.17$  mg/dl) and pregnant cows ( $5.31 \pm 0.14$  mg/dl). However, the levels were significantly ( $p < 0.01$ ) low in repeat breeder cows affected with endometritis ( $5.00 \pm 0.070$  mg/dl) and cows which were showing metestral bleeding (MEB) ( $4.70 \pm 0.145$  mg/dl) in comparison to normal cyclic ( $5.66 \pm 0.17$  mg/dl) cow. Almost similar results of high concentration were found in normal cyclic than that of repeat breeding cows in studies conducted by Kekana and Shirbhate (2015), Modi *et al.* (2017) and Kumar *et al.* (2022).

Besides, the normal values of magnesium vary from 1.50 to 2.90 mg/dl (Merck Veterinary Manual 2005). However, it does not directly play any role in reproduction as it is in antagonism with calcium levels but its deficiency can alter reproductive efficiency by altering the normal appetite of the animal (Yasothai 2014). Results of magnesium levels in present study were almost similar among different groups of cow except in pregnant cows where the levels were significantly ( $p < 0.01$ ) higher. Few workers reported higher levels of magnesium in normal cyclic cows than anestrus cows (Kalita and Sarmah 2006, Ugyen *et al.* 2016, Anushma *et al.* 2020). Any change in ratio of calcium-phosphorus-magnesium affects reproduction of farm animals (Reddy 2010).

The recorded levels of copper were significantly ( $p < 0.01$ ) lower in infertile cows suffering with endometritis ( $0.711 \pm 0.011$  ppm), anestrus ( $0.702 \pm 0.012$  ppm) and silent estrus ( $0.698 \pm 0.017$ ) in comparison to pregnant ( $0.848 \pm 0.049$  ppm) and normal cyclic ( $0.939 \pm 0.055$  ppm) cows. However, no significant difference was found between different groups for zinc. Our results were in accordance to the results of Kalita *et al.* (2015) where it was found that the average concentration of Cu was significantly ( $P < 0.01$ ) higher in normal cycling cows than in infertile cows. However, some studies had shown no significant difference in serum copper concentrations of repeat breeder and normal cows (Ahmed *et al.* 2017, Kumar *et al.* 2022). Both copper and zinc help in the progesterone

Table 1. Blood plasma concentration of minerals (Mean $\pm$ SE) in cows (n=885) with different reproductive clinical conditions

| Clinical Condition   | Calcium (ppm)         | Inorganic phosphorus (mg/dl) | Ca:P ratio | Magnesium (mg/dl)  | Copper (ppm)        | Zinc (ppm)        | Sodium (mEq/l)       | Potassium (mEq/l)   |
|----------------------|-----------------------|------------------------------|------------|--------------------|---------------------|-------------------|----------------------|---------------------|
| Endometritis (n=350) | $7.81 \pm 0.075^x$    | $5.00 \pm 0.070^x$           | 1.56:1     | $1.79 \pm 0.034^x$ | $0.711 \pm 0.011^x$ | $1.294 \pm 0.024$ | $118.802 \pm 0.72^x$ | $3.187 \pm 0.028^x$ |
| Anestrus (n=310)     | $7.96 \pm 0.079^{ax}$ | $5.11 \pm 0.058$             | 1.56:1     | $1.73 \pm 0.035^x$ | $0.702 \pm 0.012^x$ | $1.315 \pm 0.027$ | $118.39 \pm 0.73^x$  | $3.27 \pm 0.029^x$  |
| Silent estrus (n=80) | $7.45 \pm 0.15^{bx}$  | $5.14 \pm 0.147$             | 1.45:1     | $1.73 \pm 0.062^x$ | $0.698 \pm 0.017^x$ | $1.386 \pm 0.051$ | $117.67 \pm 1.71^x$  | $3.27 \pm 0.07^x$   |
| Pregnant (n=55)      | $9.06 \pm 0.13^y$     | $5.31 \pm 0.14$              | 1.71:1     | $2.74 \pm 0.08^y$  | $0.848 \pm 0.049^y$ | $1.249 \pm 0.085$ | $122.55 \pm 1.95$    | $3.418 \pm 0.127^x$ |
| Cyclic (n=40)        | $8.75 \pm 0.19^y$     | $5.66 \pm 0.017^y$           | 1.55:1     | $1.78 \pm 0.10^x$  | $0.939 \pm 0.055^y$ | $1.452 \pm 0.074$ | $129.71 \pm 3.22^y$  | $4.11 \pm 0.187^y$  |
| MEB (n=50)           | $8.07 \pm 0.25^x$     | $4.70 \pm 0.145^x$           | 1.72:1     | $1.70 \pm 0.09^x$  | $0.668 \pm 0.029^x$ | $1.272 \pm 0.072$ | $123.26 \pm 1.56$    | $3.13 \pm 0.076^x$  |

