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Evaluation of Nesfatin–1 levels in ewes with pregnancy toxemia

Evaluación de los niveles de Nesfatina-1 en ovejas con toxemia gestacional

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ABSTRACT

The goal of the present research was to investigate the effects of pregnancy toxemia (PT) on nesfatin–1 levels in ewes and to examine its relationship with spexin, another biomarker involved in energy metabolism. The study was conducted on 45 Awassi ewes that had given birth at least once and were between 120 and 150 days of pregnancy. There were three groups of ewes. The control group (Group 1, n=15) consisting healthy ewes showing β–hydroxybutyric acid (BHBA) levels < 0.8 mmol·L^{-1}, the subclinical PT group (Group 2, n=15) involved ewes with BHBA levels between 0.8 to 2.5 mmol·L^{-1}, and the clinical PT group (Group 3, n=15) contained ewes with BHBA levels > 2.5 mmol·L^{-1} (which ranged from 2.6 to 7.10 mmol·L^{-1}). We utilized a commercial kit that measured the levels of serum nesfatin–1 and spexin. Compared to the control group, the concentrations of spexin were significantly lower in both of the subclinical and clinical PT groups (P<0.001). The subclinical PT group showed lower levels of Nesfatin–1 than the control group, but the clinical PT group had larger levels (P<0.001). The subclinical and clinical PT groups had significantly higher levels of triglycerides and cholesterol in comparison to the control group (P<0.001). The subclinical and clinical PT groups had very high levels of aspartate aminotransferase (AST) and gamma–glutamyl transferase (GGT) (P<0.001). The correlation examination elucidated the ensuing relationships: There was an important positive link between spexin levels and insulin (P<0.001). Spexin levels showed an adverse correlation with nesfatin–1, BHBA, non–esterified fatty acids (NEFA), triglycerides, cholesterol, AST, and GGT (P<0.001). Nesfatin–1 levels exhibited a positive connection with glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT, and a significant negative correlation with insulin (P<0.001). In conclusion, nesfatin–1 and spexin neuropetids could act as novel biomarkers for the detection of both clinical and subclinical forms of PT in sheep.

Key words: Ewe; Nesfatin–1; pregnancy toxemia; spexin

RESUMEN

El objetivo de este estudio fue investigar los efectos de la toxemia gestacional (TP) en los niveles de nesfatina–1 en ovejas y examinar su relación con la espexina, otro biomarcador involucrado en el metabolismo energético. El estudio se realizó en 45 ovejas Awassi que habían parido al menos una vez y tenían entre 120 y 150 días de gestación. Las ovejas se dividieron en tres grupos. El grupo control (Grupo 1, n=15) consistió en ovejas sanas con niveles de BHBA (ácido β–hidroxibutírico) < 0,8 mmol·L^{-1}, el grupo PT subclínico (Grupo 2, n=15) incluyó ovejas con niveles de BHBA entre 0,8 y 2,5 mmol·L^{-1}, y el grupo PT clínico (Grupo 3, n=15) consistió en ovejas con niveles de BHBA > 2,5 mmol·L^{-1} (rango de 2,6 a 7,10 mmol·L^{-1}). Los niveles séricos de nesfatina–1 y espexina se midieron utilizando un kit comercial. Los niveles de espexina fueron significativamente menores en los grupos de PT subclínico y clínico en comparación con el grupo control (P<0,001). Los niveles de nesfatina–1 fueron menores en el grupo de PT subclínico, pero mayores en el grupo de PT clínico en comparación con el control (P<0,001). Los niveles de triglicéridos y colesterol fueron significativamente mayores en los grupos de PT subclínico y clínico en comparación con el control (P<0,001). Los niveles de aspartato aminotransferasa (AST) y gama glutamil transferasa (GGT) también fueron significativamente elevados en los grupos de PT subclínico y clínico (P<0,001). El análisis de correlación reveló las siguientes relaciones: los niveles de espexina mostraron una correlación positiva significativa con la insulina (P<0,001). Los niveles de espexina se correlacionaron negativamente con nesfatina–1, BHBA, (ácidos grasos no esterificados) NEFA, triglicéridos, colesterol, AST y GGT (P<0,001). Los niveles de nesfatina–1 se correlacionaron positivamente con la glucosa, BHBA, NEFA, triglicéridos, colesterol, AST y GGT, mientras que mostraron una correlación negativa significativa con la insulina (P<0,001). En conclusión, las neuropéptidos nesfatina–1 y espexina podrían servir como nuevos biomarcadores para el diagnóstico de las formas clínicas y subclínicas de TP en ovejas.

