



## RESEARCH ARTICLE

### Serum Mineral Profile in Various Reproductive Phases of Mares

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#### ABSTRACT

The present study was conducted to determine the trace mineral profile in fertile, subfertile and pregnant mares kept under different management conditions. For this purpose the blood samples were collected without anticoagulant from 100 field mares and 100 farm mares for serum separation. All animals were grouped according to their history and rectal examination. Serum manganese levels in pregnant mares were significantly ( $P < 0.05$ ) higher than all other mares. Serum iron levels showed no significant difference between the groups and within the groups. Pregnant mare in field conditions showed significantly ( $P < 0.05$ ) higher serum copper level than farm animals. Serum zinc levels in estrual group of mares under field conditions showed significantly ( $P < 0.05$ ) lower levels compared with rest of the three groups and from farm maintained groups. Serum zinc levels in estrual mares under farm condition were significantly ( $P < 0.05$ ) higher as compared to their counterparts under field conditions. Fertile, subfertile and pregnant mares under field conditions differed significantly ( $P < 0.05$ ) from one another, pregnant mares showed significantly ( $P < 0.05$ ) higher levels compared with rest of three groups under same condition. Pregnant mares under field conditions showed significantly ( $P < 0.05$ ) higher serum selenium levels when compared with the farm animals. It can be concluded that deficiency of manganese, iron, zinc, copper and selenium might be possible causes of infertility in mares.

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#### INTRODUCTION

Minerals have wide range of activities within the body of animals and are crucial for vitamin synthesis, enzymatic activity, hormone production, oxygen transport, tissue synthesis and several physiological processes related to reproductive performance (Bertelsmann *et al.*, 2010; Meliani *et al.*, 2011; Namini *et al.*, 2011). In different domestic species the micro minerals such as selenium, copper, zinc and molybdenum play crucial role on metabolism of proteins, carbohydrates, lipids and affect reproduction in animals due to the complex neuro-hormonal relationship (Richardson *et al.*, 2006; Vazquez-Armijo *et al.*, 2011). Deficiency of these trace elements causes severe economic loss due to increased susceptibility to oxidative stress, growth retardation in young animals, anemia, pregnancy wastage (Bureau *et al.*, 2008), nutritional myopathy, decreased fertility, premature rupture of membranes and muscular dystrophy (Pathak and Kapil,

2004; Robinson *et al.*, 2006). Ovulatory disturbance is one of the major causes of repeat breeding in crossbred dairy in India (Kutty and Ramchandran, 2003). Copper deficiency is related with rupture of uterine artery during foaling (Harper and Gill, 2006). Mineral deficiency in mares may cause disorders in growth and development, besides decreased immune function or impaired reproductive efficiency (Rink and Haase, 2007; Hussain *et al.*, 2011; Mahmood *et al.*, 2012).

Mineral imbalance or deficiencies may cause repeat breeding in cattle (Das *et al.*, 2002). Hormonal dysfunction, genetic disabilities and management factors including nutrition are the major causes of infertility in brood mares. Zinc deficiency has negative effect on growth rate, specific organs weight, hematological parameters, levels of copper and iron in serum (El-Hendy *et al.*, 2001). Manganese plays vital role in activity of certain endocrine glands. Manganese acts as a co-factor in production of cholesterol and is necessary for the synthesis of steroids such as progesterone, estrogen and

testosterone (Keen and Zidenburg-Cherr, 1990). The deficiency of manganese can cause irregular heats, silent heats, poor conception and abortion (Hackbart *et al.*, 2010). Research in chicks (Takeo *et al.*, 2005) and baby pigs (Caine *et al.*, 2001; Payne *et al.*, 2006) indicated that supplementation of zinc during the production period can increase intestinal villi growth and enhance nutrient absorption during the first few weeks of life. More than 300 enzymes, including the ribonucleic polymerases, alcohol dehydrogenase, carbonic anhydrase and alkaline phosphatase require zinc for their activities (Nowak *et al.*, 2005; Goldhaber, 2003). Copper-deficient sheep had increased mortality from bacterial infection (Chew, 2000). The supplementation of zinc, copper, manganese and selenium in horses have been reported to change in pregnancy owing to increase requirement of these elements during developing fetus (Huntington *et al.*, 2003). Effects of prostaglandin synthesis suggest that zinc deficiency has profound effects on reproductive cycle and pregnancy. Iron deficient animals become repeat breeders and require increased number of insemination per conception and occasionally may abort (Kumar *et al.*, 2011). Therefore, the present study was designed to determine the serum level of various minerals in relation to reproductive status in mares.

