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Current energy and lipid metabolism biomarkers in sheep with subclinical and clinical pregnancy toxemia*

MERVE ÖZTÜRK[1], NURI MAMAK[2]

[1]Department of Internal Diseases, Faculty of Veterinary Medicine, Bingol University, Bingol 12000, Turkey
[2]Department of Internal Diseases, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur 15030, Turkey

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Öztürk M., Mamak N.

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Summary

The aim of this study was to evaluate the relationship between existing metabolic biomarkers (asprosin, FABP1, PPARα and FGF21) and clinical parameters and to determine their role in the early diagnosis of sheep pregnancy toxemia. In the study, 70 Akkaraman sheep aged three to five years in the last three weeks of pregnancy were divided into three groups: healthy group (n = 20), subclinical group (n = 30), and clinical group (n = 20). Clinical examination (body temperature, respiratory rate, and heart rate), blood serum biochemistry, and ELISA analyses were performed. In sheep with clinical and subclinical pregnancy toxemia, serum PPARα, β-HBA, NEFA, HbA1c, HDL, triglyceride, creatinine, and phosphorus levels were statistically higher, whereas serum glucose and LDL levels were lower than in healthy sheep. However, serum FGF21, AST, ALT, and VLDL values were not statistically different between the three groups. In addition, serum asprosin and FABP1 levels were higher in the subclinical group than in the clinical group. Thus it was concluded that serum asprosin, FABP1, and PPARα findings could be useful in evaluating lipid and energy metabolism in subclinical and clinical forms of pregnancy toxemia. Since this study was based on blood samples from individually reared sheep herds, many environmental factors (e.g., housing, nutrition, and population density) could not be considered. For this reason, it is thought that there is a need for experimental studies in which environmental variables can be controlled.

Keywords: FGF21, FABP1, PPARα, asprosin, pregnancy toxemia

Pregnancy toxemia is a metabolic disease that develops in the last weeks of pregnancy or in the first weeks of the lactation period in sheep (34). Although it is associated with multiple fetuses, it can also be seen in single pregnancies because of climate change and nutritional deficiencies. Studies on pregnancy toxemia deal with pathogenesis, clinical findings, current diagnosis, and treatment methods (5). It is known that in pregnancy toxemia, the quick and accurate planning of diagnostic protocols increases the success of treatment and the chances of avoiding diseases. β-hydroxybutyric acid (β-HBA) is generally considered as the gold standard for diagnosis, since there is an increase in ketone bodies in pregnancy toxemia (2). Glucose levels often drop because of the negative energy balance that occurs towards the end of the pregnancy. However, hyperglycemia may also occur, which is associated with fetus death or increased cortisol levels (6, 8). Therefore, especially in the early diagnosis of metabolic diseases and herd treatment, current metabolic diagnostic protocols are of great importance (21, 37).

The peroxisome proliferator-activated receptor α (PPARα), which is a nuclear receptor activated by fatty acids, is regarded as an important metabolic marker. It is needed in the process of adaptation to hunger and plays a role in the regulation of ketogenesis (20). PPARα is a key biomarker in cases such as hunger, where hypoglycemia is dominant, and in the adaptation of the pancreatic islets. The protective effects of PPARα on pancreatic functions are evident especially

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in obesity and pregnancy. PPARα activation during pregnancy is important for the regulation of insulin secretion and provides protection against negative effects of fatty acids on the pancreatic islets (13). The control of lipid metabolism and energy homeostasis is mediated by FGF21 (fibroblast growth factor 21) together with PPARα during a fasting period (14). FGF21 plays a fundamental role in metabolic changes associated with ketogenesis and is regarded as an important biomarker of lipid oxidation in animals fed a ketogenic diet (7). It acts as an important regulator of energy homeostasis, glucose, and lipid metabolism, as well as insulin sensitivity (19).

Asprosin is a glycogenic hormone that is one of the factors that stimulate the functional role of the liver during hunger. To start glucose release by binding to hepatocyte surfaces, asprosin passes through the blood-brain barrier, and then stimulates orexigenic agouti-related neuropeptides (AgRP) through the cyclic adenosine monophosphate-dependent pathway (10). In the case of hunger, asprosin initiates the functional role of the liver and regulates normal neural functions between daily meals (11).

It was reported that FABP1 (fatty acid-binding protein 1) helps to identify changes in fatty acid metabolism, sheds light on several metabolic disorders, including pancreatic beta cell damage, dyslipidemia, hepatic steatosis, and metabolic syndrome, and plays a role in the intake, transport, activation, oxidation, and synthesis of fatty acids, as well as in the pathogenesis of dysfunctions, such as esterification and ketosis (36).

Current biomarkers are needed for the early diagnosis of pregnancy toxemia and to reveal the metabolic mechanism. This study investigated the usefulness of the serum levels of PPARα, FGF21, FABP1 and asprosin in the early diagnosis of lipid and energy metabolism disorders in sheep with clinical and subclinical pregnancy toxemia.