Palabras clave: Oveja; Nesfatina–1; toxemia gestacional; espexina

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INTRODUCTION

Pregnancy toxemia (PT) is a prevalent metabolic condition seen in small ruminants during the final six weeks of gestation. This condition is characterized by imbalances in energy, protein, mineral profile, and oxidative–antioxidative balance. As a disease with distinct clinical symptoms, it can lead to severe consequences, including the loss of both the fetus and the mother [1, 2]. PT can present in either clinical or subclinical forms and is predominantly detected in undernourished ewes gestating two or more fetuses [3]. The differentiation into clinical and subclinical forms is predicated on clinical manifestations and blood β–Hydroxybutyric acid (BHBA) concentrations. Blood BHBA levels indicate the severity of negative energy balance (NEB) and are thus regarded as a crucial biochemical sign for the diagnosis of PT [4].

Researchers found spexin, also called neuropeptide Q, in 2007 through an in silico analysis. It is a 14-amino acid peptide hormone [5]. This hormone is produced in large amounts in both central and peripheral tissues and is released into the bloodstream when the body is under metabolic stress. Spexin exerts its functions by binding to galanin receptors 2 and 3. While these receptors are primarily located in adipose tissue, they are also found in the testes, skeletal muscles, small and large intestines, rectum, pancreas, placenta, and liver. To date, the majority of research on spexin has focused on its roles in humans. These studies have examined its association with energy and glucose metabolism as well as its involvement in the pathogenesis of various diseases [6, 7]

Nesfatin–1 is an 82-amino acid multifunctional metabolic regulator that plays a significant role in metabolic control due to its anorexigenic and antihyperglycemic effects [8].The Nucleobindin 2 (NUCB2) messenger RNA, a precursor of nesfatin–1, serves as the basis for the synthesis of this peptide. Although the receptor for nesfatin–1 has not yet been fully identified, it is known to be widely distributed in various tissues, including the central nervous system, pancreatic islet cells, pituitary gland, adipose tissue, gastric, and intestinal mucosa. The extensive presence of NUCB2/Nesfatin–1 in the hypothalamus and brainstem indicates that this peptide is essential for the regulation of energy balance [9].

Nesfatin–1 functions as an anorectic factor that modulates the activity of certain neurons in the brain, promoting satiety and suppressing food and water consumption [10]. Studies on nesfatin–1 in veterinary medicine is lacking. Particularly, there are no studies examining the role of nesfatin–1 in PT in sheep (*Ovis aries)*.

This work aims to find out the changes in nesfatin–1 levels produced by PT in ewes and look into their correlation with spexin, a different biomarker associated in energy metabolism. The study also aims to evaluate the potential role of nesfatin–1 and spexin in the diagnosis of PT.

MATERIALS AND METHODS

This study was conducted with the approval of the Harran University Local Animal Experiments Ethics Committee (HRÜ–HADYEK), under permit number 2025/001/05.

Selecting animal material

The study involved 45 Awassi ewes, each having given birth at least once and gestating for 120 to 150 days (d), who were taken to the Veterinary Faculty Animal Hospital of Harran University. The ewes were categorized into three groups according to their BHBA levels, as determined from blood samples obtained from the jugular vein, and the clinical manifestations of PT [11]. The control group (Group 1, n=15) included healthy ewes with a BHBA level <0.8 $mmol{\cdot}L^{-1}$, the subclinical PT group (Group 2, n=15) included ewes with a BHBA level between 0.8–2.5 $mmol{\cdot}L^{-1}$ that did not show any clinical signs of PT, and the clinical PT group (Group 3, n=15) included ewes with a BHBA level >2.5 $mmol{\cdot}L^{-1}$ (2.6–7.10 $mmol{\cdot}L^{-1}$) that showed clinical signs of PT such as anorexia, lethargy, head drooping, opisthotonus, limb edema, and sternal recumbency [12]. The study exclusively used pregnant ewes in the final month of gestation exhibiting comparable body condition scores, and who were not in lactation. The sheep in the research had also not received any medicine in the preceding two weeks and shown no further indications of disease.

