INVESTIGATION OF VARIATIONS IN SERUM AND PLASMA ZN AND CU CONCENTRATIONS DURING THE OESTROUS CYCLES IN COWS*

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İneklerde Östrüs Siklusu Boyunca Kan Serumu ve Plazması Çinko (Zn) ve Bakır (Cu) Düzeylerinin Araştırılması

ÖZET

Bu çalışma, ineklerde östrüs siklusu boyunca Zn ve Cu'ın kan serumu ve plazmasındaki değerlerini incelemek amacıyla yapıldı. Ayrıca strüs siklusunun dönemlerine göre plazma ve serum arasında farklılığın olup olmadığı da araştırıldı.

Çalışmada toplam 32 Esmer ırkı inek kullanıldı. İneklerin östrüsleri PGF_2 alfa ile senkronize edilerek rastgele 2 gruba ayrıldı. Birinci grubun kan serumu, 2. grubun kan plazması incelendi. Östrüs günü başlanarak, östrüs siklusu boyunca (ortalama 21 gün), gün aşırı vena jugularisten her defasında 10 cc kan alındı. Zn ve Cu tayini atomik absorpsiyon cihazı, progesteron (P4) ise RIA ile yapıldı.

Çinkonun serum değerlerinin 0.49 ± 0.07 ile 0.68 ± 0.11 mg/L, kan plazmasının ise 0.74 ± 0.08 ile 0.98 ± 0.17 mg/L arasında değişti. Bakır değerleri ise serumda 0.67 ± 0.04 - 0.73 ± 0.05ng/L, plazmadan 0.48 ± 0.03 - 0.54 ±0.02 ng/L) olarak bulundu. Bu sonuçlar plazma Zn seviyelerinin östrüs siklusunun 4 ($P<0.01$), 10 ($P<0.0005$), 16($P<0.005$) ve 18. günlerinde ($P<0.01$) serumdan daha olduğunu gösterdi. Oysa serum Cu seviyeleri tüm günlerde kan plazmasından daha yüksek bulundu. Östrüs siklusu boyunca progesteron ile ilişki sadece Zn ile kan serumunda negatif ve önemli ($P<0.001$), bulundu.

Sonuç olarak; Zn ve Cu eksikliği durumunda hormonların kan seviyelerinin değişmesi olayının tersine olarak; organizmadaki hormonal dengelerin çok değiştiği östrüs siklusu boyunca Zn ve Cu'ın kan serumu ve plazması miktarlarının etkilenmediği kanısına varıldı.

Anahtar Kelimeler: Çinko, bakır, progesteron, östrüs siklusu, inek.

SUMMARY

This study was undertaken to examine serum and plasma Zn and Cu levels during different stages of oestrous cycle in cows. Presence of any differences in plasma and serum concentrations of these trace elements between various stages of the cycles were also investigated. A total of 32 Brown Swiss cows were used. The blood samples of 15 cows were used for serum and of remaining 17 cows for the plasma extraction. All animals were synchronised with PGF_{2a}, and then randomly allocated into two groups. Commencing on the onset of the oestrous cycle, blood samples (10ml) at

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two days intervals were collected from the jugular veins of all cows for 21 days. Zn and Cu concentrations and progesterone (P) levels were determined using atomic absorption unit and RIA, respectively.

Serum Zn levels varied from 0.49 ± 0.07 to 0.68 ± 0.11mg/L and those of plasma from 0.74 ± 0.08 to 0.98 ± 0.17 mg/L. Cu levels were found to be between 0.67 ± 0.04 - 0.73 ± 0.05ng/L in serum and between 0.48 ± 0.03 - 0.54 ±0.02 ng/L in plasma. The results showed that plasma Zn levels were higher than those obtained from the serum on the days 4, 10, 16 and 18 of the oestrous cycle. Whereas serum Cu levels were found to be higher at all sampling intervals than those of plasma. There was a negative correlation between P4 levels and serum Zn concentrations throughout the oestrous cycle. It was concluded that Zn and Cu deficiency resulted in some fluctuations in plasma P4 levels. Unlike the well-documented variations in blood hormone levels, concentrations of Zn and Cu remained unchanged during the oestrous cycle.

Key Words: Zn, Cu, progesterone, oestrous cycle, cows.

