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IGF-I in grazing dairy ewes during late pregnancy and lactation and its correlation to biochemical markers of bone metabolism

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ABSTRACT

We investigated the influence of late pregnancy and lactation on bone metabolism in grazing dairy sheep, by measuring concentrations of minerals and bone markers in blood serum. The research was performed on ten cross-breed ewes (Istrian × East-Friesian). The concentrations of total Ca, inorganic P, total Mg, the activity of total alkaline phosphatase (AP) and bone alkaline phosphatase (BAP), concentrations of carboxyterminal crosslinked telopeptide of type I collagen (CTx), insulin-like growth factor - I (IGF-I) and 17- β estradiol (E2) were determined in the blood serum. The results showed significantly lower Ca concentrations in blood during lactation and P concentrations in the period of late lactation. Late lactation was characterized by the lowest CTx concentration and higher BAP activity. The obtained results are related to a decrease in the degree of bone resorption and an increase in the deposition of minerals in the bone tissue during late lactation. The increased concentration of CTx, with the simultaneous decrease in BAP activity in the period of late pregnancy and early lactation indicate a high magnitude of bone resorption. The concentration of IGF-I in the blood serum of the ewes is positively correlated with the activities of the total AP and BAP and the total Ca and Mg, which could be related to the activity of IGF-I on the osteoblasts and the release of minerals from the bones. The results of the present study show a relationship between concentrations of IGF-I and bone remodelling markers in the blood serum of dairy ewes, and their mutual correlation with changes in estradiol concentration.

Key words: IGF-I, bone biochemical markers, dairy ewes, pregnancy, lactation

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Introduction

Pregnancy and lactation cause an increase in mineral requirements, which must be partially compensated by elevated bone resorption (LIESEGANG et al., 2000). The mechanisms of adaptation of bone remodelling to pregnancy and lactation have not been completely investigated, but they include activity of the parathyroid hormone (PTH), 1.25 $(OH)_2$ D3 vitamin (calcitriol) and parathyroid hormone-related peptide (PTHrP). These processes are influenced by substantial changes of the systemic concentration of hormones that usually occur during pregnancy and lactation, especially estrogens, prolactin and placental lactogen (HORST et al., 2005; KOVACS, 2005) and also by the somatotropic axis (UELAND, 2005; BAYLINK et al., 2007). Insulin-like growth factor (IGF-I) is an important member of the somatotropic axis that influences the growth and maturation of the skeleton. In adults, IGF-I has an important role in the maintenance of bone mass and influences bone remodelling (UELAND, 2005; BAYLINK et al., 2007; GIUSTINA et al., 2008). There are two sources of IGF-I in bone tissue: 1) IGF-I from the systemic circulation, mostly from the liver, and 2) IGF-I that is synthesised in the bone tissue, under the influence of growth hormone (GH) and PTH (GIUSTINA et al., 2008). The insulin-like growth factor takes part in the coupling of osteoclasts with osteoblasts (UELAND, 2005). Receptors for IGF-I are found on osteoblasts and osteoclasts (CENTRELLA et al., 1990; FIORELLI et al., 1996). In osteoblasts, synthesis of IGF-I is mostly regulated by PTH (McCARTHY et al., 1990), but it is also induced by estrogens (SPELSBERG et al., 1999) and thyroid hormones (LAKATOS et al., 1993), but inhibited by glucocorticoids (DELANY et al., 2001). It is believed that the main effects of IGF-I in bone tissue are mediated by the stimulated activity of mature osteoblasts: it is known that IGF-I inhibits the apoptosis of osteoblasts and their final differentiation in osteocytes (GIUSTINA et al., 2008). Insulin-like growth factor indirectly stimulates osteoclastogenesis, through its influence on the balance of the RANK/RANKL/OPG system (RUBIN et al., 2002; GORNY et al., 2004), increased IL6 production in osteoblasts (SLOOTWEG et al., 1992) and, also, through its influence on the migration of preosteoblasts through the endothelial wall (FORMIGLI et al., 1997).

