

PLASMA COPPER, IRON AND ZINC CONCENTRATIONS IN COWS WITH CYSTIC OR INACTIVE OVARIES

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ABSTRACT

Efficient reproductive performance is essential for optimal milk production and profitability of the dairy herd, whereas fertility is affected by multiple factors including nutrition. It is known that selenium may be effective on cyclicity and reproductive performance of the dairy cow, however there is also evidence for other trace minerals. A total of 44 dairy cows in the postpartum period, from commercial herds were divided into three groups Group I (n=16): Inactive ovaries: Absence of at least one luteal structure, accompanied with progesterone levels lower than 1 ng/ml.; Group II (n=12): Cystic ovaries: Presence and persistence of cystic structures with a diameter of over 2.5 cm.; Group III: Cyclic cows (n=16): Cyclic cows were selected according to ultrasonographic examination of luteal structure and serum progesterone analysis. Plasma Cu, Zn and Fe concentrations were measured at examinations performed ten days apart. Mean concentrations were calculated and compared between groups. No significant differences were observed in between group analysis and the trace element levels were normal when compared to the reference values. It was concluded that reproductive disorders (cystic ovaries, inactive ovaries) encountered in this observation did not result from a deficiency of the selected trace elements.

INTRODUCTION

The continuity of the reproductive cycle in the modern dairy herd is the key factor to ensure the productivity of the herd, while qualified milk production and sufficient number of replacement dairy heifers can be defined as the main products. To obtain efficient fertility, herdsmen and veterinarians should mainly focus on fertility parameters such as calving to first estrus, calving to conception interval and service per conception rate. However during the periparturient period, beginning from parturition itself which is followed by lactation stress, especially in high milk yield cows, availability and supplementation of nutrients in the feed, their intake and metabolism play a crucial role in the health status of the cow. Primarily adequate intake of energy is important to ensure that the cow will be able to minimize the live weight loss associated with the peak lactation period, also called the negative energy balance. It is documented that unless this is attained decreased fertility will be encountered in postpartum cows with a prolonged calving to conception interval (1, 2, 3, 4). Hidiroglou et al (5) have reviewed the effect of various minerals on reproductive performance in ruminants; reproductive failure may be induced by deficiencies of single or combined trace elements such as copper, cobalt, selenium, manganese, iodine, zinc and iron and by imbalances (5).

Increasing interest is focused on the possible relationship between postpartum fertility and trace element supplementation of dairy cows during the dry period and early lactation (4, 6, 7,

8, 9), and others have examined trace element deficiency and its effects on fertility of dairy cows during this period (10, 11). Selenium and vitamin E deficiency were postulated as one of the significant causes of retained placenta and benefical effects of the supplementation of these compounds have been advocated (10, 11). Other minerals that are thought to effect fertility but less frequently investigated are copper, zinc and iron. Underwood and Suttle (12) stated that dietary copper (Cu) deficiency could cause infertility, anemia or suppressed immune function. Supplementation of zinc in the deficient animals have caused a significant improvement in hormone levels like thyroxine triiodothyronine, oestrogen and progesterone (13). It was observed that marginal or low zinc and copper contents in pasture, soil or animal feed may be responsible for the delayed age of puberty (14). Iron also plays an important role in the immune response in the body (4).

Pathogenesis of infertility in the cow is a multifactorial condition and it is often hard to define the cause. Nutritional factors may be of great importance however their effects are frequently underestimated under field conditions. The aim of the present study is to investigate Cu, Zn and Fe levels in dairy cows with a prolonged postpartum anestrus or with diagnosed cystic ovarian disease.

MATERIALS AND METHODS

Animals and selection criteria

A total of forty-four Holstein Friesian dairy cows from three

different commercial dairy herds were used in this study. The total lactating number of animals in these herds were between 50 and 80 and all three herds had a history of infertility problems, mainly prolonged postpartum anestrus. The animals were fed a mixture of corn silage, cotton seed meal, alfalfa and hay as roughage and a commercial milking cow feed was used as concentrate. No vitamin or mineral supplements were added to the ration. The cows had free access to feed and water all the time. The cows were housed in tie stall barns and bedded with sand, and were milked twice daily at approximately 11 - 13 hours interval. The study was conducted throughout a year with visits arranged according to the requirements of the study.