INTRODUCTION

Zinc (Zn) is an essential trace element for healthy nourishment and continuation of reproduction functions in domesticated animals. It is found as metal-enzyme in the body and contributes to formations of more than 70 enzymes as either their structural units or activators (9,23). Zn, the second largest trace element after iron in animal organism is densely found in the eye, testicles, liver, pancreas, bone and skin. Sperm contains high amount of Zn (21). Its concentration varies in plasma and serum (20).

In the farm animals normal values of plasma Zn level are between 0.4-0.6 mg/L and zinc deficiency may occur when its levels fall below 0.4 mg/L (14). As previously noted, Zn plays important roles in many enzymatic activities and its deficiency may cause many disorders including retardation of growth rate, decrease in fertility rate, ovulation disorders, embryonic death, functional disorders of the endocrine glands, disruption of oestrous cycle, quite oestrous, anoestrus, abortion, severe haemorrhage at birth, secondary retention in females; testicular atrophy, reduction in spermatogenesis, delayed development of the secondary sexual characters in males, weight loss, inappetite, restlessness, swellings in limbs, allopecia, parakeratosis, dermatitis, degeneration in epithelial tissues, prolonged healing time, failure in defecation, skeletal deformities, muscular dystrophy, hepatosplenomegaly, immune deficiency in both males and females (4, 6, 8, 9,16,17-19, 22, 23).

Metabolism of Zn is closely associated with the presence of Cu, Ca, Mg and Fe in the body. Cu is an essential micro element for the continuation of several physiological and biochemical functions in animal organism. It is also a structural element for many enzymes and several proteins are known to contain Cu. It is mostly found in the liver, spinal cord, skeleton and hair, and mainly stored in the liver. Anaemia, inapetite, disposition to infections, diarrhoea, disruption of general health, irregular pigmentation, keratinisation, miscarriage, degenerative changes in brain tissue, skeletal deformities, decrease in milk production and fertility rate, retardation in growth rate, prolonged oestrous cycles in cows, irregular cycles, suboestrus, anoestrus, dystocia, foetal death and reabsorption are commonly encountered symptoms in the absence of this trace element (4, 10, 12, 15, 16, 22, 23). Furthermore, absence of Cu prevents absorption of Fe which in turn results in anaemia (9).

Several studies have investigated the roles of Zn and Cu in reproductive events due to the close functional relationships between them and metabolism of the endocrine glands (23). Saxena and Gupta (17) have reported that amounts of plasma Zn and Cu levels were

less in quite oestroused or anoestroused heifers than those having a regular oestrous cycle. It has also been shown by the same workers (18) that significant differences in Zn and Cu levels occur between the cows with and without oestrous cycles within 60 days following birth. Madhavan and Iyer (13) gave anoestroused cows and heifers some trace elements including Zn and Cu for 21 days and determined oestrous and pregnancy rates of these animals to increase respectively 80 and 51% within the next 28 days. In addition, plasma Zn concentrations have been examined at different stages of the oestrous cycles in cows (5) and buffalo (2) and different findings have been reported.

This study was undertaken to examine blood serum and plasma Zn and Cu levels during different stages of oestrous cycle in cows. Presence of any differences in plasma and serum concentrations of these trace elements between various stages of the cycles were also investigated.

MATERIALS AND METHOD

This study was undertaken at the Research and Practising farm of Firat University on a total of 32 Brown Swiss cows ageing between 3-5 years. The blood samples of 15 cows were used for serum and of remaining 17 cows for the plasma extraction. All cows were housed under the standard conditions and fed ad libitum with a ration containing hay and milk feed produced in the Elazig Feed Factory.

Following synchronisation of the oestrous cycles with intramuscular application of prostaglandin F_{2a} (PGF_{2a}; Dinolytic 25mg, Eczacıbaşı, İstanbul), the cows were randomly allocated into two groups. Serum and plasma samples were obtained from Group I and Group II, respectively. Commencing on the onset of the oestrous cycle (day 1), blood samples (10ml) at two days intervals were collected from the jugular veins of all cows for 21 days. Heparinized tubes were used for plasma collection and those for serum samples contained no anticoagulant. The blood samples for plasma extraction were immediately centrifuged at 4°C for 20 mins at 3000 RPM and stored at -20°C until assayed. The remaining blood samples were left for coagulation at room temperature for two hours. Their serum content was then transferred to fresh tubes, centrifuged (as above) and kept at 20°C until analysed.

