

# Comparative study on serum macro and micro mineral profiles during oestrus in repeat breeding crossbred cattle with impaired and normal ovulation

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## Abstract

The study was conducted on 40 clinically healthy repeat breeding crossbred cattle (15 with anovulation, 15 with delayed ovulation and 10 with normal ovulation). The serum concentrations of calcium (Ca), inorganic phosphorus (IP), magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu) and iron (Fe) during the oestrus were compared among the animals with normal and impaired ovulations. Ovulatory status of the experimental animals was determined through per rectum examination and blood samples were collected during the oestrus.

The concentrations of Ca (mg %), IP (mg %) and Zn (ppm) varied significantly ( $P < 0.01$ ) among the animals with normal ovulation ( $9.58 \pm 0.49$ ,  $5.45 \pm 0.15$  and  $1.78 \pm 0.02$ ), anovulation ( $6.48 \pm 0.13$ ,  $4.45 \pm 0.14$  and  $0.85 \pm 0.03$ ) and delayed ovulation ( $7.36 \pm 0.13$ ,  $4.96 \pm 0.09$  and  $1.41 \pm 0.04$ ). The concentrations of Mg (mg %) and Mn (ppm) were found to be significantly ( $P < 0.01$ ) lower in the animals with anovulation ( $2.64 \pm 0.04$  and  $0.14 \pm 0.01$ ) compared to the animals with normal ( $3.12 \pm 0.05$  and  $28 \pm 0.01$ ) and delayed ovulation ( $2.98 \pm 0.06$  and  $0.23 \pm 0.01$ ). In contrast, Cu and Fe concentrations did not differ significantly among the different ovulatory groups.

The results indicated that Ca, IP and Zn were probably crucial for the normal ovulatory process and Mg and Mn below the optimum level might have caused anovulation in crossbred cattle. However, Cu and Fe might have played little role in the ovulatory process in crossbred cattle.

**Key words:** Crossbred cattle, macro minerals, micro minerals, ovulatory disturbances

## Introduction

Repeat breeding is one of the major problems in dairy cattle that affects fertility and in turn incurs great economic loss to farmers. Ovulatory disturbance is one of the major causes of repeat breeding in crossbred dairy in India (Ibraheem Kutty and Ramchandran 2003). Mineral imbalance or deficiencies may cause repeat breeding in cattle (Rupde et al 1993, Das et al 2002). Variations in the level of trace elements have been found to be associated with reproductive disorders (Jain and Madan 1984, Jain 1993). The deficiency of a particular element may influence the level of other elements in the body fluid and the functional characteristics of endocrine glands, especially the hypophyseal-gonadal axis (Bhaskaran and Abdullakhan 1981).

Calcium (Ca) plays an important role in gonadotropic regulation of ovarian steroidogenesis (Carnegie and Tsang 1984). Marginal deficiency of phosphorus cause disturbance in the pituitary-ovarian-axis including ovulation (Bhaskaran and Abdullakhan 1981). Zinc (Zn) deficiency may reduce GnRH secretion that eventually leads to the arrest of ovulation (Kaswan and Bedwal 1995). Lack of manganese (Mn) may inhibit the synthesis of cholesterol and its precursors that in turn may limit the synthesis of sex hormones and possibly other steroids (Doisey 1973). Copper (Cu) has a significant role in maintaining optimum fertility as it is

involved in FSH, LH and estrogen activity (Desai et al 1982). A low level of serum iron (Fe) has been found to influence normal gonadal activity possibly by causing improper hormonal output and deterioration of general physiological function (Sharma et al 1986).

At present, the information on blood mineral attributes during the oestrus in repeat breeding crossbred cattle with impaired ovulation are scanty. The present study was conducted to compare the serum level of Ca, inorganic phosphorus (IP), magnesium (Mg), Mn, Zn, Cu and Fe during the oestrus in repeat breeding crossbred cattle with normal and impaired ovulations.