Material and methods

Sheep selection and management. In this study, Akkaraman sheep aged 3-5 years were examined in the last 2-3 weeks of pregnancy. A total of 500 sheep suspected to have pregnancy toxemia were screened. The sheep selected from 50 different herds, with 10 sheep from each one herd. The clinical ketosis, subclinical ketosis, and control groups were formed on the basis of the β-HBA levels measured with rapid test kits in blood samples from pregnant sheep showing clinical symptoms of pregnancy toxemia or poor general health. The animal material consisted of 70 sheep divided into the clinical ketosis group (n = 20), the subclinical ketosis group (n = 30), and the healthy (control) group (n = 20). The clinical ketosis group included sheep with high blood plasma ketone levels (β-HBA 3 mmol/L or higher), the subclinical ketosis group included sheep with medium blood plasma ketone levels (β-HBA 0.8-3 mmol/L), and the control group included sheep with low blood plasma ketone levels (β-HBA 0.8 mmol/L or lower).

Blood samples. The blood collected from the vena jugularis was transferred to serum gel tubes. For biochemical analyses, the samples were left in the serum gel tubes for 30 minutes and then centrifuged at 3000 rpm for 10 minutes. The sera obtained were transferred to Eppendorf tubes and stored at –20°C.

Biochemical analysis. Serum triglyceride, LDL, HDL, VLDL, AST, ALT, ALP, GGT, creatinine, phosphorus, NEFA, and Hb1Ac parameters were measured in an auto-analyzer (Randox RX Monaco, UK). Serum β-HBA and glucose parameters were measured with rapid test kits (Precision Xceed for Blood Glucose and Ketone, Abbott/Abingdon, UK) in blood plasma after blood samples were collected from the vena jugularis (31). The levels of serum asprosin (Sheep Asprosin ELISA kit, MBS7265859, USA), FABP1 (Sheep Fatty Acid-Binding Protein ELISA kit, MBS751763, USA), FGF21 (Sheep Fibroblast Growth Factor-21 ELISA kit, MBS747499, USA), and PPARα (Sheep Peroxisome Proliferator-Activated Receptor Alpha ELISA kit, MBS749650, USA) were measured with an ELISA reader (BioTek ELISA, USA) according to instructions of the kit manufacturer MyBio Source.

Statistical analysis. The tests applied to the data were determined as parametric or non-parametric according to the results of normality analyses. The inter-group comparisons of normally distributed data were carried out using one-way analysis of variance (ANOVA) (Post Hoc Multiple Comparison: Bonferroni test). The inter-group comparisons of non-normally distributed data were carried out using the Kruskal-Wallis test (Post Hoc Mann-Whitney U test for pairwise comparisons). Spearman's correlation analysis was conducted to analyze relationships between the values measured. In the interpretation of the results, the level of statistical significance was set at $p < 0.05$, and the analyses were carried out using the SPSS 22.0 program (for Windows).

Results and discussion

Clinical examination. The body temperature, heart rate, and respiratory rate of the animals from the three groups included in the study are given in Table 1. Body

Tab. 1. Clinical examination by group: clinical, subclinical, and control

Parameters		Clinical group (n = 20)	Subclinical group (n = 30)	Control group (n = 20)	p Value
Body temperature (°C)	median (min/max)*	39.00^{a} (38.50/39.40)	39.00^{a} (38.40/39.30)	38.35^{b} (37.80/38.70)	0.000
Heart rate (/min.)	mean ± SE**	79.65 ± 1.05^{a}	78.36 ± 0.69^{a}	75.00 ± 0.85^{b}	0.002
Respiration rate (/min.)	mean ± SE**	27.10 ± 0.36^{a}	25.30 ± 0.29^{b}	23.60 ± 0.43^{c}	0.000

Explanations: a, b, c – indicates that there is a statistically significant difference between values in the same row ($p < 0.05$); a – indicates that there is no statistically significant difference between values with the same letters; *shows the median (minimum/maximum) value; **shows the value of data as the mean ± standard error

temperatures and respiratory rates were significantly higher ($p < 0.000$) in the clinical group than they were in the control group. There was no statistically significant difference in the heart rate between the clinical and subclinical groups, but the heart rate was higher ($p < 0.02$) in these groups compared to the control group. In addition, the animals in the clinical group showed non-specific clinical signs, such as inattentiveness to their environment, unwillingness to remain standing, lower feed consumption, and teeth grinding. On the other hand, the results of lymph node, lung, and heart examinations were within normal ranges in all groups.

Blood metabolites. Serum β-HBA, NEFA, LDH, triglycerides, GGT, creatinine, and phosphorus values were higher in the clinical group than in the subclinical and healthy groups. On the other hand, serum glucose, ALP, and HDL values were higher in the healthy group than in the clinical and subclinical groups. AST, ALT, and VLDL values did not differ statistically between the groups. In addition, the serum HbA1c value was higher in the subclinical group than in the clinical group (Tab. 2).