Taking blood samples and testing them in a lab

Blood samples were taken from the jugular vein of the ewes in all groups following the BHBA measurement. To get serum from the blood samples, they were spun (NÜVE NF 200, Ankara, Türkiye) at 3000 rpm for 10 min. The obtained serum samples were stored at -20°C until the d of analysis. Serum nesfatin–1 levels of the ewes in the study groups were determined using a commercial kit (Sheep Nesfatin ELISA Kit, MBS735285, MyBioSource, Inc., USA). Spexin levels were also measured using a commercial kit (Sheep Spexin ELISA Kit, MBS7273093, MyBioSource, Inc., USA). Insulin levels were also measured using a commercial kit (Sheep Insulin (INS) ELISA Kit, EK10248, Signalway Antibody, USA). BHBA level was measured using rapid test kits (Free Style Optium Neo H–Abbott®). Triglycerides, cholesterol, aspartate aminotransferase (AST), gamma–glutamyltransferase (GGT), nonesterified fatty acids (NEFA) and glucose levels were evaluated using the Seamaty SMT-120V (Notavet, İzmir, Türkiye) biochemical analyzer.

Statistical analysis

Statistical analyses were done using SPSS version 26 software. We used visual approaches (histograms and probability plots) and analytical tests (Kolmogorov–Smirnov/Shapiro–Wilk tests) to determine if the variables were normal. For variables that indicated a normal distribution, descriptive analyses were provided as mean ± standard deviation. Since the measured parameters were found to follow a normal distribution, the differences between these parameters were compared using a one–way ANOVA test. In cases where significant differences were detected between groups, pairwise comparisons were performed using the Tukey test. Correlation analysis and statistical significance assessment of the data were conducted using the Pearson test. A significance level of 5% was considered for all analyses.

RESULTS AND DISCUSSION

The levels of spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT according to the clinical form of PT in ewes are presented in TABLE I.

TABLE I
The levels of spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT according to the clinical form of pregnancy toxemia (PT) in ewes (Mean ± Standard Error of Mean)

Parameters	Control	Subclinical PT	Clinical PT	*P* value
Spexin (pg·mL^{-1})	123 ± 2.99^{a}	73.14 ± 1.55^{b}	49.11 ± 2.11^{c}	<0.001
Nesfatin–1 (ng·mL^{-1})	5.28 ± 0.14^{a}	3.43 ± 0.48^{b}	12.31 ± 1.78^{c}	<0.001
Insulin (ng·mL^{-1})	7.48 ± 0.39^{a}	3.93 ± 0.24^{b}	3.36 ± 0.35^{bc}	<0.001
Glucose (mg·dl^{-1})	60.83 ± 0.5^{a}	44.44 ± 0.69^{b}	66.27 ± 0.56^{c}	<0.001
BHBA (mmol·L^{-1})	0.42 ± 0.23^{a}	1.77 ± 0.31^{b}	5.93 ± 0.25^{c}	<0.001
NEFA (umoL·L^{-1})	0.249 ± 0.007^{a}	0.631 ± 0.010^{b}	1.000 ± 0.27^{c}	<0.001
Triglycerides (mg·dL^{-1})	0.44 ± 0.013^{a}	1.14 ± 0.027^{b}	3.73 ± 0.069^{c}	<0.001
Cholesterol (mg·dL^{-1})	60.73 ± 0.44^{a}	59.66 ± 0.28^{ab}	89.78 ± 0.57^{c}	<0.001
AST (U·L^{-1})	108.77 ± 1.70^{a}	115.92 ± 2.31^{ab}	170.53 ± 2.54^{c}	<0.001
GGT (U·L^{-1})	31.04 ± 0.56^{a}	51.69 ± 0.74^{b}	81.38 ± 0.84^{c}	<0.001

^{a,b,c}: Different letters in the same column indicate a statistically significant difference. β–hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), aspartate aminotransferase (AST), gamma–glutamyltransferase (GGT)

This work offers strong evidence that spexin and nesfatin–1 hormones may exist as potential biomarkers for the diagnosis and track of pregnant toxemia in sheep. Spexin is a hormone encoded by the Cg12orf39 gene, and it is strongly vital for many things, like controlling energy levels, obesity, endocrine homeostasis, and glucose and lipid metabolism [13, 14]. Gu *et al.* [6] noticed that individuals with type 2 diabetes demonstrated substantially decreased amounts of spexin, which had a strong association with their glucose levels. A further investigation which includes people with type 1 diabetes identically revealed a significant decrease in spexin levels [7]. A study monitoring the therapeutic properties of spexin shown that its treatment resulted in decreased adipocyte hypertrophy, normalizing of metabolic profile markers, and substantial reductions in weight gain and pro–inflammatory cytokine production [15].