The extent of bone remodelling may be monitored indirectly by measuring bone biochemical markers in the blood and urine (DELMAS, 1995). Carboxyterminal crosslinked telopeptide of type I collagen (CTx) is one of the products of degradation of type I collagen (which is the main component of the organic part of the bone matrix). CTx is a very commonly used marker of bone resorption in humans (DELMAS, 1995; ROSEN et al., 2000). Bone-specific alkaline phosphatase (BAP) is an enzyme from osteoblasts, and measurement of its activity in blood serum/plasma is used as a marker of bone synthesis (DELMAS, 1995). Besides in human medicine, those markers have been recently used in studies of bone metabolism in domestic animals (LIESEGANG et al., 2000; LEPAGE et al., 2001; HOLTENIUS and EKELUND, 2005; SEEBECK et al., 2005; LIESEGANG et al., 2006 and 2007). Most of the studies on bone metabolism in dairy ewes have been performed on ewes kept indoors, but recent studies pointed to the significance of different factors such as altitude, pasture type, and sheep breed on bone metabolism in grazing lambs (WILLEMS et al., 2013) and lactating ewes (KOHLER et al., 2013).

There have been many studies of the relationship between IGF-I and energetic status in ewes during pregnancy (GLUCKMAN and BARRY, 1988; BRAMELD et al., 2000; McMULLEN et al., 2005; OLIVER et al., 2005). Nevertheless, to the best of our knowledge, the physiological changes in serum IGF-I concentration in ewes during late pregnancy and lactation have not been described, especially in relation to bone remodelling. Therefore, the aim of the present study was to investigate the dynamics of bone remodelling and the regulation of mineral metabolism in grazing dairy ewes, by measuring the concentrations/activities of the biochemical markers of bone remodelling, hormones and minerals in blood serum. In addition, the aim was to establish the possible existence of a relationship between bone metabolism and serum concentrations of IGF-I in ewes, similar to that observed in women during pregnancy and breastfeeding.

Materials and methods

Animals and blood sampling. The research was conducted on crossbreed dairy ewes (Istrian × East-Friesian; n = 10) randomly selected from a herd of dairy sheep, which consisted of 682 dairy sheep. All sheep were in their third lactation, at an average 3.5 years old. Between 8:00 to 11:00 hours and 18:00 to 21:00 hours the ewes were grazing in separate paddocks. After returning to the barn, the ewes were given concentrate and alfalfa hay. During the night the ewes were kept in the barn on deep litter. The amount of pasture consumed was determined on the basis of the difference in weight before and after leaving the pasture, and in addition by the prediction model as described by AVONDO et al. (2002). Pasture samples were taken every month (ten replications, 1 m^2). All feed samples were analyzed according to AOAC procedures (ANONYMOUS, 1999) after grinding to the size of 1 mm. Detergent fibre were analyzed according to the methods described by ROBERTSON and VAN SOEST (1981) and VAN SOEST et al. (1991), with α amylase (Sigma-Aldrich, Inc., USA) added during the NDV extraction. Sodium sulphite was not used. The ration was composed to meet or exceed NRC recommendations (ANONYMOUS, 1985). The chemical composition of the concentrate, alfalfa hay and pasture is shown in Table 1. Fresh water was available to all animals ad libitum. The amount of minerals in feed has been defined by ANONYMUS (1985) and is presented in Table 2. During the experiment, the mineral composition of the concentrate was periodically adjusted according to the predicted feed intake. The amount of milk produced by ewes before weaning was measured as described by BENSON et al. (1999), and after weaning by using recording jars in the milking parlour. The quantity of milk was determined weekly and the samples were taken for chemical analysis of milk. The concentration of protein, fat and lactose in the sheeps' milk was determined using infrared spectroscopy (FT Milcoscan 120, Foss, Denmark). The urea values were determined by the enzymatic colorimetric method using commercial kits of reagents (Herbos Dijagnostika doo, Sisak, Croatia).