## Materials and methods

### Experimental animals

The study was conducted on 40 clinically healthy repeat breeding crossbred cattle (15 with anovulation, 15 with delayed ovulation and 10 with normal ovulation). The experimental animals were selected from private farms located in Khanapara, Guwahati, Assam, after confirming the status of ovulation (normal, delayed and anovulation) through per rectum examination. The animals were examined per rectum from the onset of oestrus at 12 h interval until 72 h to detect ovulation. The animals were also examined per rectum on day 10 following the onset of oestrus to determine the presence of a mature corpus luteum (CL). Normal ovulation was considered, when corpus haemorrhagicum was detected within 36 h and a mature CL was present on day 10 following the onset of oestrus. Anovulation was considered if corpus haemorrhagicum was absent till 72 h or a mature CL was absent on day 10 following the onset of oestrus. Delayed ovulation was considered, when Graafian follicle was present till 36 h and a mature CL was present on day 10 following the onset of oestrus. The animals were free from any anatomical abnormality of reproductive organs. The animals were maintained under stallfed condition and fed adequate green fodders, straw, concentrate and *ad libitum* drinking water.

### Sample collection and analysis

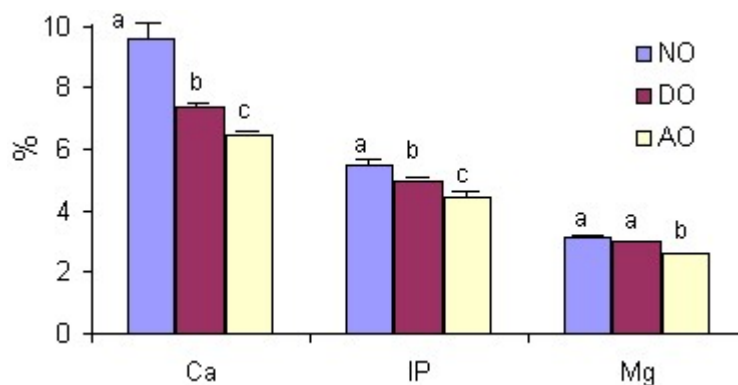
Blood samples were collected from the animals during the oestrus and serum was separated and stored at -20°C until further analysis. The concentrations of Ca (Webster 1962), IP (Gomori 1942) and Mg (Andreasen 1957) in serum were estimated colorimetrically according to previously described methods. The concentrations of Mn, Zn, Cu and Fe in serum were estimated using a Flame Atomic Absorption Spectrophotometry (Solar, AA Series Spectrometer, Thermo Electron Corporation, UK) according to previously described methods (Fick et al 1979).

### Statistical analysis

The variation in the serum concentration of different minerals was analyzed by ANOVA using general linear model procedure (SPSS 1999). The model included the different ovulatory groups as source of variation. The Student Newman Keuls test was used to isolate means when effect was found significant (SPSS 1999).

## Results and discussion

Mean serum concentrations of Ca, IP and Mg at oestrus in repeat breeding crossbred cattle with normal ovulation, delayed ovulation and anovulation are depicted in figure 1.

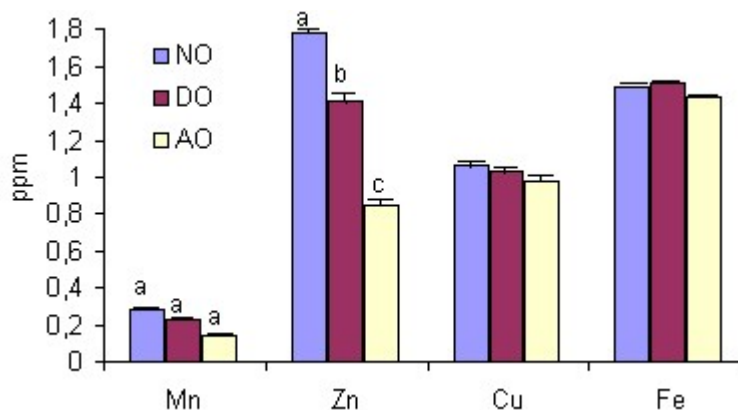


**Figure 1.** Mean  $\pm$  SE serum concentration of calcium (Ca), inorganic phosphorus (IP) and magnesium (Mg) at oestrus in repeat breeding crossbred cattle with normal ovulation, delayed ovulation and anovulation; a,b,c on Error bar indicates the values differ significantly ( $p < 0.01$ )