Because they remain relatively constant in plasma and serum, β-HBA levels are regarded as the gold standard in testing for pregnancy toxemia (22). In this study, according to the clinical examinations and observations of sheep with β-HBA values higher than 0.8 mmol/L, their energy intake was insufficient, and they were in danger of ketosis at values higher than 3 mmol/L. Sheep with β-HBA values higher than 3 mmol/L showed clinical signs, such as teeth grinding, unwillingness to remain standing, severely reduced feed consumption, and premature labor. Similar clinical findings were reported by Rodolfo et al. (27). In their study, clinical signs such as insufficient energy intake, grinding of teeth with β-HBA values over 3 mmol/L, the inability to stand, a severe decrease in feed consumption, and premature birth were detected by clinical examinations and observations when β-HBA values exceeded 0.8 mmol/L. On the other hand, in the present study, the sheep with β-HBA values over 1.6 mmol/L, which is regarded as subclinical ketosis, also showed non-specific clinical signs, such as reduced feed consumption and inattentiveness to their environment. With regard to previous studies on pregnancy toxemia in the literature, Radostits et al. (26) stated that β-HBA levels up to 0.8 mmol/L suggested sufficient energy intake, levels between 0.8 and 1.6 mmol/L indicated insufficient energy intake, and those higher than 1.6 mmol/L were an indicator of severe malnourishment. Duehlmeier et al. (8) reported that plasma β-HBA levels higher than 1.6 mmol/L indicated severe malnourishment, and these levels could exceed 3 mmol/L in sheep with pregnancy toxemia. These results show that there is no consensus on values that distinguish clinical ketosis from subclinical ketosis.

Sheep have limited capacity to provide enough glucose to the fetus from their dietary resources (8). In periods when NEB and demand for glucose increase, approximately 23% of glucose is synthesized from glycerol released from adipose tissue. With this glucogenic precursor, more fatty acids are released into circulation, which accelerates the formation of ketone bodies (16). Lima et al. (18) and Souto et al. (32) reported that they observed hyperglycemia as a result of fetal death in their studies on pregnancy toxemia in goats. Cal-Pereyra et al. (6) reported that

Table 2. Biochemical metabolites by group: clinical, subclinical, and control

Parameters		Clinical group (n = 20)	Subclinical group (n = 30)	Control group (n = 20)	p Value
β-HBA (mmol/L)	median (min/max)*	3.10^{a} (3,0/3,90)	1.90^{b} (1,10/2,30)	0.30^{c} (0,10/0,80)	0.000
Glucose (mg/dl)	mean + SE**	37,85 ± 0,77^{c}	50,30 ± 0,82^{b}	65,10 ± 1,72^{a}	0.000
HbA1c (%)	median (min/max)*	8.09^{a} (4.99/9.78)	1.60^{c} (1.04/4.01)	4.20^{b} (1.62/6.21)	0.000
NEFA (mmol/L)	median (min/max)*	2.30^{a} (1.50/2.80)	1.00^{b} (0.60/1.90)	0.25^{c} (0.10/0.70)	0.000
AST (U/L)	median (min/max)*	118.50^{a} (63/321)	97.00^{a} (71/149)	114.50^{a} (59/155)	0.525
ALP (U/L)	median (min/max)*	37.50^{b} (32.58,60)	77.50^{a} (58/241)	86.50^{a} (74±104)	0.000
ALT (U/L)	median (min/max)*	79.00^{a} (74/89)	74.50^{a} (63/81)	65.00^{a} (60/83)	0.000
GGT (U/L)	median (min/max)*	61.50^{a} (41/78)	43.00^{b} (33/64)	33.50^{b} (26/69)	0.000
LDL (mg/dl)	mean + SE**	44.75 ± 0.56^{a}	34.06 ± 0.89^{b}	28.90 ± 1.13^{c}	0.000
HDL (mg/dl)	mean + SE**	52.32 ± 1.28^{c}	71.96 ± 1.45^{b}	85.82 ± 1.85^{a}	0.000
VLDL (mg/dl)	median (min/max)*	1.41^{a} (1,30/1,56)	1.40^{a} (1,22/1,56)	1.38^{a} (1,24/1,78)	0.726
Triglyceride (mg/dl)	mean + SE**	44,90 ± 0,99^{a}	21,13 ± 0,92^{b}	13,15 ± 0,62^{c}	0,000
Creatinine (mg/dl)	median (min/max)*	1.6^{a} (1.50/1.70)	1.50^{b} (1.40/1.60)	1.60^{ab} (1.40/1.70)	0.006
Phosphorus (mg/dl)	mean + SE**	8.57 ± 0.19^{a}	7.10 ± 0.29^{b}	4.20 ± 0.12^{c}	0.000

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hyperglycemia occurred due to the increasing cortisol concentration in plasma towards the end of pregnancy. The blood levels of glucose in pregnant animals with pregnancy toxaemia have varied and this give rise to the idea that hypoglycaemia might indicate that the fetuses are alive and hyperglycemia might indicate that the fetuses are dead (33). In this study, the detection of lower glucose levels in sheep with clinical and subclinical symptoms compared to healthy sheep supports the conclusion that glucose reserves are insufficient and that hypoglycemia occurs with pregnancy toxemia.

NEB increases the mobilization of fats that are stored in the body in the form of triglycerides, which leads to an increase in serum NEFA levels. It is one of the earliest developed and important metabolic indicators of negative energy balance in pregnant sheep. In the present study, however, NEFA levels in sheep with subclinical and clinical pregnancy toxemia were higher than they were in healthy pregnant sheep. This finding supports studies reporting that lipolysis increases with hypoglycemia in pregnancy toxemia (2).