There were big variations between groups in the levels of spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT (P<0.001). Spexin levels were significantly reduced in the subclinical and clinical PT groups relative to the control group (P<0.001).

A study investigating changes in spexin concentrations at different times during the transition period in dairy cows revealed a significant decrease in spexin levels during the 21–day prepartum phase, leading up in the lowest concentrations seen on the day previous calving. Furthermore, spexin concentrations showed a significant negative connection with NEFA and BHBA levels [16]. In our study, the concentrations of spexin in ewes with PT were significantly lower than those in healthy animals, promoting the results of Gu *et al.* [6], Karaca *et al.* [7], and Uztimur and Ünal [17]. This findings confirm the significant negative correlations between spexin concentrations and BHBA and NEFA levels reported by Mikula *et al.* [16] and Uztimur and Ünal [17].

Compared to the control group, the subclinical PT group had lower levels of Nesfatin–1 while the clinical PT group had greater levels (P<0.001). Insulin levels were reduced in both the subclinical and clinical PT groups relative to the control group (P<0.001). The serum insulin levels in sheep with subclinical and clinical PT were similar (P>0.05). The subclinical PT group had lower glucose levels than the control group, whereas the clinical PT group had higher glucose levels than the control group (P<0.001). The levels of triglycerides and cholesterol were greater in both the subclinical and clinical PT groups than in the control group (P<0.001). Serum cholesterol concentrations were comparable in sheep with subclinical and clinical PT (P>0.05). AST and GGT levels were higher in both the subclinical and clinical PT groups compared to the control group (P<0.001). Serum AST levels were analogous in sheep with control and subclinical PT (P>0.05).

Nesfatin–1 is a peptide derived from the NUCB2 precursor molecule [18]. It has been identified in both the central nervous system and peripheral tissues, playing a significant role in appetite regulation within the central nervous system [19]. The appetite–suppressing function of nesfatin–1 has been demonstrated through its activating effect on melanocortin–3/4 receptors in the hypothalamus [18]. Some studies suggest that nesfatin–1 may play a regulatory role in energy and homeostasis. Individuals diagnosed with anorexia nervosa and those experiencing chronic food restriction were found to have significantly lower plasma nesfatin–1 levels compared to healthy controls [20]. Plasma nesfatin–1 levels have been shown to be associated with insulin resistance, fasting blood glucose, fasting insulin levels, body weight, and fat mass [21, 22]. Data suggest that nesfatin–1, particularly when derived from adipose tissue, is crucial for metabolism and food intake regulation [23].

The correlation findings between spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT levels according to the clinical form of PT in ewes are presented in TABLE II. Strong correlations were determined between spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT levels (P<0.001). Spexin levels showed a significant positive correlation with insulin (r=0.950, P<0.01). Spexin levels were negatively correlated with nesfatin–1, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT (r=-0.569, P<0.01; r=-0.833, P<0.01; r=-0.928, P<0.01; r=-0.837, P<0.01; r=-0.701, P<0.01; r=-0.769, P<0.01; r=-0.913, P<0.01, respectively). Nesfatin–1 levels were

TABLE II
The correlation findings between spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT levels according to the clinical form of pregnancy toxemia in ewes

