Kafkas Univ Vet Fak Derg
22 (5): 679-683, 2016
DOI: 10.9775/kvfd.2016.15103

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Serum Iron Status and Its Relation with Haematological Indexes Before and After Parturition in Sheep

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Article Code: KVFD-2016-15103 Received: 25.01.2016 Accepted: 22.04.2016 Published Online: 22.04.2016

Abstract

Erythrocyte indices, total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) values provide important information for the diagnosis of anaemia in iron deficiency conditions and inflammatory diseases. The aim of this study was to evaluate the serum iron status and its relation with haematological indexes before and after parturition in sheep. Blood samples were collected from pregnant (n=30) and non-pregnant aged-matching Ivesi sheep (n=30), housed under the same conditions, at 1 week (wk) (±2 days) before expected parturition, in parturition (baseline), at day 1 and at 1, 2, 3, 4, 6, 8 and 12th wk subsequent to the parturition, to determine the serum levels of iron (SI), TIBC and UIBC as well as the haematological parameters. Erythrocyte (RBC) count, haematocrit (Hct) and haemoglobin (Hb) values dropped at 12 wk (P<0.05) according to 1 wk (±2 days) before expected parturition and during parturition. Mean corpuscular volume concentrations were also found lower (P<0.05) at 4-12 wk after parturition, in comparison with their baselines. After the parturition, SI concentration decreased at day 1 (P<0.05) and then remained low (P<0.05) during the study. TIBC was higher for the first 2 wk, but was lower at 6-12 wk compared with its baseline (P<0.05). UIBC values were in parallel to TIBC values after the parturition. In conclusion; negative effects on leukocytes of metabolic stress after delivery should be taken into consideration, as it can trigger the potential problems during this period. In postpartum period, SI levels along with erythrocyte indices can be useful parameters to follow the iron loss by fetus and colostrum. Incompatible results of TIBC and UIBC levels with SI and erythrocyte indices in this study showed that TIBC and UIBC may not be useful biomarkers to evaluate serum iron values, particularly in postpartum period.

Keywords: *Iron, Binding Capacity, Unsaturated, Erythrocyte Indices, Sheep*

Koyunlarda Doğum Öncesi ve Sonrası Serum Demir Durumu ve Hematolojik Endekslerle İlişkisi

Özet

Eritrosit belirteçleri, total demir bağlama kapasitesi (TIBC) ve demir bağlama kapasitesi (UIBC) değerleri demir eksikliği durumları ve inflamatuar hastalıklarda, aneminin tanısı için önemli bilgiler sağlar. Bu çalışmanın amacı, doğum öncesi ve sonrası koyunlarda serum demir durumu ve hematolojik endekslerle ilişkisini değerlendirmektir. Serum demir seviyesi (SI), TIBC, UIBC ve hematolojik parametreleri belirlemek üzere kan örnekleri aynı yaş ve barındırılma koşullarındaki gebe (n=30) ve gebe olmayan (n=30) İvesi koyunlarından doğumdan tahmini 1 hafta (±2 gün) önce, doğum zamanı ve doğumdan 1 gün sonra ve 1, 2, 3, 4, 6, 8 ve 12. haftalarda toplandı. Eritrosit (RBC), hematokrit (Hct) değer ve hemoglobin (Hb) seviyeleri postpartum dönemin 12. haftasına doğru (P<0.05) doğumdan 1 hafta (±2 gün) önceki ve doğum sırasındaki değerlerine oranla daha düşüktü. Ayrıca doğum sonrası 4-12. haftalardaki ortalama eritrosit hacmi konsantrasyonları da doğum sırasındaki değerlerine oranla düşük bulundu (P<0.05). Doğum sonrası SI seviyesi 1. günde düşmüş (P<0.05) ve çalışma boyunca da düşmeye devam etmiştir (P<0.05). TIBC doğum sonrası ilk 2 hafta boyunca yüksek iken, doğum anındaki değere kıyasla 6-12. haftalarda daha düşüktü (P<0.05). UIBC değerleri doğumdan sonraki TIBC değerlerine paralel seyretmiştir. Sonuç olarak, potansiyel problemleri tetikleyebileceği için, doğum sonrası metabolik stresin lökositler üzerindeki negatif etkisi bu periyodda dikkate alınmalıdır. Postpartum dönemde eritrosit indeksleri ile birlikte serum demir düzeyinin belirlenmesi fetüs ve kolosturum aracılığı ile meydana gelen demir kaybının takip edilebilmesi açısından faydalı parametrelerdir. Bu çalışmada saptanan TIBC ve UIBC düzeyleri ile SI ve eritrosit indeksleri arasındaki uyumsuzluk, TIBC ve UIBC'nin özellikle postpartum dönemde SI düzeylerinin değerlendirilmesinde yararlı biyobelirteçler olmayabileceğini göstermiştir.