Zn and Cu concentrations were determined using atomic absorption spectrophotometry according to the method of Saxena and Gupta (17). Plasma progesterone (P4) levels were measured by the use of RIA (Coated-A-Count®, Diagnostic Products Corporation, DPC, USA) as previously described (1,11).

Student's t-test and correlation regression were performed on the data using StatView 512+TM package program (7).

RESULTS

Serum and plasma Zn and Cu concentrations, plasma P4 levels and relationships between these parameters are illustrated in Table 1 and Figure 1. The relationship between serum Zn and plasma P4 levels throughout the oestrous cycles is shown in Figure 2.

Zn levels were found to be between 0.49 ± 0.07 and 0.68 ± 0.11mg/L in serum and between 0.74 ± 0.08 and 0.98 ± 0.17 mg/L in plasma. Serum Cu levels varied from 0.67 ± 0.04 to 0.73 ± 0.05ng/L and those of plasma between 0.48 ± 0.03 to 0.54 ±0.02 ng/L. The results showed that plasma Zn levels were higher than those obtained from the serum on the days 4 ($p<0.01$), 10 ($p<0.001$), 16 ($p<0.005$) and 18 ($p<0.01$) of the oestrous cycle (Table 1). Serum Cu levels were found to be higher at all sampling intervals than those of plasma.

There was a negative correlation ($p<0.001$) between P4 levels and serum Zn concentrations throughout the oestrous cycle. No significant association was observed between Zn and Cu concentrations at different stages of the oestrous cycle in cows.

Table 1. Values of plasma and serum Zn, Cu and progesterone levels throughout the oestrous cycles in cows (serum samples n=15, plasma samples n=17). **t :** Student's t-test.

Days of oestrous cycle	Serum Zn (mg/L)	Serum Cu (mg/L)	Plasma Zn (mg/L)	Plasma Cu (mg/L)	SerumProgest (ng/ml)	PlasmaProg. (ng/ml)	Ser/ plasma Zn t	Serum/ plasma Cu t
1	0.68 ± 0.11	0.73 ± 0.05	0.87 ± 0.09	0.53 ± 0.04	0.33 ± 0.09	0.14 ± 0.00		P<0.05
4	0.51 ± 0.05	0.68 ± 0.05	0.81 ± 0.08	0.54 ± 0.02	0.6 ± 0.12	0.10 ± 0.00	P<0.01	P<0.01
6	0.60 ± 0.10	0.68 ± 0.04	0.87 ± 0.09	0.51 ± 0.03	1.09 ± 0.34	0.33 ± 0.08		P<0.01
8	0.56 ± 0.07	0.71 ± 0.03	0.74 ± 0.08	0.49 ± 0.04	2.08 ± 0.43	0.88 ± 0.11		P<0.001
10	0.49 ± 0.05	0.70 ± 0.04	0.87 ± 0.07	0.51 ± 0.03	2.99 ± 0.62	1.75 ± 0.14	P<0.001	P<0.001
12	0.54 ± 0.07	0.67 ± 0.04	0.78 ± 0.07	0.51 ± 0.03	3.71 ± 0.69	2.02 ± 0.16		P<0.001
14	0.49 ± 0.07	0.71 ± 0.05	0.84 ± 0.10	0.53 ± 0.03	3.75 ± 0.72	2.21 ± 0.14		P<0.001
16	0.56 ± 0.09	0.69 ± 0.04	0.92 ± 0.11	0.48 ± 0.03	3.49 ± 0.52	2.20 ± 0.23	P<0.01	P<0.001
18	0.50 ± 0.06	0.70 ± 0.05	0.88 ± 0.06	0.54 ± 0.04	1.95 ± 0.41	1.33 ± 0.29	P<0.01	P<0.001
21	0.59 ± 0.10	0.71 ± 0.03	0.98 ± 0.17	0.50 ± 0.04	0.29 ± 0.06	0.18 ± 0.05		P<0.001