The sheep with clinical ketosis in this study had higher serum HbA1c levels than did the healthy sheep and the sheep with subclinical ketosis. It has been reported that pregnancy toxemia in sheep is similar to preeclampsia in humans, but this similarity does not constitute a precise model. Preeclampsia (in humans) is an endocrine condition characterized by increased HbA1c levels, liver dysfunction, insulin resistance, and calcium imbalance in the late period of pregnancy (30). Furthermore, pregnancy toxemia in sheep has a negative effect on the secretion of insulin from pancreatic beta cells as a result of high rates of lipid mobilization and an increase in plasma NEFA levels associated with these rates (1). One of the predisposing factors in pregnancy toxemia in sheep is insulin resistance similar to that of gestational diabetes mellitus (GDM). Moreover, HbA1c was reported to be a potential simple marker of insulin resistance in the early part of the process (3). This observation provides a reason why the higher HbA1c levels in the clinical ketosis group compared to the subclinical ketosis and control groups may be associated with increased lipid mobilization, disruptions in glucose metabolism, and insulin resistance. The HbA1c levels in the subclinical group were also lower than those in the control group. During a normal pregnancy, new erythrocytes are formed as a result of low fasting blood glucose levels, and since they are exposed to lower glucose concentrations and since the lifespan of erythrocytes is shorter during pregnancy, low HbA1c levels can be observed (23). This information from the literature suggests that the low HbA1c levels in the subclinical group could have originated not only from the presence of hypoglycemia caused by subclinical ketosis, but also from lower HbA1c glycation levels due to pregnancy. Therefore, in sheep pregnancies, HbA1c measurements can be used for hypoglycemia follow ups and as an early diagnostic marker for insulin resistance in pregnancy toxemia.

In pregnancy toxemia, as a result of hypoglycemia and hyperketonemia, the intracellular and extracellular concentrations of minerals are affected, which results in an acid-base imbalance (29). In the present study, serum phosphorus levels were significantly higher in the clinical ketosis group (Tab. 2). In diabetes cases that may lead to ketosis, metabolic acidosis, and insulin resistance, hyperphosphatemia can develop (24). This finding shows that the exacerbation of ketosis and increased phosphorus concentrations in pregnancy toxemia can be related to disrupted mineral metabolism.

Endocrine and energy metabolites. Serum PPARα levels were higher in the clinical group than in the subclinical and control groups (Tab. 3). Serum PPARα levels were strongly and negatively correlated with ALT levels ($r = –0.620^{**}$; $p = 0.004$) and moderately and positively correlated with VLDL levels ($r = –0.386^{*}$; $p = 0.035$) in the clinical group. In the subclinical group, PPARα levels were moderately and positively correlated with AST levels ($r = 0.518^{**}$; $p = 0.003$) and ALP levels ($r = 0.389^{*}$; $p = 0.034$), while they were moderately and positively correlated with VLDL levels ($r = –0.386^{*}$; $p = 0.035$) (Tab. 4).

PPARα is regarded as a key biomarker in the adaptation of the pancreatic islets to conditions such as hunger, where hypoglycemia is dominant. Its protective effects on pancreatic functions can be seen especially in obesity and pregnancy. During pregnancy, PPARα activation has a role in the regulation of insulin secretion, and it provides protection from the negative effects of fatty acids in the pancreatic islets (13). In the present study, the higher PPARα levels in the clinical and subclinical groups compared with the control

Tab. 3. Endocrine and energy metabolites by group: clinical, subclinical, and control

Parameters		Clinical group (n = 20)	Subclinical group (n = 30)	Control group (n = 20)	p Value
Asprosin (pg/ml)	median (min/max)*	3031^{b} (866/4316)	3936^{a} (2246/5806)	2631^{b} (276/5646)	0.001
PPARα (pg/ml)	median (min/max)*	3961^{a} (2111/4471)	3548.50^{b} (1936/4691)	3318.50^{bc} (1301/4461)	0.032
FGF21 (pg/ml)	median (min/max)*	1555.20^{a} (467.20/1755.20)	1506.20^{a} (649.20/1977.20)	1387.20^{a} (725.20/2011.20)	0.716
FABP1 (ng/ml)	median (min/max)*	12.67^{b} (2.42/15.77)	14.78^{a} (7.74/18.04)	12.05^{b} (6.67/19.88)	0.044

Explanations: a, b, c – indicates that there is a statistically significant difference between values in the same row ($p < 0.05$); a – indicates that there is no statistically significant difference between values with the same letters; * shows the median (minimum/maximum) value

Tab. 4. Correlation between clinical findings and biochemical and endocrine metabolites by group