The concentrations of Ca and IP were found to vary significantly ( $p < 0.01$ ) among the groups. The concentrations of Ca and IP were found to be lower in the animals with anovulation and delayed ovulation compared to the animals with normal ovulation. Ca plays an important role in the gonadotropic regulation of ovarian steroidogenesis (Carnegie and Tsang 1984) and regulation of the membrane potential of oocytes. It is also suggested that Ca is involved in the disruption of cumulus cell cohesiveness by regulating the number of gap junctions between the cells (Peracchia 1978), which contributes to the process of ovulation. Bhaskaran and Patil (1982) have suggested that marginal deficiency of phosphorus could cause disturbances in the pituitary-ovarian axis including ovulation. Moreover, a disturbed calcium-phosphorus ratio has a blocking action on the pituitary gland and consequently on the ovarian function (Herrick and John 1977). The results of this study indicate that the low Ca and IP levels might be related to the ovulatory disturbances in crossbred cattle.

The concentration of Mg was found to be significantly ( $p < 0.01$ ) lower in the animals with anovulation. Though the concentration did not differ among the animals with delayed and normal ovulation, the concentration was found to be lower in the animals with delayed ovulation. Although Mg does not have any direct role in reproduction, it is involved in many of the enzymatic reactions catalyzed by ATP associated enzymes. Moreover, it influences the absorption of Ca and P (Sharma et al 2004). Therefore, Mg imbalance may influence reproductive efficiency indirectly. The results of our study also revealed that the low Mg level was associated with anovulation.

Variations in the serum concentration of Mn, Zn, Cu and Fe at oestrus in repeat breeding crossbred cattle with normal ovulation, delayed ovulation and anovulation are depicted in figure 2.



**Figure 2.** Mean  $\pm$  SE serum concentration of manganese (Mn), zinc (Zn), copper (Cu) and iron (Fe) at oestrus in repeat breeding crossbred cattle with normal ovulation, delayed ovulation and anovulation; a,b,c on Error bar indicates the values differ significantly ( $p < 0.01$ )

The concentrations of Mn and Zn were found to vary significantly ( $p < 0.01$ ) among the different ovulatory groups. The concentration of Mn was found to be significantly lower in the animals with anovulation. Though the concentrations did not differ among the animals with delayed and normal ovulation, these concentrations were found to be lower in the animals with delayed ovulation. Mn has a role in steroid synthesis (Keen and Zidenberg-cheer 1990) and it is associated with impaired reproductive functions such as anoestrus, delayed ovulation and repeat breeding (Corah 1996). Delayed ovulation has been induced experimentally in dairy cows by withholding Mn over a long period (Rojas et al 1965). The results of the present study indicate that Mg and Mn might have played some role in the normal ovulatory process. The concentration of Zn was found to be significantly ( $p < 0.01$ ) lower in the animals with anovulation and delayed ovulation compared to the animals with normal ovulation. Zn deficiency may lead to the reduction in GnRH secretion by hypothalamus and eventually lead to decreased LH and FSH levels and arrest of ovulation (Kaswan and Bedwal 1995). Apagar (1985) also has demonstrated that an optimum Zn level is essential for maintaining the activity of LH to induce normal ovulation. The results of the present study indicate that the low Zn level might have caused ovulatory disturbances in crossbred cattle.

The concentrations of Cu and Fe did not differ significantly among the various ovulatory groups. It is evident that in ruminant, Fe deficiency is rare in grazing and well managed animals (Hidiroglou 1979, Puls 1994). Previous reports also indicate that Cu deficiency does not influence the normal fertility in cattle (Hunter 1977, Whitaker 1982). The results of the present study indicate that probably Cu and Fe did not play a crucial role in the ovulatory process of the experimental animals.

## Conclusions

- The concentrations of Ca, IP and Zn varied significantly among the different ovulatory groups. The concentrations of Mn and Mg were found to be significantly lower in the animals with anovulation, but did not differ in the animals with normal and delayed ovulation. In contrast, the concentrations of Cu and Fe did not differ significantly among the different ovulatory groups. The results indicated that in crossbred cattle, Ca, IP and Zn were probably crucial for the normal ovulatory processes, and Mg and Mn below the optimum level might have caused anovulation. However, Cu and Fe might have played a little role in the ovulatory process in crossbred cattle.

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[Go to top](#)