Table 1. Chemical composition of concentrate, alfalfa hay and pasture (dry matter)

Composition	Concentrate	Alfalfa hay hay	Pasture
Dry matter (%)	88.1	89.0	13.0
Ash (%)	7.5	7.2	7.0
Ether extract (%)	3.0	2.3	3.8
Crude protein (%)	18.3	15.9	9.1
Neutral detergent fiber (%)	16.9	52.3	59.0
Acid detergent fiber (%)	7.1	38.6	36.0

Table 2. Daily mineral intake

Mineral	Late pregnancy	Lactation
Calcium (g/day)	10.04	14.73
Phosphorus (g/day)	5.23	8.73
Magnesium (g/day)	3.08	4.83
DCAB (mEq/kgDM)	253.90	250.84

Samples for blood analyses were taken in five experimental periods: 10 days before lambing, and on the 10^{th}, 20^{th}, 90^{th} and 130^{th} days after lambing (+10, +20, +90 and +130, respectively). All procedures with animals were approved by the Ethics Committee of the Faculty of Veterinary Medicine and the Ministry of Agriculture, Forestry and Water Management of the Republic of Croatia (Class UP/I 322-01/10-01/85, No 525-06-1-0255/10-3). Blood was taken from the v. jugularis externa and collected in plain BD Vacutainer® tubes (BD Diagnostics, Plymouth, UK). Samples were centrifuged at 2000 g for 20 minutes at 4 °C. Aliquot samples were stored at -80 °C (for determination of BAP) or at -20 °C for determination of the other parameters, until analysed.

Reagents and analysis procedures. The concentrations of Ca, Pi and Mg were determined by using the automatic analyzer (SABA 18, AMS, Rome, Italy) and commercial kits. The concentration of Ca and Mg was determined by the complexometric method (Herbos Dijagnostika, Sisak, Croatia). The concentration of Pi was determined by the colorimetric method (Randox, UK).

The concentration of Carboxyterminal crosslinked telopeptide of type I collagen (CTx) was determined by using the commercial ELISA immunoassay Serum CrossLaps® Elisa (Nordic Bioscience Diagnostics, Denmark). Crossreactivity for ovine CTx was established by the manufacturer. The declared Intra Assay coefficient of variability (CV) was <6%, and Inter Assay CV was <9%.

The activity of bone-specific alkaline phosphatase (BAP) was determined using the commercial kit Metra BAP EIA Kit (Quidel Corporation, San Diego, USA), as described earlier for use in sheep (MacLEAY et al., 2004; LIESEGANG and RISTELI, 2005). The sensitivity of the assay is 0.7 U/L. The intra-assay coefficient of variation was 3.5%. The declared Intra Assay CV was <6%, and Inter Assay CV <8%.

The concentration of Insulin-like growth factor (IGF-I) was determined using the commercial kit IGF-I equine ELISA (DRG Instruments GmbH, Marburg, Germany). The sensitivity of the test was 4.9 ng/mL. Crossreactivity for IGF-II was <0.01%, for insulin <0.1 %, and for growth hormone <0.1%. The declared Intra Assay CV was <9.8%, and Inter Assay CV <13.8%. Mean Intra Assay CV in our experiment was 5.55%.

The concentration of 17-β estradiol was determined by using the commercial kit 17beta-Estradiol ELISA (IBL, Hamburg, Germany). The sensitivity of the test was 9.714 pg/mL. Crossreactivity for other steroid-hormones was less than 0.1%. The declared Intra Assay CV was <7%, and Inter Assay CV <10%.

Statistical analyses. The obtained data were analyzed using the software package Statistica 9. (StatSoft, Inc., Tulsa, SAD). The Kolmogorov - Smirnov - test of normality, ANOVA (Repeated measures) and post hoc Tukey HSD test, and calculation of Pearson´s correlation coefficient were used for analysis. Differences and correlations on the level $P<0.05$ were considered statistically significant.

Results

Average milk yield and composition are presented in Table 3. Concentrations/activities of the investigated parameters are presented in Fig. 1 (A-G). Correlations between the investigated parameters are presented in Table 4. Concentrations of IGF-I in the blood serum of the investigated ewes were the highest 10 days before lambing, while the lowest values were recorded on the 10th day of lactation (Fig. 1A). Concentrations of serum IGF-I positively correlated with the activity of total- and bone-alkaline phosphatase and also with concentrations of total serum Ca and Mg (Table 4). A weak negative correlation was observed between the concentrations of IGF-I and E2 (Table 4).