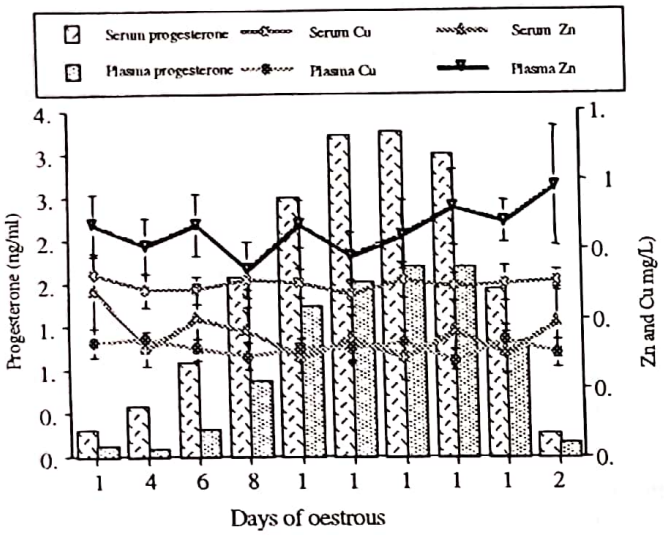

Figure 1 Profiles of plasma and serum Zn and Cu levels and plasma progesterone concentrations throughout the oestrous cycles in cows (serum samples n=15, plasma samples n=17).

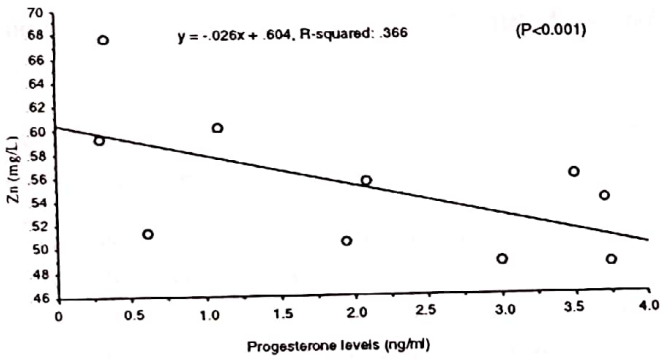

Figure 2. Relationship between serum Zn and plasma progesterone levels throughout the oestrous cycles in cows (serum samples n=15, plasma samples n=17).

DISCUSSION

There are conflicting findings on the serum Zn and Cu levels between different studies. Dufty et al (5) have reported significant variations while Chandolia and Verma (2) found no significant changes in the concentrations of these trace elements at different stages of the oestrous cycle. Serum Cu levels have been shown to be lower around ovulation and on oestrus than those values seen during dioestrus (3). In the present study, no significant variations were observed between the Zn and Cu levels during the oestrous cycle in cows. A significant negative correlation was seen between fluctuating serum P4 and Zn concentrations at various stages of the oestrous cycle.

It has been suggested that concentrations of Zn and Cu vary in plasma and serum (20). Our results confirm these findings since we have also shown that plasma Zn levels were higher than those detected in the serum on the days 4, 10, 16 and 18 of the oestrous cycle. Whereas plasma Cu concentrations were found to be lower at all sampling intervals than those of serum. No significant variations in the plasma Zn levels were seen between the different stages of the oestrous cycle. There was also no significant correlation between the plasma Zn levels and fluctuating P4 profiles.

It has previously been reported that Zn deficiency results in decreases in plasma P4 and oestrogen and an increase in plasma PGF_{2a} levels in rabbits (6). The present results have shown that variations in hormone levels did not seem to significantly affect the Zn and Cu levels. In other words, no converse correlation between P4 levels and Zn and Cu values was observed in our experiments. In a recent study, decreases in plasma Cu and Zn concentrations have been attributed to elevated levels of PGF_{2a} levels in cows and mares (8). If this postulation was the case, similar reductions in Zn and Cu values would be expected on the days 16, 17 and 18 of the oestrous cycle in our study as well. Because, it is known that PGF_{2a} levels increase at around these stages of the oestrous cycle, which plays a pivotal role for luteal regression and hence commencing of a new cycle in nonpregnant cows.

In conclusion, we suggest that both plasma and serum values of Zn and Cu are not affected by the fluctuating hormonal changes at various stages of the oestrous cycle. The present study has also shown that Zn and Cu tend to be present in higher concentrations in plasma and serum, respectively.

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