Correlation analysis		Spexin	Nesfatin–1	Insulin	Glucose	BHBA	NEFA	Triglycerides	Cholesterol	AST	GGT
Spexin	r	1									
Nesfatin–1	r	-0.569**	1								
Insulin	r	0.827**	-0.393**	1							
Glucose	r	-0.029	0.763 **	0.167	1						
BHBA	r	-0.833**	0.843**	-0.686**	0.454**	1					
NEFA	r	-0.928**	0.706**	-0.807**	0.227	0.888**	1				
Triglycerides	r	-0.837**	0.891**	-0.667**	0.508**	0.966**	0.922**	1			
Cholesterol	r	-0.701**	0.944**	-0.507**	0.696**	0.932**	0.821**	0.959**	1		
AST	r	-0.769**	0.874**	-0.577**	0.570**	0.932**	0.870**	0.948**	0.955**	1	
GGT	r	-0.913**	0.778**	-0.787**	0.317	0.953**	0.965**	0.963**	0.886**	0.918**	1

** P<0.01. β–hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), aspartate aminotransferase (AST), gamma–glutamyltransferase (GGT)

positively correlated with glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT (r=0.763, *P*<0.01; r=0.843, *P*<0.01; r=0.706, *P*<0.01; r=0.891, *P*<0.01; r=0.944, *P*<0.01; r=0.874, *P*<0.01; r=0.778, *P*<0.01, respectively). Nesfatin–1 levels were negatively correlated with insulin (r=-0.393, *P*<0.01).

There were substantial variances between groups in the levels of spexin, nesfatin–1, insulin, glucose, BHBA, NEFA, triglycerides, cholesterol, AST, and GGT (*P*<0.001). The subclinical and clinical PT cohorts exhibited reduced levels of spexin in comparison to the control group (*P*<0.001).

This study discovered significant negative correlations between spexin and the energy metabolism biomarkers NEFA and BHBA, indicating that spexin may be an extremely useful diagnostic tool for pregnant toxemia. This hormone can be necessary for the prompt identification of pregnancy toxemia in sheep and for the control of glucose and lipid metabolism.

In a study conducted by Başar *et al.* [24] on humans, a negative correlation was found between serum nesfatin–1 levels and fasting blood glucose. In the present study, the decreased nesfatin–1 levels in ewes with subclinical PT were associated with increased appetite and food intake. This phenomenon is thought to be a homeostatic mechanism developed by the body to prevent disease progression. It is known that diabetic polyphagia is caused by reduced circulating nesfatin–1 levels [25].

Nesfatin–1 has been reported to exhibit antihyperglycemic effects under conditions of impaired glucose metabolism [8]. Additionally, it may act by increasing insulin sensitivity in the brain [26] and enhancing insulin secretion from beta cells in response to hyperglycemia [27]. A study on patients with type 2 diabetes found that plasma nesfatin–1 levels were lower compared to healthy controls and patients with type 1 diabetes [25]. Another study reported that serum nesfatin–1 levels were lower in women with gestational diabetes compared to healthy controls [28]. Type 2 diabetes is often associated with obesity, impaired insulin sensitivity [29], and eating disorders [30].

In contrast to the literature, the present study found that nesfatin–1 levels were higher in ewes with clinical PT compared to the control group. During the late stages of pregnancy in ewes, significant adaptations occur in maternal energy metabolism to meet the increased energy demand. These adaptations involve reducing glucose utilization by peripheral maternal tissues to preserve energy for fetal growth. Key adaptations include decreased insulin production and physiological peripheral insulin resistance (IR), characterized by reduced insulin secretion, decreased insulin sensitivity, or both [31].

Peripheral IR limits glucose supply to maternal adipose and skeletal muscle tissues, promoting the mobilization of lipid reserves. In this study, clinical pregnancy toxemia ewes were brought to the clinic in advanced stages of the disease, with high average BHBA levels, suggesting a higher degree of insulin resistance. This may explain the elevated nesfatin–1 levels observed in these animals. Additionally, all ewes in the clinical pregnancy toxemia group exhibited anorexia, which may indicate that the increased nesfatin–1 levels observed in PT could be related to anorexia, a common symptom of the disease. Nesfatin–1 plays a role in inhibiting food intake within the central nervous system [32]; however, further studies are needed to clarify its specific mechanism in PT.