<sup>a,b</sup>values with different superscripts for the same parameter and condition within a column differ significantly ( $p < 0.05$ ). <sup>x,y</sup>values with different superscripts for the same parameter and condition within a column differ significantly ( $p < 0.01$ ).

synthesis from luteal cells under the control of superoxide dismutase (Sales *et al.* 2011). Deficiency of these minerals in animals can cause low conception rate, anestrus and non-functional ovaries. Deficiency of copper can cause reduced conception rate even though the estrus found normal (Chesworth 1992). It is found to cause increased retention of fetal membrane, early embryonic death, embryonic resorption and placental necrosis. The animals suffering with its deficiency can cause anemia which ultimately results in weak and silent heat in such animals, and is further responsible for reduced fertility and increase day open due to inactive ovaries (Mudgal *et al.* 2014, Anushma 2020).

Similarly, zinc is required for sexual maturity, onset of estrus and for reproductive capacity. It is also required for endometrial repair and maintenance after calving and help in return to normal reproductive functioning and estrus manifestation (Greene *et al.* 1998). The concentrations of zinc were found higher in normal cyclic cow than repeat breeder cows (Ahmed *et al.* 2017). But contrary results of higher concentration were reported by Parmar *et al.* (1986) in repeat breeder than normal cyclic cows. Lower concentrations of Cu and Zn were also reported in infertile cows suffering with silent heat in comparison to normal cyclic cows (Das *et al.* 2002). In general, almost half of animals diagnosed as anestrus were deficit in serum Zn (Mishra 2006) and when it is supplemented in repeat breeder buffaloes helped in improving the fertility rate up to 80% (Marai *et al.* 1992). In our study, the levels of zinc were found non-significantly low in anestrus and silent estrus animals than normal cyclic cows.

The normal concentration of blood sodium in cow recorded was 121-152 mEq/l (Pandey *et al.* 2009). The supplementation of salt in low quantity in animals can affect the efficiency of digestion and indirectly reduces the reproductive performance of cows (Elrod and Butler 1993). In our study, the concentrations of sodium recorded were  $118.39 \pm 0.73$ ,  $118.80 \pm 0.72$  and  $117.67 \pm 1.71$  mEq/l in cows suffering with anestrus, endometritis and silent estrus, respectively where these estimated levels were significantly ( $p < 0.01$ ) lower than normal cyclic ( $129.71 \pm 3.22$  mEq/l) cows. The results in present study were comparable to the results recorded by Pandey *et al.* (2009) where significant low levels were recorded in repeat breeder ( $100.5 \pm 0.11$  mEq/l) cows than normal cyclic cows ( $125.8 \pm 6.3$  mEq/l).

Similarly, the deficiency of potassium can affect the normal reproductive process as it is responsible in maintaining the muscle integrity (Yasothai 2014). However, feeding of high levels of potassium can increase the incidence of anestrus in heifers, poor formation of CL and delays onset of puberty and ovulation (Bindari *et al.* 2013). The levels of potassium in present study were found almost similar in all categories of cows. But in a study conducted by Pandey *et al.* in 2009, the plasma concentration of this mineral was significantly higher in repeat breeder cows ( $4.7 \pm 0.3$  mEq/l) than normal cyclic cows ( $3.1 \pm 0.4$  mEq/l).

In the present study, the levels of different minerals were

estimated in different categories of infertile animals and were compared with normal cyclic and pregnant animals as the minerals are required for various reproductive functions which control metabolism, growth and maintenance of animals. The deficiencies of few trace minerals and other major minerals like calcium and phosphorus are responsible for causing various reproductive disorders. So to check their deficiencies, time to time estimation should be carried out in blood. The present study is helpful for assessing the role of different minerals for animal reproduction.

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