Anahtar sözcükler: *Demir, Bağlama Kapasitesi, Doymamış, Eritrosit Belirteçleri, Koyun*

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INTRODUCTION

Iron is an essential constituent of the haem portion of haemoglobin (Hb), and as the Hb in aged RBCs is broken down and fresh Hb synthesized, so the iron in the body is continually re-cycled. Its transport within the body is in the plasma, attached to a beta-1 globulin known as transferrin [1].

Iron derived from the degradation of Hb by the mono-nuclear phagocyte system (MPS) may be stored in the MPS (liver, spleen and bone marrow) in the form of ferritin or hemosiderin [2]. However, most of the iron passes into the plasma, becomes bound to transferrin and is placed within newly formed RBCs and released into the circulation [1].

The total iron binding capacity (TIBC) is a measure of the total serum transferrin concentration. TIBC is the amount of iron that serum transferrin can bind when all iron - binding sites are saturated. The concentration of the iron standard minus the unbound iron is called the unbound iron-binding capacity (UIBC), which is used to calculate the TIBC (UIBC plus total serum iron concentration). TIBC is increased in iron deficiency in humans, horses, cattle, and pigs, but does not appear to be increased in clinical iron deficiency in dogs [2,3]. In a study by [4], TIBC levels in sheep exposed to dietary limitation were higher than that of control group. Also, TIBC decreased or in the low - normal range in inflammatory diseases [2].

Iron is required for Hb synthesis. Both true and functional iron deficiency result in deficient Hb synthesis. Because Hb concentration signals cessation of cell division, iron deficient cells undergo one or two more cell divisions resulting in formation of microcytes [2]. Both mean corpuscular volume (MCV) and mean corpuscular Hb concentration (MCHC) tend to be decreased in iron deficiency [5], but in some cases MCHC may be normal when the MCV is mildly decreased [2]. Although it can be artefactual, microcytosis in routine practice is considered as a hallmark of iron deficiency, usually associated with hyposideremia, while it can also occur in other pathophysiological conditions [6]. To authors' best knowledge, there is no data yet regarding iron status and its related parameters, TIBC and UIBC, in a controlled randomized long-term study with healthy pregnant and non-pregnant sheep. Thus, the aim of this study was to evaluate serum levels of iron, TIBC and UIBC, as well as to compare between erythrocyte indices and iron status before and after parturition in sheep.

MATERIAL and METHODS

Admission of Animals

This study was conducted in the town of Nilufer in Bursa, Turkey. The herd consisted of totally 150 Ivesi sheep. In different compartments of the same pen 85 sheep, 65 goats, all animals were fed with alfalfa, as forage wheat bran, sunflower meal and soybean meal, as well as a commercial mixture for lambs as concentrate which consists of ground corn, barley, calcium carbonate, and vitamin premix. All sheep had free access to water. Blood sampling and treatments were performed under the control of the farm health coordinator in accordance with the Animal Welfare Guidelines [7]. The study was approved by Committee of the Animal Experimentation of Uludag University (1.06.2004/2).

Collection of Samples

Animals were divided into two groups: pregnant (n=30) (during second breeding season) and non-pregnant (n=30) (four months after second parturition), 2-3 years old Ivesi sheep. All animals were housed under the same conditions. Synchronization was not applied and sheep were mated by ram. Pregnancy examinations were performed by conducting an ultrasound of the abdominal area, and rectally during the 3rd month post-mating. According to the examination results, clinically healthy pregnant sheep were included to the study. All ewes were let to graze on pasture during periods when seasonal conditions were appropriate. When the ewes returned to the sheep corral, appropriate forage and concentrated feed were provided after the nutritive value of the pasture was taken into consideration. Routine health controls and deworming were applied to all animals after mating.