Pregnant ewes with PT have problems with lipid metabolism and liver function because they don't get enough energy. This affects fatty acid oxidation (FAO), acetyl–CoA metabolism (ACM), and triglycerides synthesis (TGS). These metabolic abnormalities are exacerbated in ewes exhibiting clinical PT. In the current study, it is significant that nesfatin–1 exhibited a positive association with triglyceride and cholesterol levels. The mechanism by which elevated nesfatin–1 diminishes intracellular lipid accumulation is believed to entail the facilitation of triglyceride mobilization through the upregulation of hormone–sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) [33]. The inverse relationship between spexin and AST/GGT, together with the direct relationship between nesfatin–1 and AST/GGT, indicates that these hormones may operate as crucial indicators for the diagnosis of lipid and hepatic dysfunction related to PT. Nesfatin–1 and spexin are vital for retaining blood sugar levels stable and breaking down fats. Others think that both hormones can activate the AMP–activated

protein kinase (AMPK) route, which speeds up the decomposition of fatty acids. This might be a way for the body to fix issues with lipids. This method has been shown to improve nesfatin–1 and spexin levels in people with type 2 diabetes mellitus (T2DM) [34].

The oxidative decarboxylation of pyruvate and the β–oxidation of fatty acids develop acetyl–Coenzyme A (Acetyl–CoA). Acetyl–CoA serves as a metabolic intermediary and second messenger, affecting essential liver functions such as fatty acid synthesis, cholesterol generation, and ketone body synthesis [35]. The hepatic mitochondrial matrix mostly transforms Acetyl–CoA into molecules of ketone, including acetoacetic acid (AcAc) and BHBA, by ketogenesis [36]. Glucose, lipids, and proteins enter the tricarboxylic acid (TCA) cycle and oxidative phosphorylation via Acetyl–CoA, where they are completely oxidized into carbon dioxide and water, producing ATP for energy synthesis. Nutritional restriction leads to decreased glucose levels and a reduction in glycolytic metabolism. This condition enhances lipolysis and ketogenesis in adipose tissue, stimulating mitochondrial oxidation of non–glucose energy substrates into Acetyl–CoA and activating the TCA cycle for energy production [37].

The low nesfatin–1 levels observed in the subclinical PT group are thought to exhibit a protective effect against the progression of PT by inhibiting cAMP production and insulin signaling, thereby suppressing adipocyte differentiation [38]. Spexin plays a crucial role in energy homeostasis by regulating lipid metabolism in mammals. Walewski et al. reported that spexin expression in human white adipose tissue inhibits the uptake of long–chain fatty acids, leading to weight loss in rat models [13]. Spexin promotes lipolysis by phosphorylating HSL while inhibiting lipid synthesis [39]. In conclusion, spexin regulates lipid metabolism through multiple mechanisms, including blocking fat uptake, inhibiting lipid synthesis, and promoting lipolysis. The decreased spexin levels in ewes with PT may act as a protective mechanism to limit lipolysis and prevent further disease progression.

There are some limiting factors in our study. The fact that the clinical group consisted only of clinical cases resulted in higher average BHBA levels in this group compared to the subclinical group. This limitation prevented us from determining the exact stage at which the increase in nesfatin–1 levels began. Additionally, since long–term follow–up of the ewes was not possible, it was not feasible to assess the response of nesfatin–1 and spexin levels to treatment.

CONCLUSIONS

Nesfatin–1 and Spexin are considered potential biomarkers for the diagnosis of both clinical and subclinical forms of pregnancy toxemia in ewes. However, to fully elucidate the roles of these biomarkers throughout the course and treatment of pregnancy toxemia, studies in which the disease is experimentally induced are required. It is believed that such studies, supported by molecular–level analyses, will allow for a more detailed understanding of the effects of Nesfatin–1 and Spexin in pregnancy toxemia.

Conflict of interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

BIBLIOGRAPHIC REFERENCES

[1] Rook JS. Pregnancy toxemia of ewes, does and beef cows. Vet. Clin. N. Am. Food A. [Internet]. 2000; 16(2):293–317. doi: https://doi.org/njpx

[2] Souto RJC, Afonso JAB, Mendonça CL, Carvalho CCD, Filho S, Cajueiro FP, Lima EHF, Soares PC. Biochemical, electrolytic and hormonal findings in goats afected with pregnancy toxaemia. Pesq. Vet. [Internet]. 2013; 33(10):1174-1182. doi: https://doi.org/pzwx

[3] Lacetera N, Bernabucci U, Ronchi B, Nardone A. Efects of subclinical pregnancy toxemia on immune responses in sheep. Am. J. Vet. Res. [Internet]. 2001; 62(7):1020-1024. doi: https://doi.org/b4bffg

