EVALUATION OF SERUM AND PLASMA Zn AND Cu CONCENTRATIONS WITH RESPECT TO PREGNANCY DURING 21 DAYS FOLLOWING INSEMINATION IN COWS*

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İneklerde Tohumlama Sonrası 21 Gün Boyunca Kan Serumu Ve Plazması Çinko (Zn) Ve Bakır (Cu) Düzeylerinin Gebelik Bakımından Değerlendirilmesi

ÖZET

Bu çalışmada, ineklerde tohumlama sonrası 21 gün boyunca kan serumu ve plazması çinko ve bakır düzeyleri bakımından, erken gebelik döneminde, gebelerle gebe kalamayanlar arasında bir ilişkinin olup olmadığının araştırması amaçlanmıştır. Serum için 5 gebe, 5 gebe olmayan; plazma için de 5 gebe, 5 gebe olmayan olmak üzere toplam esmer ırkı 20 inek kullanıldı. İneklerden, tohumlama günü başlanarak, 21 gün boyunca haftada 3 kez olmak üzere, vena jugularisten her defasında 10 cc kan alındı Çinko ve bakır düzeyi atomik absorpsiyon cihazı ile, progesteron (P4) düzeyi ise RIA ile tayin edildi.

Kan serumu çinko değerlerinin 0.33 ± 0.13 ile 0.75 ± 0.14 mg/L, kan plazması çinko değerlerinini ise 0.72 ± 0.15 ile 1.23 ± 0.17 mg/L arasında değiştiği ve plazmanın, daima serumdan daha yüksek oranda çinko bulundurduğu tespit edildi. Bakır konsantrasyonu da serum ve plazmada sırasıyla 0.64 ± 0.08 - 0.77 ± 0.02, 0.46 ± 0.02 - 0.64 ± 0.02 olarak Zn'nun tersine serumda daha yüksek oranda bulundu. Gebe kalan ve kalamayan hayvanların kan serumu Zn seviyeleri, aynı günler arasında karşılaştırıldığında 4. günler ($P<0.025$) dışında fark önemli bulunmadı. Plazmada ise gebe kalanlarla gebe kalamayanların Zn seviyeleri aynı günler arasında 9 ($P<0.025$), 18 ($P<0.05$) ve 21. ($P<0.005$) günlerde farkın önemli olduğu görüldü. Gebe kalanlarda kan serumu Zn değerleri düzensiz olarak seyrederken plazma Zn değerinde 18 ve 21. günler önemli artışlar tespit edildi. Yirmibir gün boyunca alınan örneklerin toplam Zn değerleri, serumu incelenenlerde gebe kalanlarla kalamayanlar arasındaki fark önemli bulunmazken, kan plazmasında farkın önemli ($P<0.05$) olduğu görüldü. Cu ise sadece plazmada gebe kalanların 1.gününün kalamayanların 4 ve 5.günlerinden önemli derecede ($P<0.05$) yüksek bulundu. Cu serum ve plazma düzeylerinin ise gebe kalanlarla kalamayanlar arasında istatatistiksel anlamda farklı olmadığı tespit edildi. Zn ve Cu arasında da plazma ve serumda ilişki bulunmazken erken gebelik teşhisinde önemli bir kriter kabul edilen, progesteron ile Zn arasında serumda gebe kalamayanlarda, Cu ile plazmada gebe kalanlarda ilişki negatif ve önemli bulundu. Sonuç olarak; Zn gebeliğin ilk döneminde, gebe olmayanların aynı günlerine göre daha yüksek olduğu ve gebeliğin erken safhasında önemli rol oynayabileceği, Cu'ın ise gebeliğin başlangıcında önemli bir değişiklik göstermediği kanaatına varıldı.

Anahtar kelimeler: Zn, Cu, gebelik, serum, plazma ve inek.

SUMMARY

This study was undertaken to examine serum and plasma Zn and Cu levels during 21 days after insemination. Presence of any relationship in plasma and serum concentrations of these trace elements between pregnant and non-pregnant cows was also investigated. The study was conducted on a total of 20 Swiss Brown cows. Equal number of pregnant and non-pregnant cows were used for serum and plasma extraction. Blood samples (10 ml) were collected

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three times a week from the jugular vein during 21 days after insemination. Zn and Cu concentrations and progesterone (P4) levels were determined using atomic absorption unit and RIA, respectively.