Blood samples were collected at 1 week (wk) (±2 days) before expected parturition, at parturition (baseline), day 1, and 1, 2, 3, 4, 6, 8 and 12th wk subsequent to the parturition. Blood was sampled one time from non-pregnant group. Samples were collected from all sheep in the study between Fabruary and May. The heparinized blood (Vacusera 4 mL, lithium heparin tube, Turkey) obtained through jugular vena puncture was centrifuged to separate the plasma and stored at -20°C until biochemical analysis. Additionally, blood for haematological evaluation was collected into 2 mL vacutainer tube with EDTA (Hema&Tube®, Turkey) from the same vein and analysed within 2 h. The haematological parameters (complete cell counts with 5-parts leucocyte differentials) were analysed using an automatic analyser (Cell-Dyne 3500®, Abott Inc., USA), and white blood cell (WBC) count, red blood cell (RBC) count, Hct, Hb and MCV were recorded for this study.

Serum iron (SI), UIBC and TIBC concentrations were measured on Architect ci8200 System (Abbott Laboratories, IL, USA) according to the manufacturer's instructions.

Statistical Analysis

Data between non-pregnant and pregnant sheep (before parturition) was analysed by student t test. Data obtained 1 wk before parturition, during parturition (baseline), 1 day after parturition and weekly until 12 wk was analysed by one-way analysis of variance (SigmaStat 3.1, GmbH, Germany). Tukey test was used as post-hoc test. Results were expressed as mean ± standard deviation (SD), and a P value less than 0.05 was considered as statistically significant.

RESULTS

A statistical significance was detected for WBC between week 2 and baseline, day 1 and week 8 (P<0.05). Erythrocyte indices and SI, TIBC and UIBC in non-pregnant and in sheep 1 week before (± 2 days) parturition, and during parturition (baseline) and post-partum periods are shown in *Table 1* and *Table 2* respectively. In non-pregnant and pre-partum sheep, there were statistically significant differences in RBC, Hct, Hb and MCV (P<0.05). In post-partum period, RBC, Hct, Hb levels in baseline levels were higher than values period after week 3 (P<0.05). MCV, TIBC and UIBC values in day 1 were different from values in period after week 6. There were also statistically differences between baseline for UIBC value (P<0.05). For serum iron, there were statistically differences (P<0.05) in all the time (except week 1 and week 3) compared with baseline.

Table 1. *Erythrocyte indices, serum iron (SI), TIBC and UIBC results in non-pregnant and in sheep 1 week before parturition*

Tablo 1. *Gebe olmayan ve doğumdan bir hafta önceki koyunlarda eritrosit belirteçleri, serum demir, TIBC ve UIBC sonuçları*

Parameters	Non-pregnant (n=30)	1 Week (± 2 days) Before Parturition (n=30)
WBC ($x10^{9}$/L)	8.4±3.3^{a}	8.6±3.7^{a}
RBC (10^{12}/L)	7.1±8.1^{a}	7.6±1.1^{b}
Hct (%)	35.2±0.4^{a}	38.5±0.5^{b}
Hb (g/L)	12±0.1^{a}	13±0.^{b}
MCV (fl)	31±0.3^{a}	36±0.3^{b}
Iron (µg/dL)	187.2±15.4^{a}	183.1±10.1^{a}
TIBC (µg/dL)	477.8±13.6^{a}	466.3±11.7^{a}
UIBC (µg/dL)	265.7±26.3^{a}	317.3±17.7^{a}

^{a,b} Differences between the values involving different letters in the same row were found to be statistically significant at P<0.05

DISCUSSION

WBC counts may increase in post parturient period due to various causes, such as ketosis, retentio secundinarum, infectious disorders, mastitis, metritis and the combination of disorders [8,9]. Increasing numbers of leucocytes toward the time of delivering [10,11] were also found in the sheep studied herein, which were with normal clinical findings. In the present study, baseline WBC counts markedly increased in 2 weeks after parturition. None of pregnant sheep examined showed any clinical manifestations during the observation periods. Reproductive examinations of sheep were normal during after parturition. New born lambs were also apparently healthy, grew and were weaned normally. Thus, observed increases in WBC count in the study might be the result of the metabolic stress [10-12] associated with parturition and lactation.