Table 3. Milk yield and composition from dairy ewes during the study

Parameters	Day in lactation			
	10	20	90	130
Milk (g/day)	848 ± 169	1197 ± 185	1107 ± 33	925 ± 239
DM (%)	14.71 ± 2.10	15.25 ± 1.22	15.06 ± 0.52	15.95 ± 1.37
Non-fat DM (%)	8.99 ± 0.83	9.02 ± 0.57	10.20 ± 0.27	10.21 ± 0.58
Milk fat (%)	5.72 ± 1.69	6.23 ± 1.11	4.86 ± 0.50	5.74 ± 1.01
Milk protein (%)	4.30 ± 0.31	4.14 ± 0.29	5.46 ± 0.17	5.54 ± 0.30
Lactose (%)	4.69 ± 0.58	4.88 ± 0.34	4.73 ± 0.22	4.67 ± 0.33

Table 4. Linear correlation between bone biochemical markers, minerals and hormones in blood serum of ewes during late pregnancy and lactation.

	CTx (ng/mL)	BAP (U/L)	Ca (mmol/L)	P (mmol/L)	Mg (mmol/L)	E2 (pg/mL)
IGF-I (ng/mL)	-0.093	0.273*	0.508*	-0.136	0.448*	-0.338*
CTx (ng/mL)		-0.427*	0.457*	0.557*	0.278*	0.214
BAP (U/L)			-0.305*	-0.430*	-0.086	-0.295*
Ca (mmol/L)				0.345*	0.764*	0.217
P (mmol/L)					0.173	0.023
Mg (mmol/L)						0.314*

*correlations are significantly different (P<0.05).

The mean values of calcium concentrations in the serum of the investigated ewes Ca significantly decreased in the period of early lactation and remained low throughout lactation, with an additional decrease on Day 90 of lactation (Fig. 1B). A similar trend was observed for the concentrations of phosphates in the serum of the investigated ewes. The concentration of total Mg in the blood serum of ewes was significantly higher during late pregnancy, in comparison with all investigated periods during lactation (Fig. 1D). A small increase was observed on the 20^{th} day of lactation, with a decrease on the 90^{th} day of lactation. A significant correlation between concentrations of serum Ca and Mg was observed during the present study, as well as a correlation between serum concentrations of Mg and CTx (Table 4).

The concentration of CTx in the blood serum of the investigated ewes was high during late pregnancy and it decreased in late lactation (Fig. 1E). A significant increase in the activity of BAP was observed during late lactation in the ewes (Fig. 1F). The extent of bone resorption and synthesis were inversely proportional during lactation in ewes.