Serum Zn levels ranged from 0.33 ± 0.13 to 0.75 ± 0.14 mg/L and plasma Zn levels from 0.72 ± 0.15 to 1.23 ± 0.17 mg/L. The plasma Zn levels were always higher than those seen in the serum. Cu levels were found to be between 0.64 ± 0.08 - 0.77 ± 0.02ng/L in serum and between 0.46 ± 0.02 - 0.64 ±0.02 ng/L in plasma. The serum Cu levels were always higher than those of the plasma. The only significant differences ($p<0.05$) in serum Zn levels were observed on the day 4 between pregnant and non-pregnant cows. The results showed that plasma Zn levels significantly differed between the pregnant and non-pregnant cows on the days 9 ($p<0.05$), 18 ($p<0.05$) and 21 ($p<0.005$). In the pregnant cows, there were fluctuations in the serum Zn concentrations, but increases in those of the plasma on the days 18 and 21. The difference between the total serum Zn values of the blood samples collected during 21 days from pregnant and non-pregnant cows was found to be non-significant, but difference between the total plasma Zn values of the samples collected for the same period from other pregnant and non-pregnant cows was significant ($P<0.05$). Day 1 plasma Cu levels of the pregnant cows were found to be significantly higher than those detected on the days 4 and 5 of the non-pregnant cows ($p<0.05$). Serum and plasma Cu concentrations were not significantly different between the pregnant and non-pregnant cows. There was no correlation between serum and plasma Cu and Zn levels. Negative correlations were found in the serum Zn and P4 levels of the non-pregnant cows and plasma Cu and P4 levels of the pregnant cows.

In conclusion, the findings that plasma Zn levels were higher in early period of the pregnant cows than non-pregnant ones indicates that Zn has an important role in early pregnancy in cows. However, Cu concentrations did not show any significant variations in the early period of pregnancy.

Keywords: Zn, Cu, pregnancy, serum, plasma and cow.

INTRODUCTION

Zinc (Zn) and copper (Cu) are essential trace elements for healthy nourishment and continuation of reproduction functions in domesticated animals. Reduced fertility rate, disruption of ovulation, failure in fertilisation, foetal developmental disorders, embryonic death, dysfunction of endocrine glands, suppression of different stages of the oestrous cycle, suboestrus, anoestrus, dystocia, excessive hemorrhage during parturition, foetal death in rats, reabsorption of foetus and retained placenta are commonly encountered symptoms in the absence of these trace elements (1, 4, 5, 7, 10, 12-14, 16-18, 20, 23- 26, 30, 31).

Although there have been several studies on plasma Zn and Cu levels before and late in pregnancy, few attempts have been made during early pregnancy in cows (7, 22, 28). Dufty et al (8) have shown that Zn levels fluctuate before pregnancy, but remain relatively stable following pregnancy in cows. It has also been reported that Zn and Cu levels are higher in pregnant buffaloes throughout the year than those of non-pregnant animals (22).

Madhavan and Iyer (21) gave anoestrus cows and heifers some trace elements including Zn and Cu for 20 days and determined oestrous (81.25% and 72.5%, respectively) and pregnancy rates of these animals to increase 51% in both groups within the next 28 days. In a previous study, repeat breeder cows were given 5.5 mg/kg $ZnSO_4$ for 21 days and 51% pregnancy rates was recorded (27). In addition, several abortion cases in cows, sheep and mares have been attributed to Zn and Cu deficiency (13, 30). Detection of Zn and Cu levels may provide information in predicting foetal losses (13). Doyle et al (7) have suggested that Zn, Cu and Mn play important roles in fertilisation and embryonal survival.

This study was undertaken to examine serum and plasma Zn and Cu levels during 21 days after insemination in pregnant and non-pregnant cows. Presence of any relationship in plasma and serum concentrations of these trace elements between pregnant and non-pregnant cows was also investigated.