Parameters		ASPROSIN			FABP1			FGF21			PPARα		
		clinical	subclinical	control	clinical	subclinical	control	clinical	subclinical	control	clinical	subclinical	control
β-HBA	r	–0.393	–0.144	–0.092	–0.207	–0.113	–0.038	–0.453*	0.115	–0.086	–0.210	0.083	–0.158
	p	0.087	0.447	0.698	0.382	0.552	0.873	0.045	0.545	0.718	0.373	0.663	0.506
HbA1C	r	0.177	–0.450*	–0.302	0.272	–0.133	–0.278	0.193	–0.197	–0.214	0.062	–0.047	–0.102
	p	0.456	0.013	0.195	0.245	0.485	0.235	0.414	0.297	0.366	0.794	0.805	0.668
NEFA	r	0.474*	–0.126	–0.496*	0.595**	–0.388*	–0.619**	0.313	–0.091	–0.333	0.112	–0.229	–0.270
	p	0.035	0.507	0.026	0.006	0.034	0.004	0.178	0.632	0.151	0.638	0.223	0.249
Glucose	r	0.085	0.024	0.053	–0.009	0.014	–0.206	0.191	–0.170	0.073	0.207	–0.280	–0.025
	p	0.723	0.899	0.823	0.970	0.940	0.383	0.420	0.370	0.760	0.382	0.134	0.917
AST	r	–0.244	0.419*	0.093	–0.458*	0.231	0.135	–0.123	0.544**	–0.021	0.156	0.518**	0.023
	p	0.299	0.021	0.698	0.042	0.219	0.571	0.607	0.002	0.930	0.512	0.003	0.922
ALT	r	–0.236	–0.269	0.466*	–0.051	–0.297	0.575**	–0.523*	–0.077	0.261	–0.620**	–0.220	0.150
	p	0.317	0.151	0.038	0.832	0.111	0.008	0.018	0.685	0.267	0.004	0.244	0.529
ALP	r	0.111	–0.069	0.279	0.126	0.381*	0.228	0.154	0.188	0.082	–0.095	0.389*	–0.185
	p	0.642	0.717	0.234	0.597	0.038	0.334	0.517	0.320	0.730	0.690	0.034	0.435
GGT	r	0.067	–0.025	–0.418	–0.118	0.066	–0.262	0.440	0.272	–0.291	0.268	0.107	–0.028
	p	0.779	0.897	0.067	0.619	0.729	0.265	0.052	0.145	0.213	0.253	0.574	0.907
LDL	r	0.060	0.152	–0.112	0.324	–0.102	–0.357	–0.229	0.189	0.016	–0.245	0.145	–0.252
	p	0.803	0.422	0.638	0.164	0.593	0.122	0.332	0.316	0.947	0.297	0.446	0.285
HDL	r	0.250	–0.429*	–0.029	0.239	0.084	0.168	0.211	–0.242	–0.229	0.141	–0.112	–0.175
	p	0.287	0.018	0.902	0.310	0.661	0.480	0.373	0.197	0.331	0.552	0.557	0.460
VLDL	r	–0.112	–0.199	–0.072	–0.270	–0.084	–0.135	0.562**	–0.514**	–0.059	0.459*	–0.386*	0.153
	p	0.637	0.291	0.762	0.249	0.660	0.570	0.010	0.004	0.804	0.042	0.035	0.520
Triglycerides	r	0.181	–0.082	0.405	0.116	–0.320	0.321	–0.155	0.001	–0.017	0.005	0.064	–0.274
	p	0.445	0.668	0.077	0.627	0.085	0.168	0.513	0.996	0.942	0.985	0.735	0.243
Creatinine	r	0.315	0.056	–0.443	0.233	–0.079	–0.507*	0.123	–0.077	–0.105	0.123	–0.049	0.050
	p	0.176	0.767	0.050	0.323	0.678	0.022	0.604	0.685	0.661	0.604	0.796	0.833
Phosphorus	r	–0.001	–0.414*	–0.110	0.011	–0.200	–0.093	0.252	–0.425*	0.142	0.385	–0.286	0.259
	p	0.997	0.023	0.645	0.965	0.291	0.698	0.285	0.019	0.552	0.094	0.125	0.270
Body temperature	r	–0.429	–0.053	0.347	–0.426	0.307	0.237	0.205	0.020	0.411	0.348	–0.196	0.211
	p	0.059	0.781	0.134	0.061	0.098	0.314	0.387	0.917	0.072	0.133	0.298	0.371
Heart rate	r	0.664**	–0.285	–0.011	–0.259	–0.278	0.195	0.026	–0.319	0.086	0.161	–0.123	–0.043
	p	0.001	0.127	0.962	0.270	0.137	0.410	0.913	0.085	0.718	0.497	0.517	0.856
Respiration rate	r	–0.135	–0.148	0.197	0.579**	–0.357	–0.100	–0.187	–0.238	–0.218	–0.070	–0.156	–0.413
	p	0.570	0.434	0.405	0.008	0.052	0.675	0.429	0.205	0.355	0.769	0.409	0.070

group were an indicator that this outcome may be a protective response against ketosis occurring during pregnancy toxemia.

The serum asprosin values were higher in the subclinical group compared to the clinical group (Tab. 3). In comparison to the control group, the clinical and subclinical groups had higher serum asprosin levels ($p < 0.001$). Asprosin levels in the clinical group were strongly and positively correlated with respiratory rates ($r = 0.664^{**}$; $p = 0.001$). In the subclinical group, the asprosin levels were moderately and negatively correlated with HbA1c levels ($r = –0.450^{*}$; $p = 0.013$), moderately and positively correlated with AST levels ($r = 0.419^{*}$; $p = 0.021$), moderately and negatively correlated with HDL levels ($r = –0.429^{*}$; $p = 0.018$), and moderately and negatively correlated with phosphorus levels ($r = –0.414^{*}$; $p = 0.023$). In the control group, asprosin levels were moderately and positively correlated with ALT levels (Tab. 4).