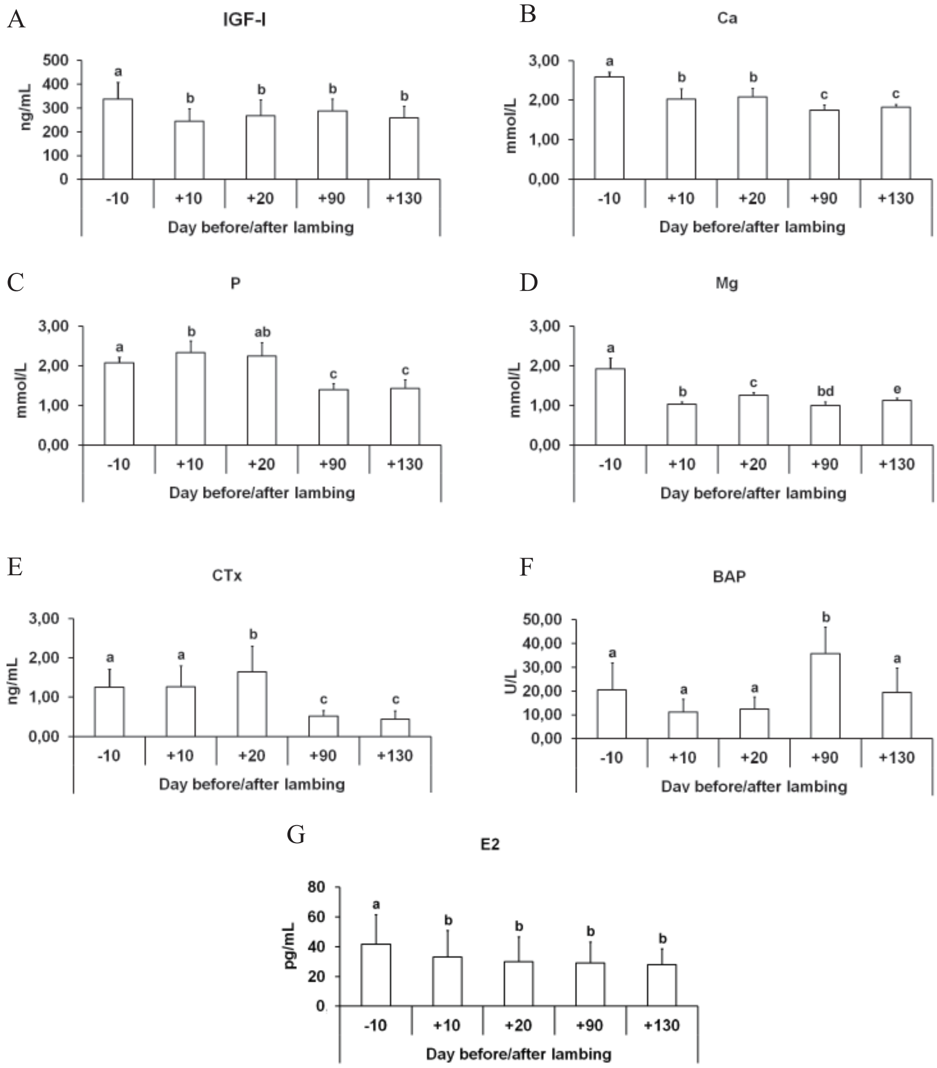

Fig. 1. Means (□) and standard deviations (I) for serum; A) insulin-like growth factor I (IGF-I); B) Ca; C) P; D) Mg; E) carboxyterminal crosslinked telopeptide of type I collagen (CTx); F) bone-specific alkaline phosphatise (BAP); and G) estradiol 17β (E2) values in dairy ewes during late pregnancy and lactation. Values with different superscripts are significantly different ($P<0.05$).

E2 concentrations in the blood serum of the investigated ewes decreased after lambing (Fig. 1G). A significant negative correlation was observed between the concentration of E2 on the one hand, and IGF-I and BAP activity on the other (Table 4).

Discussion

In the present study, serum concentrations of IGF-I during pregnancy and lactation in grazing dairy ewes were investigated, along with their relation to the biomarkers of bone metabolism. The highest concentrations of IGF-I were found 10 days before lambing, and there was a significant decrease on day 10 of lactation. Most of the plasma IGF-I originates from the liver (YAKAR et al., 1999), and its systemic secretion is dependent on many factors, such as the nutritive and metabolic status of the subject (VICINI et al., 1991), the estrus cycle, reproductive status and lactation (ROBERTS et al., 1997; COHICK, 1998; ĐURIČIĆ et al., 2011). Therefore, the decrease in IGF-I concentrations observed at the beginning of lactation could be mostly related to the negative energetic balance and the decrease in plasma estrogens. Considering the substantial presence of IGF-I in bone tissue and its role in regulation of bone remodeling, the observed correlation between concentrations of IGF-I and the activity of BAP is in accordance with the previously described role of IGF-I in coupling osteoclasts with osteoblasts (UELAND, 2005; GIUSTINA et al., 2008). Namely, the main effects of IGF-I in bone tissue are directed at stimulating the activity of mature osteoblasts, and one of their functions is the synthesis and secretion of BAP (GIUSTINA et al., 2008). It has been assumed that IGF-I inhibits the apoptosis of osteoblasts and their final differentiation to osteocytes (GIUSTINA et al., 2008). Hence, IGF-I could, indirectly through an increase in the number of mature osteoblasts, contribute to the increased secretion of BAP. During the present study on ewes, we did not observe any correlation between concentrations of IGF-I and the marker of bone resorption - CTx, although we expected it according to the previously explained role of IGF-I in the stimulation of osteoclastogenesis, secretion of proresorptive cytokines in osteoblasts and recruitment of preosteoclasts (SLOOTWEG et al., 1992; FORMIGLI et al., 1997; UELAND, 2005). We also expected the increased release of IGF-I into the blood stream from the bone matrix during the observed extensive bone resorption. Those discrepancies could be explained by the prevailing influence of other factors on concentrations of IGF-I in plasma. Similar to the present results in ewes, it has been observed in women that there is a relationship between calcium metabolism and circulatory concentrations of IGF-I during lactation (O'BRIEN et al., 2006).