#### MATERIALS AND METHODS

The present study was conducted on total of 200 mares including 100 each at intensively managed farms and under field conditions of Chenab breeding area in Pakistan. Animals were grouped on the basis of their reproductive status as estrual, fertile, sub-fertile and pregnant mares after rectal examination and by taking history. All the mares in present study were fed seasonal fodders and offered clean fresh water *ad libitum*.

The blood samples were directly collected from jugular vein (Akhtar *et al.*, 2012) in clean sterile glass test tubes without anticoagulant (EDTA) from each mare early in the morning before feeding. The test tubes were allowed to stand for approximately three hours in slanting position at room temperature for serum separation. Serum samples were also separated by direct centrifugation techniques. Sterilized labeled plastic vials with clear serum were used and stored at -20°C until analysis. For

wet digestion a quantity of 0.5 ml stored serum was taken into a digestion flask and 5 ml of nitric acid was added. The content of the flask were placed on hot plate at 80-100°C for 10-15 minutes until all fumes were evaporated from the flask and approximately 1-2 ml material in the flask was remained. After cooling 2.5 ml perchloric acid was added. The contents of the flask were heated intensively until the volume was reduced to 1-2 ml. After that the remaining volume was filtered and stored in labeled plastic bottles. These digested samples were used for estimation of copper, selenium and manganese by atomic absorption spectrophotometer. Iron and zinc level analyzed by using commercial kits in chemistry analyzer (Map lab plus), by dually calibrated procedures as specified by the respective kit manufacturer (Akhtar *et al.*, 2012). For the studied parameters, data were arranged in 2 x 4 x 5 categories. For each categories standard deviation were also determined for mean.

**Statistical analysis:** The data of various categories collected in present study were subjected to two sampled t-test and the probability level was set at or below ( $P \leq 0.05$ ).

#### RESULTS AND DISCUSSION

The results on various trace minerals in mares are presented in table 1. The results revealed that the serum manganese levels in pregnant mares under farm conditions were significantly ( $P < 0.05$ ) higher than subfertile and fertile mares. In field conditions pregnant mares had significantly higher serum manganese levels as compared to all mares in present study. However, sub-fertile mares had significantly ( $P < 0.05$ ) higher serum manganese levels as compared to fertile mares (Table 1). Significantly higher ( $P < 0.05$ ) serum manganese levels were found in estrual mares kept at farm level as compared to those kept under field conditions.

Serum manganese levels determined in present study were reduced than those reported earlier (Naheed, 2004) for fertile and sub fertile mares but close to those reported for pregnant mares. Sever deficiency of manganese can give rise to resorption in utero or death at birth, manganese deficiency can also result in irregular estrous cycle in mares (Harper and Gill, 2006). According to Corah and Ives (1991) deficiency of manganese may be

**Table 1:** Serum mineral profiles in mares of different reproductive phases kept under different management conditions.

Minerals/ Area	Reproductive Phases			
	Estrual	Fertile	Sub-fertile	Pregnant
Manganese (mg/l)				
Field	0.86±0.65Bb	0.55±0.53Ca	0.95±0.53Bb	1.76±0.12Aa
Farm	1.68±0.18Aa	0.66±0.50Ba	0.82±0.20Ba	1.70±0.09Aa
Iron (mg/l)				
Field	3.63±0.29Aa	3.64±0.20Aa	3.70±0.14Aa	3.69±0.12Aa
Farm	3.64±0.34Aa	3.65±0.41Aa	3.70±0.11Aa	3.71±0.16Aa
Copper (µg/dl)				
Field	78.46±2.1AB	76.41±1.71AB	76.35±1.79Ba	84.90±1.29Ab
Farm	81.00±1.66Aa	77.40±1.69Aa	78.46±1.66Aa	76.22±1.29Aa
Zinc (µg/dl)				
Field	136.50±39.78Ab	167.62±28.08Ba	168.50±19.68Ba	168.70±17.68 Ba
Farm	171.10± 29.53Aa	160.93±25.47Aa	159.63±16.04Aa	164.36±20.99Aa
Selenium (µg/ml)				
Field	0.39±0.05Aa	0.35±0.05Aa	0.21±0.05Ca	0.27±0.04Bb
Farm	0.38±0.05Aa	0.37±0.07Aa	0.20±0.03Ca	0.24±0.03Ba