Because pregnancy toxemia is frequently associated with clinical signs, such as hypo- and hyperglycemia, lipemia, and hyperketonemia, individual- or breed-dependent insulin resistance is believed to be the main predisposing factor to this condition (9). In the present study, statistically significant differences in asprosin levels were found between all groups of

animals (Tab. 3). The increased serum asprosin levels combined with the exacerbation of hypoglycemia in the sheep with subclinical ketosis compared with the healthy sheep in the control group ($p < 0.001$) was compatible with the report by Romere et al. (28) that asprosin levels increase in hypoglycemia. This finding suggests that asprosin modulates the glucose mechanism regardless of the effect of insulin and maintains energy homeostasis. While asprosin has a direct effect on liver glucose levels, it has a mild effect on the insulin compensation mechanism (11). Likewise, Wang et al. (35) found that asprosin levels were higher in prediabetic patients than they were in diabetic patients, which suggested that asprosin could be a decisive biomarker for early diagnosis. In this study, it is thought that the presence of higher serum asprosin levels in the subclinical group (compared to the clinical group) and glucose metabolism (such as insulin resistance or prediabetic form mechanisms) can be used to evaluate the subclinical form of pregnancy toxemia. However, although the clinical group showed lower asprosin levels than did the subclinical group, hypoglycemia in the clinical group was more severe. Similarly, in their study on type 1 DM patients, Groener et al. (12) did not observe the expected increase in serum asprosin levels despite severe hypoglycemia and suggested that hypoglycemia occurred as a consequence of a blunted asprosin response. Moreover, GDM, in which insulin resistance develops, was reported as one of the predisposing factors of pregnancy toxemia (9). GDM is characterized by dysfunctions in insulin sensitivity and pancreatic β cells (4). Insulin resistance is characterized by dysglycemia, lipemia, and hyperketonemia, which are also typical signs in sheep with pregnancy toxemia (17). Thus, the lower serum asprosin levels in the clinical group compared with the subclinical group in the present study, despite hypoglycemia, suggest that the sheep could have had insulin resistance, which may have resulted in a blunted asprosin response.

Data from many experimental animal models and clinical studies indicate that increased lipid levels in circulation emerging due to increased ectopic fat depots and lipotoxicity in obesity are primary factors triggering the development of metabolic diseases. Specifically, ectopic fat accumulating in the liver plays a critical role in the development of dyslipidemia (25). Groener et al. (12) found an increase in asprosin concentrations during hypoglycemia in patients with high LDL and low HDL levels. Zhang et al. (38) also reported a relationship between plasma asprosin and triglyceride levels (in type 2 DM patients and non-diabetic individuals) and HDL levels (in non-diabetic individuals). The negative correlation between asprosin and HDL in the present study is compatible with the results of previous studies, revealing the presence of dyslipidemia ($p < 0.001$). As decreased HDL levels and increased LDL and triglyceride levels are factors that trigger insulin resistance, the simultaneous evaluation of lipid profiles and asprosin levels in sheep with clinical ketosis is important according to data obtained in this and other studies.

Serum FABP1 values were higher in the subclinical group compared to the clinical group (Tab. 3). Serum FABP1 levels were moderately and positively correlated with the heart rate ($r = 0.579^{**}$; $p = 0.008$) and moderately and negatively correlated with AST levels ($r = –0.458^{*}$; $p = 0.042$) in the clinical group. In the subclinical group, FABP1 and ALP levels were moderately and positively correlated ($r = 0.381^{*}$; $p = 0.038$). In the control group, FABP1 levels were moderately and positively correlated with ALT levels ($r = 0.575^{**}$; $p = 0.008$), while they were moderately and negatively correlated with creatinine levels ($r = –0.507^{*}$; $p = 0.022$) (Tab. 4).

Pregnancy toxemia has a mechanism similar to ketosis in type 2 DM in humans or in cattle, and the reduced serum FABP1 levels in the sheep with clinical ketosis in this study agreed with the results of previous studies. FABP1 is a molecule group that coordinates lipid reactions in the cell. It facilitates the transport of lipids into the cell (15). In the present study, no correlation was found between FABP1 and lipid profile parameters, i.e. LDL, HDL, VLDL, and triglyceride levels. Nonetheless, this result was compatible with the statistically significant increase in serum LDL and triglyceride levels in the subclinical group compared with the healthy control group in this study.

FGF21 is known as a metabolic regulator that regulates fatty acid oxidation and lipid metabolism throughout NEB. Chen et al. (7) reported that serum FGF21 levels in dairy cows with subclinical or clinical ketosis were positively correlated with β-HBA in a subclinical ketosis group. In addition, they determined that the serum FGF21 level increased until the β-HBA level was > 1.6 mmol/L. Therefore, FGF21 may be a metabolic parameter that can be evaluated like β-HBA in cows with subclinical ketosis. In the present study, serum FGF21 levels were higher in the sheep with clinical or subclinical signs than they were in the healthy sheep. However, no statistically significant difference was found.