The mean values of calcium concentrations in the serum of the investigated ewes during late pregnancy were within the referral values for adult ewes (2.88-3.20 mmol/L; KANEKO et al., 1997). Values for Ca significantly decreased in the period of early lactation and remained low throughout lactation (Fig. 1B). These findings could be explained by the substantial loss of calcium through milk. Although we observed adaptation to substantial loss of calcium by increased bone resorption in ewes (Fig. 1E), it is obvious that this adaptation is not sufficient, and concentrations of calcium in the ewes' serum remain at a lower level. The concentrations of phosphates in the serum of the investigated ewes during late pregnancy and early lactation were within the referral range for adult ewes (1.62-2.36 mmol/L; KANEKO et al., 1997) (Fig. 1C). Values for phosphates significantly decreased and remained lower than referral values on day 90 and 130 of lactation. The similar courses of the changes in the concentration of phosphates and CTx, and also their mutual correlation lead to the conclusion that the decrease in phosphate concentration, which was observed during late lactation, is connected with the lower extent of bone resorption and, consequently, the decreased release of phosphates from the bones. Similar to the present investigation, higher serum phosphate concentrations during early lactation were observed in mares (FILIPOVIĆ et al., 2010) and cows (STOJEVIĆ et al., 2004). Increases in the concentration of serum phosphates, observed in female rats and women during lactation, are assumed to be due to elevated bone resorption and lower excretion of phosphates by the kidneys (KOVACS, 2005). Similar to the present investigation in ewes, the highest phosphate concentrations in the serum of mares (FILIPOVIĆ et al., 2010) corresponded with the highest concentrations of bone resorption markers. Calcium and phosphorus share common regulatory mechanisms. They are connected in the skeleton as calcium-phosphate. During the mobilization of calcium, phosphorus is simultaneously released into the blood (ROSOL and CAPEN, 1997; EKELUND, 2003). The significant correlation between the concentrations of these two minerals in the serum of ewes observed in the present investigation is in agreement with the above.

The concentration of Mg was within the referral values for ewes throughout (0,90-0,31 mmol/L; according to KANEKO et al., 1997). The concentration of total Mg in the blood serum of ewes was the highest during late pregnancy (Fig. 1D), and a decrease of serum Mg concentration was observed up to the 90th day of lactation. Similar to our results in ewes, ROMO et al. (1991) observed an increase of Mg concentration in the serum of cows, from the sixth day before calving, with the highest concentration of Mg on the day of calving, and a steady decrease to the 8th and 9th days of lactation. The correlation between serum concentrations of Mg and CTx points to a connection between Mg concentration in the blood serum and its release from the skeleton, along with the release of Ca (Table 4).

Changes in the concentration of CTx in the blood serum of the investigated ewes point to the high extent of bone resorption during late pregnancy that decreases in late lactation (Fig. 1E). This is in agreement with the data about the increase in bone resorption markers, established at the beginning of lactation in dairy cows, ewes and goats (LIESEGANG et al., 2000; LIESEGANG et al., 2006; LIESEGANG et al., 2007; HOLTENIUS and EKELUND, 2005; STOJEVIĆ et al., 2007; STOJEVIĆ et al., 2009; FILIPOVIĆ et al., 2008).