MATERIAL AND METHOD

This study was undertaken at the Research and Practising farm of Firat University on a total of 20 Brown Swiss cows ageing between 3-5 years. The blood samples of 5 pregnant and 5 non-pregnant cows were used for serum and of remaining pregnant (n=5) and non-pregnant (n=5) cows for the plasma extraction. All cows were housed under the standard conditions and fed with a ration containing hay and milk feed produced in the Elazig Feed Factory. Commencing on the day of insemination, blood samples (10ml) were collected from the jugular veins of all cows three times a week for 21 days. Heparinised 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 spectrophotometer according to the method of Saxena and Gupta (26). Plasma P4 levels were measured by the use of RIA (Coated-A-Count®, Diagnostic Products Corporation, DPC, USA) as previously described (2, 19). P4 is an important hormone in determination of pregnancy and various reproductive functions (3). On the day 21, the cows showing P4 levels 1 ng/ml and <1 ng/ml were considered as pregnant and non-pregnant, respectively. These findings were corrected with rectal palpation of the cows at the end of two months.tudent's t-test and correlation regression were performed on the data using StatView 512+TM package program (11).

RESULTS

Serum and plasma Zn and Cu values and their relationships in the pregnant and non-pregnant cows during 21 days following insemination are summarised in Tables 1, 2 and 3. No significant variations were found in serum Zn levels between the pregnant and non-pregnant cows (Table 1). Plasma Zn levels were significantly different between the pregnant and non-pregnant animals ($p<0.05$; Table 2).

The only significant differences ($p<0.05$) in serum Zn levels were observed on the day 4 between pregnant and non-pregnant cows (Figure 1).

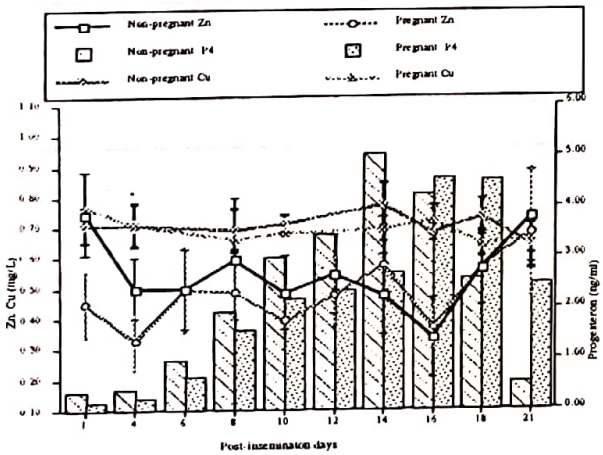

Figure 1. Serum Zn and Cu levels during 21 days following insemination. * : P<0.05, student's t test.

The results showed that plasma Zn levels significantly differed between the pregnant and non-pregnant cows on the days 9 ($p<0.05$), 18 ($p<0.05$) and 21 ($p<0.005$; Figure 2). Correlations between the serum Zn and P4 concentrations and plasma Cu and P4 levels are shown in Figures 3 and 4, respectively.

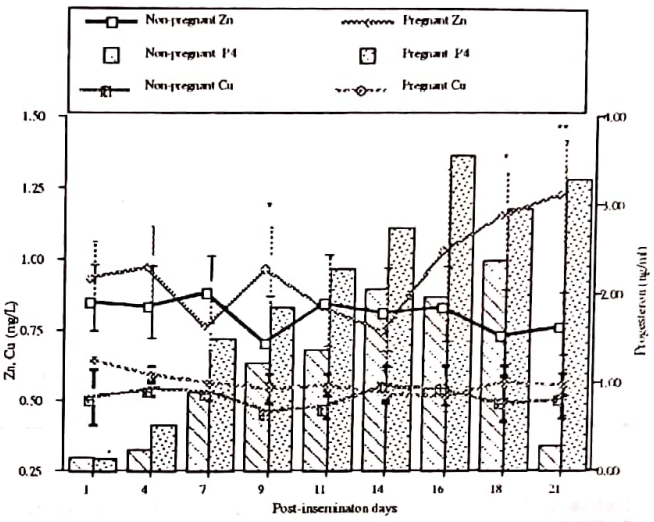

Figure 2. Plasma Zn and Cu levels in the pregnant and non-pregnant cows.
*: P<0.05; ** : P<0.01 using student's t test

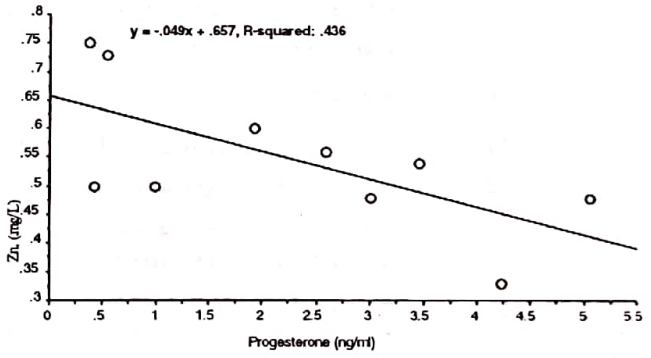

Figure 3. Correlation between serum Zn and P4 levels in the non-pregnant cows.

