

Investigation of Serum Cu, Zn, Fe and Se Concentrations during Pregnancy (60, 100 and 150 Days) and after Parturition (45 Days) in Single and Twin Pregnant Sheep

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Abstract: This study was conducted to determine the serum Cu, Zn, Se and Fe concentrations at 60, 100, and 150 days of pregnancy and at 45 days of parturition in twin and single pregnant sheep. A total of 30 pregnant Akkaraman sheep in 2 groups (15 single pregnancies, 15 twin pregnancies), aged 3-5 years and weighing 40-50 kg were used. The blood samples were analyzed for serum Cu, Zn, Se and Fe concentrations. No statistically significant difference was found for Se, Cu, Fe and Zn concentrations between the twin and single pregnancy groups. Insignificant decreases ($P > 0.05$) in serum Zn and Cu concentrations and significant decreases in serum Fe and Se concentrations were determined in the twin ($P < 0.01$, $P < 0.01$) and single ($P < 0.05$, $P < 0.01$) groups respectively at 60, 100 and 150 days of pregnancy. Except for Cu, the element concentrations increased steadily at 45 days of parturition compared to the pregnancy periods in both groups.

Key Words: Pregnancy, copper, zinc, selenium, iron, sheep, after parturition

Tekli ve İkiz Gebe Koyunlarda, Gebelik Süresince (60., 100., 150. günler) ve Doğum Sonrasında (45. gün) Serum Cu, Zn, Fe ve Se Konsantrasyonlarının Araştırılması

Özet: Bu çalışma, tekli ve ikiz gebe koyunlarda gebeliğin 60, 100 ve 150. günleri ile doğum sonrası 45. gündeki serum bakır, çinko, demir ve selenyum konsantrasyonlarının tespit edilmesi amacıyla yapılmıştır. Çalışmada 3-5 yaşlarında ve 40-50 kg canlı ağırlığında, iki grupta toplam 30 gebe Akkaraman koyunu (15 tekli gebe, 15 ikiz gebe) kullanılmıştır. Kan örnekleri, serum Cu, Zn, Fe ve Se düzeylerini saptamak amacıyla analiz edilmiştir. Tekli ve ikiz gebe grupları arasında serum Cu, Zn, Fe ve Se düzeyleri bakımından istatistiksel olarak önemli bir farklılık bulunmamıştır. Gebeliğin 60, 100 ve 150. günlerinde, serum Zn ve Cu düzeylerinde önemsiz artışlar ($P > 0,05$) ile sırasıyla serum Fe ve Se düzeylerinde ikiz ($P < 0,01$, $P < 0,01$) ve tekli ($P < 0,05$, $P < 0,01$) gebe gruplarında önemli artışlar bulunmuştur. Her iki grupta gebelik dönemlerine göre doğumdan sonraki 45. günde, serum Cu düzeyi dışındaki söz konusu iz elementlerinde hafifçe bir artış saptanmıştır.

Anahtar Sözcükler: Gebelik, bakır, çinko, selenyum, demir, koyun, doğum sonrası

Introduction

Trace elements are needed for vitamin synthesis, hormone production, enzyme activity, collagen formation, tissue synthesis, oxygen transport, energy production, and other physiological processes related to growth, reproduction and health. The importance of an appropriate trace element balance is still underestimated, even though diligent research demonstrates the critical role of trace elements in the fertility of ewes (1). These elements function as activators of enzyme systems or as

constituents of organic compounds and their deficiencies, excesses or malabsorption contribute to or cause several diseases such as cardiac conditions, immune and hormonal dysfunctions, and a host of other maladies (2). Trace element deficiencies have all been shown to have negative effects on reproductive efficiency (3,4). Balakrishnan and Balagopal (5) suggested that a mineral imbalance could be a cause of infertility in repeat breeders. In ruminants, maternal copper deficiency can cause infertility, abortion, and stillbirth (4,6). During