According to the herd records the cows that had not shown oestrus for at least six months following parturition were selected to determine the cause of anestrus. These animals were examined transrectally twice with a B-mode ultrasonography scanner (Concept M/C) for functional luteal structures, ten days apart. Following each examination two blood samples were collected and plasma and sera were prepared for determination of progesterone concentrations for confirming anoestrus animals. According to ultrasonography and serum progesterone levels, the cows were divided into three groups as follows: Group I (n=16): Inactive ovaries: Absence of at least one luteal structure, accompanied with progesterone levels lower than 1 ng/ml.; Group II (n=12): Cystic ovaries: Presence and persistence of cystic structures with a diameter of over 2.5 cm.; Group III: Cyclic cows (n=16): Presence of at least one luteal structure, together with progesterone levels higher than 1 ng/ml.

Trace element (Cu, Zn, Fe) concentrations were determined in plasma were previously obtained during the ultrasonographic examinations.

Analyses of trace elements

The concentrations of iron (Biomedical Systems®, Barcelona, Spain), copper and zinc (Randox®, Antrim, UK) in plasma were determined using commercial test kits according to the manufacturer's protocol, and carried out with a UV-spectrophotometer (Schimadzu, UV-1601).

Statistical Analysis

Animals were grouped as Group I (n = 16), Group II (n = 12) and Group III (n = 16). The mean of two samplings taken ten days apart were calculated as one value. Inter-group relationships for trace elements were measured by Kruskal - Wallis test.

RESULTS

Mean plasma Cu, Zn, Fe concentrations in Group I (Inactive ovaries) were $254.25 \pm 13.53 \mu\text{g/dL}$, $117.18 \pm 6.19 \mu\text{g/dL}$, $114.85 \pm 6.11 \mu\text{g/dL}$; in Group II (Cystic ovaries) were $245.48 \pm 19.01 \mu\text{g/dL}$, $121.31 \pm 8.83 \mu\text{g/dL}$, $121.49 \pm 6.73 \mu\text{g/dL}$ and in Group III (Cyclic cows) were $239.39 \pm 21.65 \mu\text{g/dL}$, $115.99 \pm 2.79 \mu\text{g/dL}$, $134.88 \pm 8.82 \mu\text{g/dL}$ respectively. Mean plasma concentrations of trace elements in between groups did not show any statistical significance. The results were summarized in Table 1.

Table 1: Mean plasma concentrations of trace elements in all Groups

	Group I (Inactive ovaries)	Group II (Cystic ovaries)	Group III (Cyclic cows)
Cu ($\mu\text{g/dL}$)	254.25 ± 13.53	245.48 ± 19.01	239.39 ± 21.65
Zn ($\mu\text{g/dL}$)	117.18 ± 6.19	121.31 ± 8.83	115.99 ± 2.79
Fe ($\mu\text{g/dL}$)	114.85 ± 6.11	121.49 ± 6.73	134.88 ± 8.82
	(n = 16)	(n = 12)	(n = 16)

DISCUSSION

In the present study there were no significant differences in between groups for any of the parameters. Obtained plasma Cu, Zn and Fe levels were compared with the results of the previous studies and reviews (11, 15) and both Cu (50 – 250 $\mu\text{g/dL}$) and Fe (57 – 162 $\mu\text{g/dL}$) levels were within normal ranges. However plasma zinc levels were deficient in all groups compared to two of the previous findings ($319 \pm 34 \mu\text{g/dL}$) (11, 15) and was in accordance with Meglia et al., (10). They have observed Zn status of cows from the beginning of the dry period until 28 days after parturition and indicated that a cow with a Zn serum level of over 80 $\mu\text{g/dL}$ was adequate.