Values with different superscripts in the same row (small letters) and in the same column (capital letters) in each parameter differ significantly ( $P \leq 0.05$ ).

related to suppression of estrus, silent estrus, irregular estrus cycle, cystic ovary, poor follicular development with delayed ovulation, increased in embryonic mortality and reduced conception rate.

Serum copper levels were not differed significantly among different groups of mares having different phases of reproductive cycle under farm conditions. Pregnant mares under field conditions showed significantly ( $P<0.05$ ) higher levels than that of sub fertile mares and non-significantly higher than those in estrual and fertile group of mares. The results of present study revealed that the pregnant mares under field conditions had significantly ( $P<0.05$ ) higher serum copper levels than farm pregnant mares. Serum copper levels in all mares under both management conditions were lower than the reference values reported earlier (Inoue *et al.*, 2002) but higher than reported by Abou-Zeina *et al.* (2008). Previously it has been reported that copper-deficient sheep had increased mortality from bacterial infection (Chew, 2000). Pregnant mares under field conditions showed closest values with the reported ones. Copper deficiency in cattle delayed onset of puberty, repeat breeding, low conception, early embryonic mortality and increased incidence of retention of placenta (Nix, 2002). Slight difference in copper levels among different group of mares in our study could be due to variability of digestibility of copper from diet, ranging from 9-48%. Serum zinc levels in mares under farm conditions showed no significant difference between various groups. Estrual mares under field condition had significantly ( $P<0.05$ ) lower serum zinc levels compared with rest of the three groups. Estrus mares under farm conditions had significantly ( $P<0.05$ ) higher serum zinc levels compared with their counterparts under field conditions. Serum zinc levels in all mares under both conditions, except the field mares were higher than reported earlier (Abou-Zeina *et al.*, 2008). Due to the diversity of proteins and enzymes containing Zn, Cu, Mn and Se these trace minerals are essential for a wide variety of physiological processes regulating growth, production, reproduction and health (Tanya, 2009). In the current study mares in all groups under both conditions had adequate serum zinc levels except the sub-fertile mares under farm condition that appeared to be marginally deficient. Only on the basis of these levels it is unfair to declare zinc to be cause of subfertility in these mares.

Serum selenium levels in estrual and fertile groups under farm and field conditions were non significantly different. However, the significant ( $P<0.05$ ) difference was recorded from sub fertile and pregnant mares. In these animals the levels of selenium were significantly reduced. Similar results were also observed in mares kept under field conditions. The mares in estrual and fertile groups had significantly higher serum selenium levels compared with those of sub fertile and pregnant mares. Serum selenium levels in sub fertile and pregnant mares differed significantly ( $P<0.05$ ) from one another. Serum selenium levels in all groups under both management conditions did not indicate deficiency of this element and were higher than reported earlier in horses (Abou-Zeina *et al.*, 2008). The lower values of selenium in mares in present study could be due to the reason of differences in concentrations of selenium in silage pasture and hay given

to these animals. Similar results have been also reported (Muller *et al.*, 2012). The levels estimated in this study are in agreement with those reported (Richardson *et al.*, 2006). Serum iron levels were not significantly different among all the mares kept under field and farms conditions. Similarly the serum iron values were not significantly different in mares at various stages of reproductive cycle, both at farm and field levels. Serum levels in present study were higher than those reported earlier (Biricik *et al.*, 2005; Kavazis, 2002).

**Conclusion:** From the findings of present study it can be concluded that the variations in concentrations of trace minerals are influenced by the reproductive stages of animals and play crucial role in fertility and pregnancy. The lower values could be related to the fodder and soil situations. Further large scale studies involving higher number of animals should be carried out to explain the role of supplementations with trace minerals to achieve the optimum level of fertility in mares.

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