RBC indices are the most important parameters in the evaluation of iron deficiency anaemia and total iron status. Our results were consistent with those found in a study which was conducted on Tsigain sheep with varying physiological states, such as healthy non-pregnant, pregnant, and lactating, and determined that Hb values were within physiological ranges in all 3 groups, while showing a decrease in the postpartum period as compared to the prepartum period [13]. On the contrary, no any statistically significant difference between prepartum and postpartum RBC indices were observed in another study [14]. However, in the same study [14], the finding of higher RBC and Hct values in the postpartum period as compared to the prepartum period. On the other hand, in another study, the authors reported elevated postpartum Hb and Hct values as compared to the prepartum period [15]. In this study, postpartum RBC indices (RBC, Hct, Hb, and MCV) were found to be lower than levels in 1 wk (±2 days) before expected parturition. Hemodilution during prepartum period can cause decrease in Hct and RBC volume [16-18]. However, this condition may continue within a week after parturition [18]. After this period, during a decrease in hemodilution, an increase in Hct and RBC count can

Table 2. *Erythrocyte indices, serum iron (SI), TIBC and UIBC results in sheep during parturition and postpartum periods*

Tablo 2. *Doğum sırasında ve postpartum dönemdeki koyunlarda eritrosit belirteçleri, serum demir, TIBC ve UIBC sonuçları*

Parameters	Baseline (n=30)	Day 1 (n=30)	Week 1 (n=30)	Week2 (n=30)	Week 3 (n=30)	Week 4 (n=30)	Week 6 (n=30)	Week 8 (n=30)	Week 12 (n=30)
WBC ($x10^{9}$/L)	8.1±3.6^{b}	8.1±7.6^{b}	8.6±6.8^{a,b}	12.5±7.4^{a,b}	9.2±3.4^{a}	9±4.0^{a,b}	8.6±3.3^{a,b}	8.3±3.5^{b}	9.3±4.2^{a,b}
RBC (10^{12}/L)	7.6±1.1^{a}	7.3±1.06^{a,b}	7.2±1.5^{a,b}	7.4±1.5$^{a, b}$	7.1±1.0^{b}	7.0±1.1^{b}	6.9±1.1^{b}	6.7±1.2^{b}	6.6±1.3^{b}
Hct (%)	38.5±0.5^{a}	36.4±0.4^{a,b}	34.4±0.5^{b}	36.1±0.6$^{a, b}$	35.2±0.5^{b}	34.5±0.6^{b}	34.3±0.5^{b}	31.6±0.8^{b}	30.4±0.7^{b}
Hb (g/L)	12.8±0.2^{a}	12.2±0.1^{a,b}	12±0.2^{a,b}	12.2±0.2$^{a, b}$	11.8±0.2^{b}	11.6±0.2^{b}	11.5±0.2^{b}	10.7±0.2^{b}	10.2±0.2^{b}
MCV (fl)	36±0.3^{a,b}	36±0.4^{a}	35±0.5^{a,b}	36±0.4$^{a, b}$	35±0.3^{a,b}	34±0.3$^{a, b}$	34±0.2^{b}	34±0.3^{b}	33±0.3^{b}
Iron (µg/dL)	183.1±10.1^{a}	122.7±17.1^{b}	137.9±6.1^{a,b}	120.4±15.2^{b}	138.4±7.4	125.2±7.7^{b}	133.8±8.0^{b}	126.4±13.2^{b}	118.4±8,5^{b}
TIBC (µg/dL)	466.3±11.7^{a,b}	532.8±15.9^{a}	532.7±20.3^{a,b}	520.0±10.6^{a,b}	478.4±15.8^{a,b}	476.8±18.1^{a,b}	430.2±10.1^{b}	410.3±11.0^{b}	389.7±13.6^{b}
UIBC (µg/dL)	317.3±17.7^{b}	412.5±30.2^{a}	398.4±23.2^{a,b}	403.6±14.9^{a,b}	339.1±18.7^{a,b}	351.5 ±15.3^{a,b}	308.7±13.1^{b}	264.9±13.4^{b}	280.7±25.1^{b}

^{a,b} Differences between the values involving different letters in the same row were found to be statistically significant at P<0.05

be detected. In the present study, while the presence of a decrease in RBC and Hct levels due to hemodilution within one week was observed, a slight increase in these values were detected in second week. However, this rise is expected to continue in the other coming weeks [18,19], a decrease was observed in the present study. In case of the decreases in these parameters were within reference intervals, it was thought that decreases in erythrocytes and Hct in lactation period can be caused by lactation stress which can cause increases in osmotic fragility [20].