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pregnancy copper deficiency can result in parturition of offspring with congenital disease of the nervous system (4). Animal studies have indicated that all phases of reproduction in the female, from oestrus to parturition and lactation, are affected adversely by zinc deficiency (7). Maternal zinc deficiency also has species-dependent effects on the course of pregnancy and delivery. Premature delivery may be the most likely complication of maternal zinc deficiency in the ewe and guinea pig (3). Supplementation of trace elements (Cu, Mn, Zn, Fe, Co and Se) have improved lambing rates in deficiency situations (8), but only for Se is there strong evidence that embryo survival during implantation is affected (9). Salewski and Seegers (10) reported that selenium supplementation improved insemination results and decreased fertility disturbances. Plasma Fe markedly decrease during late pregnancy and continue to decrease for 3 weeks postpartum (11). The recorded decrease in plasma Fe during late pregnancy could be related to the great demand for this element by the foetus and/or the increase of adrenocortical hormones during late pregnancy might decrease plasma iron (12). The purpose of this study was to determine the serum Cu, Zn, Fe and Se concentrations during pregnancy and at 45 days of parturition in twin and single pregnant sheep.

Materials and Methods

In the present study, a total of 30 apparently healthy pregnant Akkaraman sheep (15 single pregnancies, 15 twin pregnancies), aged 3-5 years and weighing 40-50 kg were used. The experiment was conducted between October and March. Between 5 and 10 days after oestrus, each ewe was treated with 2 intramuscular injections of PGF₂ a (5 mg) (Dinoprost Tromethamine, Dinolytic; Eczacıbaşı İlaç San. ve Tic. A.Ş., Istanbul), 4 hours apart, to synchronize oestrus. Ewes were observed for oestrus every 12 hours, and those exhibiting oestrus were mated with 2 different fertile rams. Both groups of animals were fed a constant diet composed of mineral and vitamin premixes, wheat straw and a concentrate feed consisted of 14% crude protein, 15% fibres and 3% fat to secure intake of nutrients required for maintenance in accordance with NRC (13). About 0.5 kg/animal of concentrate was divided twice a day in addition to 2 kg of wheat straw. Water was available ad libitum in the shed. On day 30, pregnancy and number of foetuses was determined on each ewe by transabdominal

ultrasonography using a B mode ultrasound (Scanner 200 Vet) console fitted with a 5 MHz linear transducer and the ewes were assigned to 2 groups according single or twin pregnancy (14).

Blood samples were collected at 60, 100 and 150 days of pregnancy and at 45 days after parturition by jugular venipuncture. Jugular blood samples were collected in vacuum tubes, Venoject® (Sterile Terumo Europe, Leuven, Belgium), early in the morning before feeding.

The serum was separated by centrifugation (500 g, 10 min) and kept under refrigeration (-20 °C) until needed for analysis. The samples were analyzed for Cu, Zn, Se and Fe concentrations using an Olympus AU 600 model atomic absorption spectrophotometer, following established literature procedures (15).

Statistical differences were determined with t-test and analysis of variance (16).

Results

Blood serum concentrations of Cu, Zn, Fe and Se at 60, 100 and 150 days of pregnancy and single and twin pregnancy of sheep in postpartum day 45 are shown in the Table.

Discussion

In this study, serum Cu, Zn, Fe and Se were maintained within the normal range for all sheep (17,18) and no statistically significant difference was determined between single and twin pregnancy groups at 60, 100 and 150 days of pregnancy or 45 days after parturition.

Serum Cu concentration at 60, 100 and 150 days of pregnancy was insignificantly higher ($P > 0.05$) than that at postpartum day 45 in both groups in this study. In a similar study (19), serum Cu concentration in the pregnancy period was found to be higher than that in the lactation period. The high serum Cu levels in pregnant animals could be related to increased Cu in the form of ceruloplasmin enzyme in response to increased blood oestrogen or progesterone (20,21) or a decrease in serum Cu concentration during lactation because of being thrown out with milk (22) and being stored in the liver before being excreted into milk (23).

Table. Blood serum concentrations of some trace minerals at 60, 100 and 150 days of pregnancy and day 45 postpartum in single and twin pregnancy of sheep (n = 15).