Negative energy balances occurring during pregnancy toxemia adversely affect lipid and energy metabolism. This study suggests that serum asprosin, FABP1, PPARα, and HbA1c are biomarkers that can be used to evaluate negative energy balances during clinical and subclinical pregnancy toxemia. Since the loss of the mother and her offspring results from the clinical form of pregnancy toxemia, the subclinical form is often overlooked, which may lead to serious yield losses. It is thought that these biomarkers will make it possible to determine metabolic disorders and implement protective measures. However, this study had some limitations, as the animal materials were col-

lected by scanning under field conditions. In particular, environmental factors can affect the secretion of adipokines. Therefore, it remains unclear whether the high concentrations of metabolic biomarkers evaluated in the study were a consequence or a cause of pregnancy toxemia. More comprehensive investigations involving advanced techniques and analyses are needed to confirm the outcomes of this study.

References

1. *Akbari H., Dalir-Naghadeh B., Asri-Rezaei S., Hadian M., Boston R. C.*: Experimental hyperlipidemia induces insulin resistance in sheep. Domestic Animal Endocrinology 2015, 53, 95-102.
2. *Allen M. S., Piantoni P.*: Metabolic control of feed intake: Implications for metabolic disease of fresh cows. Veterinary Clinics: Food Animal Practice 2013, 29, 279-297.
3. *Borai A., Livingstone C., Abdelaal F., Bawazeer A., Keti V., Ferns G.*: The relationship between glycosylated haemoglobin (HbA1c) and measures of insulin resistance across a range of glucose tolerance. Scandinavian Journal of Clinical and Laboratory Investigation 2011, 71, 168-172.
4. *Buchanan T. A., Xiang A. H.*: Gestational diabetes mellitus. J. Clin. Invest. 2005, 115, 485-491.
5. *Cal-Pereyra L., Benech A., González-Montaña J. R., Acosta-Dibarrat J., Da Silva S., Martín A.*: Changes in the metabolic profile of pregnant ewes to an acute feed restriction in late gestation. New Zealand Veterinary Journal 2015, 63, 141-146.
6. *Cal-Pereyra L., Acosta-Dibarrat J., Benech A., Silva S. D., Martin A., González-Montaña J. R.*: Ewe pregnancy toxemia. Review. Revista Mexicana De Ciencias Pecuarias 2012, 3, 247-264.
7. *Chen Y., Dong Z., Li R. X. C.*: Changes in selected biochemical parameters (including FGF21) during subclinical and clinical ketosis in dairy cows. Med. Weter. 2018, 74, 727-730.
8. *Duehlmeier R., Fluegge I., Schwert B., Parvizi N., Ganter M.*: Metabolic adaptations to pregnancy and lactation in German Blackheaded Mutton and Finn sheep ewes with different susceptibilities to pregnancy toxaemia. Small Ruminant Research. 2011, 96, 178-184.
9. *Duehlmeier R., Noldt S., Ganter M.*: Pancreatic insulin release and peripheral insulin sensitivity in German black headed mutton and Finish Landrace ewes: evaluation of the role of insulin resistance in the susceptibility to ovine pregnancy toxemia. Domestic Animal Endocrinology 2013, 44, 213-221.
10. *Duerrschmid C., He Y., Wang C., Li C., Bournat J. C., Romere C., Jia P.*: Asprosin is a centrally acting orexigenic hormone. Nature Medicine 2017, 23, 1444.
11. *Elnagar A., El-Belbasi H. I., Rehan I. F., El-Dawy K.*: Asprosin: a novel biomarker of type 2 diabetes mellitus. Veterinary Medicine In-Between Health & Economy 2018, 55, 333-347.
12. *Groener J. B., Valkanou A., Kender Z., Pfeiffenberger J., Kihm L., Fleming T., Kopf S.*: Asprosin response in hypoglycemia is not related to hypoglycemia unawareness but rather to insulin resistance in type 1 diabetes. PloS one. 2019, 14, e0222771.
13. *Holness M. J., Greenwood G. K., Smith N. D., Sugden M. C.*: Peroxisome Proliferator-Activated Receptor-α and Glucocorticoids Interactively Regulate Insulin Secretion During Pregnancy. Diabetes 2006, 55, 3501-3508.
14. *Inagaki T., Dutchak P., Zhao G., Ding X., Gautron L., Parameswara V., Kliewer S. A.*: Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. Cell Metabolism 2007, 5, 415-425.
15. *Ishimura S., Furuhashi M., Watanabe Y., Hoshina K., Fusey T., Mita T., Miura T.*: Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. PloS one 2013, 8, e81318.
16. *Jones A. K., Gately R. E., Kellogg T. D., Zinn S. A., Govoni K. E., Reed S. A.*: Evaluation of the Nova Vet Meter for sheep-side monitoring of β-hydroxybutyric acid (β-HBA) and description of ewe β-HBA during late gestation in three flocks from the Northeastern US. Research in Veterinary Science 2018, 118, 491-497.