In studies conducted in cows and buffaloes, were reported to have lower serum iron levels in postpartum according to prepartum period [21,22]. Miltenburg et al.[21] conducted a study on the level of plasma iron in dams and calves and found that during parturition and early in postpartum period dams had lower plasma iron level than prepartum period in contrast with the calves' plasma iron levels. One of the recent study indicated that the low level of iron in postpartum period of cattle can be hardly increased by mineral injection including iron chloride [23]. In the present study, postpartum serum iron levels between 1-12 weeks were lower than those in non-pregnant sheep and those in 1 wk before parturition. This study revealed decreased iron levels in sheep after parturition as seen in cattle and buffaloes [21-23]. Miltenburg et al.[21] suggested that the use of dam's iron stores by fetus at delivery was associated with these low iron levels in dams. Also, in the same study, it was noted that calving and expansion of colostrum intake can lower the plasma iron level in dams. In our study, the tendency of SI to decrease was parallel to the tendency of RBC indices to fall. Moreover, several complications such as; hemorrhage during parturition in sheep with high body score and acute/chronic infections, which were not observed in our study, might also lead to reduced SI status. However, the cause of fluctuations in serum iron levels was not fully understood, their levels are known to be affected with the amount of iron taken together with last food intake before sampling [24].

In the present study, TIBC value did not differ statistically between non-pregnant and pregnant sheep. Rising TIBC levels within the first 2 weeks after delivery may be associated with elevated transferrin level. Although transferrin levels were not evaluated in the study, transferrin, secreted as an acute phase reactant from the liver, is known to rise in the presence of acute inflammation and 1 week after parturition [25,26]. In a study, performed on Anatolian Black Goats in Siirt Province, Turkey, it was found that serum transferrin levels in blood samples taken 1 week after delivery were statistically significantly higher than those measured before delivery [26], which was a result consistent with our explanations. As compared to the baseline values, TIBC started to drop 2 weeks after the delivery and continued to decrease until the end of the study, indicating the physiological variation in SI and TIBC in the postpartum period. The factors behind this response may be reduced iron level in the ration along with decreased levels of serum transferrin and its tissue receptors. Serum transferrin value is an important parameter that affects the TIBC level [2]. Similarly, in a study, conducted on healthy pregnant sheep, was observed physiological variation between prepartum and 2-week postpartum TIBC levels [2].

In the present study, UIBC was 265.7±26.3 μg/dL in non-pregnant animals and 317.3±17.7 μg/dL in sheep 1 wk before parturition. In a study on healthy Awassi sheep and those with iron deficiency anaemia, UIBC value was found 149±5 μg/dL, 175±7 μg/dL and 154±9 μg/dL in the non-pregnant sheep, and in sheep in prepartum and postpartum periods, respectively [27]. In another study, the mean prepartum UIBC value was 157.2±5.3 μg/dL in Akkaraman sheep [28]. In the present study, the UIBC values were higher than those found in both of the above mentioned studies [27,28], which may be associated with variation between the sheep, or factors influencing the transferrin and iron levels.

In conclusion; although data detected from the study were within the reference ranges, negative effects on leukocytes of metabolic stress after delivery should be taken into consideration, as it can trigger the potential problems during this period. In postpartum period, serum iron levels along with erythrocyte indices can be useful parameters to follow the iron loss by fetus and colostrum. Incompatible results of TIBC and UIBC levels with serum iron and erythrocyte indices in this study showed that TIBC and UIBC may not be useful biomarkers to evaluate serum iron values, because particularly in postpartum period, they can be effected by factors such as involution, inflammation and malnutrition.

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