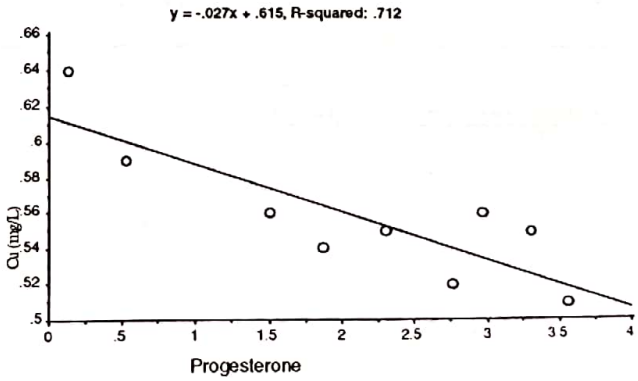

Figure 4. Correlation between plasma Cu and P4 levels in the pregnant cows.

Table 1. Mean ± SEM serum zinc, copper and progesterone levels during 21 days following insemination, (n=5).

Post-insemination days	Serum nonpregnant Zn	Serum nonpregnant Cu	Serum pregnant Zn	Serum pregnant Cu	Serum progesterone nonpregnant	Serum progesterone pregnant
1	0.75 ± 0.14[a]	0.71 ± 0.06	0.45 ± 0.11[a,b]	0.77 ± 0.02	0.37 ± 0.13	0.16 ± 0.04
4	0.50 ± 0.10[b]	0.71 ± 0.07	0.33 ± 0.10[a]	0.71 ± 0.02	0.41 ± 0.14	0.22 ± 0.58
6	0.50 ± 0.13	-	0.50 ± 0.14	-	0.99 ± 0.57	0.66 ± 0.14
8	0.60 ± 0.20[c]	0.69 ± 0.07	0.49 ± 0.09[b]	0.66 ± 0.03	1.93 ± 0.57	1.58 ± 0.45
10	0.48 ± 0.12	0.71 ± 0.03	0.39 ± 0.09[d]	0.68 ± 0.02	3.02 ± 1.24	2.18 ± 0.74
12	0.54 ± 0.13[d]	-	0.48 ± 0.12	-	3.46 ± 0.98	2.37 ± 0.45
14	0.48 ± 0.13	0.77 ± 0.07	0.57 ± 0.25	0.69 ± 0.04	5.05 ± 1.27	2.72 ± 0.64
16	0.33 ± 0.13[e]	0.68 ± 0.03	0.37 ± 0.07	0.72 ± 0.05	4.24 ± 1.29	4.57 ± 1.62
18	0.56 ± 0.12[a-f]	0.73 ± 0.06	0.57 ± 0.16[d]	0.64 ± 0.04	2.59 ± 0.96	4.52 ± 1.13
21	0.73 ± 0.15	0.64 ± 0.08	0.68 ± 0.21	0.66 ± 0.05	0.54 ± 0.12	2.51 ± 0.67
t value	Zn: P>0.05,	Cu: P>0.05				

a, b, c, e, f : P<0.05; d : P<0.005 using student's t test

Table 2. Mean ± SEM plasma zinc, copper and progesterone levels during 21 days following insemination, (n=5).