In a large scale study with dairy and beef herds, Enjalbert et al., (11) investigated the effects of copper, zinc and selenium status on performance and health. They classified the animals for their copper and zinc status in plasma as low, medium or high. It was reported that in cows that had a plasma copper level below 8 $\mu\text{mol/L}$, or as copper deficient, no increased risk of low fertility or other reproductive disorders were observed. It was also stated that in the scope of the previous knowledge about nutritional imbalances affecting fertility, there is poor evidence of subfertility that can be directly attributed to low plasma copper levels. Moreover Enjalbert et al., (11) have added that measurement and evaluation of plasma caeruloplasmin levels accompanied by Cu levels would be more accurate to estimate a condition of excess molybdenum which causes secondary copper deficiency in cows. Zinc is an essential component of numerous enzymes involved in the synthesis of DNA and RNA thus may effect immunity during cell replication and proliferation in addition to its two functions in the antioxidant system (16).

Effects of zinc deficiency in heifers have been reported as disturbed immune functions and zinc supplementation in calves caused enhanced humoral immune responses. Enjalbert et al. (11) observed a significant relationship between diarrhea in calves and metritis and mastitis in dams. Moreover low zinc levels in cows with metritis were also associated with an increased incidence of retained placenta (11). No direct relationship between subfertility and zinc deficiency has been found, however an increased metritis occurrence may cause a delay in fecundation (11).

Zinc deficiency can cause parakeratosis, hyperkeratosis and hoof epidermal deformities (3). Baggott et al. (17) have reported that the harder the keratin of the hoof wall, a greater zinc concentration would be expected than in a hoof wall with a softer keratin content. Gabarino et al. (18) have reported that cows classified as moderately lame showed an increased risk of delayed cyclic activity. Thus it may be possible that, zinc deficiency and infertility may have an indirect relationship, however the results of this study showed no significant difference in plasma zinc concentrations between cyclic cows and cows with delayed postpartum oestrus.

Iron plays an important roles in many biochemical reactions such as anti-oxidant defence system, energy and protein metabolism, as a haem respiratory carrier, oxidation-reduction reactions and in electron transport system (4). Thus it may be expected to have indirect effects on fertility.

There have been a number of studies performed on the relationship between dairy cow health in general including fertility and trace mineral supplementation in which iron was also included with Cu, Mg, Se, Zn, Mo, Co, Mn (2, 9). In a previous study, iron, manganese, copper and zinc were found to be significantly higher in the sera of regular breeders than in repeat breeders (19). However we could not find any studies on the direct relationship between cow fertility and blood iron levels or iron supplementation possibly due to very low frequency of encountering iron deficiency in ruminants (5). Another relationship between trace minerals and fertility can be considered as fetal losses. It is stated that abnormal fetal development may cause structural and metabolic defects. Graham et al. (20) have investigated the relationship between maternal and fetal liver copper, iron, manganese and zinc concentrations and fetal development in dairy cows and they have concluded that although there was a relationship between abortion and decreased Cu, Fe, Mn and Zn in the liver of fetus it is most likely that this fall is a consequence of abortion but not its cause. In the light of the previous findings and our data it can be concluded that a deficiency in blood Fe level is unlikely to indicate an existing subclinical or clinical reproductive disorder.

The effect of iodine on the occurrence of ovarian cysts was postulated by Dzhambazov (21) from the immunological measurements of iodine concentrations in cyst fluids and sera of the cows with ovarian cysts. The formation of the ovarian cysts is a result of iodine deficiency resulting in the hypofunction of the thyroid gland, and hormonal imbalances leading to functional and structural changes in the ovaries as well as to changes in the antigenic composition of the follicles and their walls.

Hidiroglou et al (22) have investigated a manganese deficiency in the blood of dairy cows with cystic ovaries, however they stated that blood Mn levels were no different from those of cycling cows and normal literature values; thus it is unlikely that cows with cystic ovaries were Mn deficient.

Increased risk of retained placenta and prolonged postpartum anestrus as well as cystic ovaries have been reported in dairy cows with selenium deficiency. In this study we were unable to measure selenium levels (23, 24).

We did not review any study investigating the relationship between

cystic ovaries and blood Cu, Zn, Fe levels in dairy cows. In the present study it was observed that in cows with ovarian cysts, plasma trace mineral concentrations were similar to cyclic cows and these levels were in accordance with previous studies. In conclusion our results indicate that postpartum anestrus and cystic ovarian disease most probably are not directly related to plasma Cu, Zn and Fe concentrations of the affected cows, and that further experimental deficiency trials are important for determining the effects of trace mineral deficiency on fertility.

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