		Pregnancy (day)			Postpartum (day)	F-values
		60 (Mean ± SEM)	100 (Mean ± SEM)	150 (Mean ± SEM)	45 (Mean ± SEM)	
Cu (µg/dl)	Single	135.80 ± 6.13	138.07 ± 5.73	132.46 ± 5.01	128.07 ± 4.70	0.642 ⁻
	Twin	132.67 ± 3.79	135.93 ± 4.03	131.00 ± 3.49	127.80 ± 3.29	0.856 ⁻
	t-test	0.598 ⁻	0.364 ⁻	0.365 ⁻	0.071 ⁻	
Zn (µg/dl)	Single	106.53 ± 6.23	98.07 ± 5.78	94.80 ± 4.63	106.53 ± 5.60	1.146 ⁻
	Twin	102.13 ± 4.88	94.27 ± 5.45	89.53 ± 4.61	103.93 ± 4.58	2.031 ⁻
	t-test	0.817 ⁻	0.541 ⁻	0.974 ⁻	0.471 ⁻	
Fe (µg/dl)	Single	126.67 ± 4.12 ^{ab}	123.33 ± 5.05 ^b	115.26 ± 4.63 ^b	136.13 ± 3.96 ^a	4.142 ⁺
	Twin	124.83 ± 3.62 ^b	120.53 ± 2.58 ^b	108.27 ± 2.14 ^c	134.00 ± 3.18 ^a	6.504 ⁺⁺
	t-test	0.620 ⁻	0.656 ⁻	1.910 ⁻	0.575 ⁻	
Se (ng/ml)	Single	104.47 ± 2.15 ^a	103.07 ± 1.90 ^a	97.47 ± 1.60 ^b	107.00 ± 1.97 ^a	4.417 ⁺⁺
	Twin	105.67 ± 2.32 ^a	104.73 ± 2.65 ^a	96.26 ± 2.02 ^b	107.07 ± 2.05 ^a	4.856 ⁺⁺
	t-test	0.389 ⁻	0.547 ⁻	0.509 ⁻	0.026 ⁻	

⁻ : P > 0.05, ⁺ : P < 0.05, ⁺⁺ : P < 0.01, ⁺⁺⁺ : P < 0.001

^{a,b,c} values with different superscripts within a row varied significantly.

In this study, serum Zn concentration in both groups tended to decrease at 100 and 150 days of pregnancy and the decrease was significant in the twin pregnancy group compared with the single pregnancy group. However, these decreases were insignificant ($P > 0.05$). Zn concentration was insignificantly higher ($P > 0.05$) in both groups at postpartum day 45 than at 100 and 150 days of pregnancy. Mehta and Gangwar (24) related the low Zn level obtained in pregnant animals compared with lactating because of the foetus requirement. Due to the high Zn levels being released during involution of the uterus, a high serum Zn concentration was observed 45 days after parturition (25).

There were statistically significant decreases in serum Fe concentrations in the twin and single pregnancy groups, especially at 100 and 150 days of pregnancy. However, serum Fe concentration was increased significantly 45 days after parturition. The recorded decline in serum Fe during late pregnancy could be related to the great demand for this element by the foetus (12), because the iron concentration in the foetus liver

increases continuously with the advance of pregnancy and reaches high levels in the fifth month in sheep (26). An increase in the number of fetuses stimulates this accumulation (27). Similarly, serum Fe concentration was low in the twin pregnancy group compared with the single pregnancy group in this study.

There were statistically significant decreases in serum Se concentrations, especially at 150 days of pregnancy, in both groups. This decline could be related to the demand for Se by the foetus in late pregnancy (28). Similarly in another study (29), serum Se concentration was found to decrease with the advance of pregnancy in sheep. Hamliri et al. (30) reported that pregnant ewes deficient in vitamin E and/or selenium may increase the incidence of stillborn progeny, or weak lambs, which only survive for a few days before dying because of acute heart failure. However, the decreases in serum Se concentrations at 150 days of pregnancy in both groups of animals were maintained within the normal range, and so these symptoms were not observed for the lambs in this study.

The results of the present study show that, staying within the normal range, serum Se, Cu, Fe and Zn concentrations decreased gradually at 60, 100 and 150 days of pregnancy and except for Cu, the element concentrations increased steadily at 45 days of

parturition. No statistically significant difference was determined between the single and twin pregnancy groups but serum concentrations of Se, Cu, Fe and Zn in the twin pregnancy group were slightly lower than those in the single pregnancy group.

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