Post-insemination days	Plasma nonpregnant Zn	Plasma nonpregnant Cu	Plasma pregnant Zn	Plasma pregnant Cu	Plasma progesterone nonpregnant	Plasma progesterone pregnant
1	0.86 ± 0.12	0.51 ± 0.1[a,b]	0.94 ± 0.12[a,b]	0.64 ± 0.02	0.14 ± 0.04	0.13 ± 0.01
4	0.85 ± 0.13	0.54 ± 0.03	0.98 ± 0.13[c]	0.59 ± 0.03	0.24 ± 0.09	0.52 ± 0.22
7	0.89 ± 0.12	0.53 ± 0.03	0.77 ± 0.10[a,d,e]	0.56 ± 0.02	0.89 ± 0.28	1.50 ± 0.37
9	0.72 ± 0.15	0.46 ± 0.02[a]	0.97 ± 0.13	0.54 ± 0.05	1.23 ± 0.34	1.86 ± 0.24
11	0.85 ± 0.16	0.48 ± 0.05[b]	0.84 ± 0.16	0.55 ± 0.04	1.36 ± 0.18	2.29 ± 0.38
14	0.82 ± 0.14	0.56 ± 0.06	0.75 ± 0.15	0.52 ± 0.03	2.06 ± 0.18	2.75 ± 0.61
16	0.84 ± 0.13	0.55 ± 0.07	1.04 ± 0.27	0.51 ± 0.03	1.96 ± 0.17	3.56 ± 0.56
18	0.74 ± 0.15	0.50 ±0.08	1.16 ± 0.14[b,c,d]	0.56 ± 0.06	2.37 ± 0.38	2.96 ± 0.51
21	0.77 ± 0.11	0.51 ± 0.08	1.23 ± 0.17[e]	0.55 ± 0.04	0.27 ± 0.13	3.29 ± 0.65
t value	Zn: P<0.05		Cu: P>0.05			

a, b, c, e : P<0.05; d : P<0.01 using student's t test.

Table 3. Comparison of serum and plasma zinc levels between the pregnant and non-pregnant cows during 21 days following insemination.

Serum			Plasma		
Non-pregnant days	Pregnant days	t value	Non-pregnant days	Pregnant days	t value
1	4	P<0.05	1	21	P<0.05
1	16	P<0.05	4	21	P<0.05
4	4	P<0.05	7	21	P<0.01
12	4	P<0.05	9	1,4,9,18,21	P<0.05
12	10	P<0.05	11	18,21	P<0.05
14	4	P<0.05	14	18	P<0.05
16	1	P<0.05	14	21	P<0.01
18	4	P<0.01	16	18,21	P<0.05
21	1	P<0.05	18	18,21	P<0.05
21	4	P<0.05	21	18	P<0.01
21	16	P<0.05	21	21	P<0.01

DISCUSSION

Ratio and deficiency of Zn and Cu are known to disrupt reproductive functions. Furthermore, it is proposed that they may play important roles in early pregnancy. In the present study, serum Zn levels ranged from 0.33 ± 0.13 to 0.75 ± 0.14 mg/L and plasma Zn levels from 0.72 ± 0.15 to 1.23 ± 0.17 mg/L during 21 days following insemination. The plasma Zn levels were always higher than those seen in the serum. These results appear to support the concept that Zn levels may vary in various tissue and body fluids (29). Cu levels were found to be between 0.64 ± 0.08 - 0.77 ± 0.02ng/L in serum and between 0.46 ± 0.02 - 0.64 ±0.02 ng/L in plasma. In contrast to Zn findings, the serum Cu levels were always higher than those of the plasma.

It has previously been reported that plasma and serum concentrations of Zn and Cu are higher in the pregnant animals than non-pregnant ones (8, 22, 29). In camels, Cu levels (55.6 mg/100 ml) increase to 99.7 mg/100 ml in the beginning of pregnancy (9). However, Bostedt and colleagues (6) have shown that Cu levels are not significantly different between pregnant and non-pregnant animals. In our study, day 1 plasma Cu levels of the pregnant cows were significantly higher than those detected on the days 4 and 5 of the non-pregnant cows. Serum and plasma Cu concentrations were not significantly different between the pregnant and non-pregnant cows. No significant correlation was determined between the serum and plasma Cu and Zn levels in the pregnant and non-pregnant cows. Plasma Cu concentrations were negatively correlated with P4 levels. Negative correlations were also found between the serum Zn and P4 levels of the pregnant and non-pregnant cows. Plasma Zn levels were significantly increased on the days 18 and 21 of the pregnant cows compared to the values seen in the non-pregnant animals throughout the sample collection period. Previously, abortion cases were shown to be associated with low concentrations of Zn (13, 30). These findings appear to support the concept (7, 15) that Zn may play an important role in fertilisation and embryonal survival.

In conclusion, plasma Zn levels were higher in early period of the pregnant cows than non-pregnant ones. Therefore, it is suggested that Zn has an important role in early pregnancy in cows. However, Cu concentrations did not show any significant variations in the early period of pregnancy.

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