During the present investigation, a significant increase in the activity of BAP, an indicator of bone synthesis, was observed during late lactation in ewes (Fig. 1F). Similarly, in women, an increase in the serum concentrations of bone synthesis markers was observed during lactation, in comparison with pregnancy (KOVACS, 2005). Similar data also exist for dairy ewes and goats (LIESEGANG et al., 2006, LIESEGANG et al., 2007). Significant changes in the activity of BAP were not established during late pregnancy and early lactation in the blood serum of dairy cows (TAINTURIER et al., 1984; FILIPOVIC et al., 2008; STOJEVIĆ et al., 2009). However, by measuring other indicators, the most intensive synthesis of bone tissue in cows was established from mid-lactation to the end of lactation (EKELUND, 2003). This was in accordance with the present investigation in ewes. The extent of bone resorption and synthesis were inversely proportional during lactation in ewes.

As we expected, E2 concentrations in the blood serum of the investigated ewes decreased after lambing (Fig. 1G). A significant negative correlation was observed between the concentration of E2 on the one hand, and IGF-I and BAP activity on the other (Table 4). Similarly, in our study on mares during late pregnancy and early lactation, we also observed a negative correlation between concentrations of E2 and BAP (FILIPOVIĆ et al., 2010). This is in agreement with the effects of estrogens on the decreased dynamics of bone remodeling (MANOLAGAS et al., 2002). In light of the fact that estrogens inhibit self-renewal of preosteoblasts (MANOLAGAS et al., 2002), it may be concluded that high concentrations of E2 in blood of ewes during late pregnancy may cause a decrease in the number of osteoblasts, which may be a possible origin of the observed negative correlation between E2 and BAP in the blood serum.

The observed changes in concentrations/activities of bone remodeling markers are in agreement with the data from the literature for East-Friesian ewes kept indoors, but, in the present study, the extent of bone resorption decreased earlier during lactation. This is probably related to the shorter lactation and earlier decrease in milk yield, connected with nutritional management and breed characteristics. The observed influence of lactation on bone resorption was similar to data from the literature for dairy cows. During the dry-period, the extent of bone resorption was high in ewes, pointing to the relatively high need for minerals during late pregnancy in ewes.

Conclusions

The results of the present study show a relationship between concentrations of IGF-I and bone remodeling markers in the blood serum of dairy ewes, and their mutual correlation with changes in estradiol concentration. In addition, the results confirm the high extent of mobilization of minerals from the bones during late pregnancy and early lactation in grazing dairy ewes.

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SAŽETAK

Istražen je učinak kasne gravidnosti i laktacije na koštani metabolizam mliječnih ovaca mjerenjem koncentracije minerala i biokemijskih pokazatelja koštane pregradnje. Istraživanje je provedeno na deset križanih ovaca (istarska × istočnofrizijska). Mjerene su koncentracije ukupnog kalcija, anorganskog fosfora, ukupnog magnezija, c-terminalnog telopeptida kolagena tipa I (CTx), inzulinu sličnog faktora rasta - I (IGF-I) i 17-β estradiola (E2) te aktivnost ukupne (AP) i koštano specifične alkalne fosfataze (BAP) u krvnom serumu. Rezultati su pokazali značajno niže koncentracije kalcija u krvnom serumu tijekom laktacije i fosfora tijekom kasne laktacije. Kasna laktacija pokazala je najnižu vrijednost CTx i višu aktivnost BAP. Dobiveni rezultati upućuju na smanjenje opsega koštane razgradnje i povišeno odlaganje minerala u koštano tkivo tijekom kasne laktacije. Viša koncentracija CTx, uz istovremeno smanjenje aktivnosti BAP u serumu tijekom kasne gravidnosti i rane laktacije upućuju na visoki opseg razgradnje koštanog tkiva. Koncentracija IGF-I u krvnom je serumu bila u pozitivnoj korelaciji s aktivnošću AP, BAP, te koncentracijama ukupnog kalcija i magnezija, što može biti povezano s učinkom IGF-I na osteoblaste i otpuštanje minerala iz kostiju. Dobiveni rezultati pokazali su povezanost između koncentracije IGF-I i biomarkera koštane pregradnje u krvnome serumu mliječnih ovaca, kao i njihovu zajedničku korelaciju s koncentracijama estradiola.

Ključne riječi: IGF-I, koštani biokemijski biljezi, mliječne ovce, gravidnost, laktacija