17. *Kahn B. B.*: Glucose transport: pivotal step in insulin action. Diabetes 1996, 45, 1644-1654.
18. *Lima M. S., Pascoal R. A., Stilwell G. T.*: Glycaemia as a sign of the viability of the foetuses in the last days of gestation in dairy goats with pregnancy toxaemia. Irish Veterinary Journal 2012, 65, 1-6.
19. *Lin Z., Tian H., Lam K. S., Lin S., Hoo R. C., Konishi M., Li* X.: Adiponectin mediates the metabolic effects of FGF21 on glucose homeostasis and insulin sensitivity in mice. Cell Metabolism 2013, 17, 779-789.
20. *Lundasen T., Hunt M. C., Nilsson L. M., Sanyal S., Angelin B., Alexson S. E., Rudling M.*: PPARα is a key regulator of hepatic FGF21. Biochemical and Biophysical Research Communications 2007, 2, 437-440.
21. *Mamak N., Devrim A. K., Aksit H., Aytekin I., Yildiz R.*: Levels of antioxidant substances, acute phase response and lipid peroxidation in the left and right abomasum displacement in cows. Polish Journal of Veterinary Sciences 2013, 16, 731-733.
22. *Marutsova V., Binev R., Marutsov P.*: Comparative clinical and haematological investigations in lactating cows with subclinical and clinical ketosis. Mac. Vet. Rev. 2015, 2, 1-7.
23. *Nielsen L. R., Ekbom P., Damm P., Glümer C., Frandsen M. M., Jensen D. M., Mathiesen E. R.*: HbA1c levels are significantly lower in early and late pregnancy. Diabetes Care. 2004, 27, 1200-1201.
24. *Palmer B. F., Clegg D. J.*: Hyperkalemia. Jama 2015, 22, 2405-2406.
25. *Paneni F., Costantino S., Cosentino F.*: Insulin resistance, diabetes, and cardiovascular risk. Current Atherosclerosis Reports 2014, 16, 419.
26. *Radostits O. M., Gay C. C., Hinchcliff K. W., Constable P. D.*: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Veterinary Medicine 2007, 10, 2045-2050.
27. *Rodolfo S. J. C., Augusto A. J. B., Mendonça C. L., Cleyton C. C. D., Alonso S. F. P., Jobson C. F. P., Elizabeth L. H. F., Pierre S. C.*: Biochemical, electrolytic and hormonal findings in goats affected with pregnancy toxemia. Pesqui. Vet. Bras. 2014, 33.
28. *Romere C., Duerrschmid C., Bournat J., Constable P., Jain M., Xia F., Chopra A. R.*: Asprosin, a fasting-induced glucogenic protein hormone. Cell 2016, 165, 566-579.
29. *Roubies N., Polızopoulou Z., Minas A., Papasteriadis A.*: A pre- and postpartum study of selected biochemical parameters in ewes for the early detection of pregnancy toxemia. Journal of the Hellenic Veterinary Medical Society 2003, 54, 11-20.
30. *Shafi N., Bano F., Uraneb S.*: The role of novel hormone asprosin in insulin resistance during preeclampsia. Pak. J. Pharm. Sci. 2021, 34, 1039-1043.
31. *Simpson K. M., Taylor J. D., Streeter R. N.*: Evaluation of prognostic indicators for goats with pregnancy toxemia. Journal of the American Veterinary Medical Association 2019, 7, 859-867.
32. *Souto R. J., Afonso J. A. B., Mendonça C. L., Carvalho C. C., Silva Filho A. P., Cajueiro J. F., Soares P. C.*: Biochemical, electrolytic and hormonal findings in goats affected with pregnancy toxemia. Pesquisa Veterinária Brasileira 2013, 33, 1174-1182.
33. *Uma Rani R., Palanichamy V., Muruganandan B.*: Clinical and Serobiochemical Studies on Pregnancy Toxaemia in Does. International Journal of Current Innovation Research 2015, 1, 102-104.
34. *Vasava P. R., Jani R. G., Goswami H. V., Rathwa S. D., Tandel F. B.*: Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. Veterinary World 2016, 9, 869.
35. *Wang Y., Qu H., Xiong X., Qiu Y., Liao Y., Chen Y., Zheng H.*: Plasma asprosin concentrations are increased in individuals with glucose dysregulation and correlated with insulin resistance and first-phase insulin secretion. Hindawi Mediators of Inflammation 2018, 1-7.
36. *White H. M.*: The role of TCA cycle anaplerosis in ketosis and fatty liver in periparturient dairy cows. Animals (Basel) 2015, 5, 793-802.
37. *Yıldız R., İder M., Ok M.*: Beta hidroksi bütirik asit düzeyinin diğer metabolik test parametreleri üzerine etkisi. Veteriner Hekimler Derneği Dergisi 2019, 90, 15-21.
38. *Zhang L., Chen C., Zhou N., Fu Y., Cheng X.*: Circulating asprosin concentrations are increased in type 2 diabetes mellitus and independently associated with fasting glucose and triglyceride. Clinica Chimica Acta 2019, 489, 183-188.

Corresponding author: Merve Öztürk, PhD, Department of Veterinary Internal Medicine, faculty of Veterinary Medicine, Bingol University, Bingol 12000, Turkey; e-mail: merveozturk@bingol.edu.tr