[4] Soares GSL, Ribeiro ACS, Paula JFC, Souto RJC, Oliveira Filho EF, Soares PC, Mendonça CL, Afonso JAB. Cardiac biomarkers and blood metabolites in cows with clinical ketosis. Semina Ciênc. Agrár. [Internet]. 2019; 40(6 Supl. 3):3525–3540. doi: https://doi.org/pzwz

[5] Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, Birney E, Rosenthal N, Gross C. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. Genome Res. [Internet]. 2007; 17(3):320–327. doi: https://doi.org/bzkfsx

[6] Gu L, Ma Y, Gu M, Zhang Y, Yan S, Li N, Wang Y, Ding X, Yin J, Fan N, Peng Y. Spexin peptide is expressed in human endocrine and epithelial tissues and reduced after glucose load in type 2 diabetes. Peptides [Internet]. 2015; 71:232–239. doi: https://doi.org/f7r6cz

[7] Karaca A, Bakar Ates F, Ersoz Gulcelik N. Decreased spexin levels in patients with type 1 and type 2 diabetes. Med. Princ. Pract. [Internet]. 2019; 27(6):549–554. doi: https://doi.org/pzw2

[8] Öztürk Özkan G. Efects of nesfatin–1 on food intake and hyperglycemia. J. Am. Coll. Nutr. [Internet]. 2020; 39(4):345–351. doi: https://doi.org/pzw3

[9] Alotibi MN, Alnoury AM, Alhozali AM. Serum nesfatin–1 and galanin concentrations in the adult with metabolic syndrome. Relationships to insulin resistance and obesity. Saudi Med. J. [Internet]. 2019; 40(1):19–25. doi: https://doi.org/pzw4

[10] Chen X, Shu X, Cong ZK, Jiang ZY, Jiang H. Nesfatin–1 acts on the dopaminergic reward pathway to inhibit food intake. Neuropeptides [Internet]. 2015; 53:45-50. doi: https://doi.org/f7wmnz

[11] Andrews A. Pregnancy toxaemia in the ewe. In Pract. [Internet]. 1997; 19(6):306-314. doi: https://doi.org/cqddsn

[12] Iqbal R, Beigh SA, Mir AQ, Shaheen M, Hussain SA, Nisar M, Dar AA. Evaluation of metabolic and oxidative profile in ovine pregnancy toxemia and to determine their association with diagnosis and prognosis of disease. Trop. Anim. Health Prod. [Internet]. 2022; 54:338. doi: https://doi.org/g5ggb8

[13] Kolakowski LF, O'Neill GP, Howard AD, Broussard SR, Sullivan KA, Feighner SD, , Sawzdargo M, Nguyen T, Kargman S, Shiao LL, Hreniuk DL, Tan CP, Evans J, Abramovitz M, Chateauneuf A, Coulombe N, Ng G, Johnson MP, Tharian A, Khoshbouei A, George SR, Smith RG, O'Dowd BF. Molecular characterization

and expression of cloned human galanin receptors GALR2 and GALR3. J. Neurochem. [Internet]. 1998; 71(6):2239–2251. doi: https://doi.org/d887kh

[14] Walewski JL, Ge F, Lobdell IV, Levin N, Schwartz GJ, Vasselli JR, Pomp A, Dakin G, Berk PD. Spexin is a novel human peptide that reduces adipocyte uptake of long chain fatty acids and causes weight loss in rodents with diet–induced obesity. Obesity [Internet]. 2014; 22(7):1643–1652. doi: https://doi.org/f58whj

[15] Gambaro SE, Zubiría MG, Giordano AP, Portales AE, Alzamendi A, Rumbo M, Giovambattista A. Spexin improves adipose tissue inflammation and macrophage recruitment in obese mice. Biochim. Biophys. Acta, Mol. Cell Biol. Lipids [Internet]. 2020; 1865(7):158700. doi: https://doi.org/g672b2

[16] Mikuła R, Pruszyńska–Oszmałek E, Pszczola M, Rząsińska J, Sassek M, Nowak KW, Nogowski L, Kołodziejski PA. Changes in metabolic and hormonal profiles during transition period in dairy cattle–the role of spexin. BMC Vet. Res. [Internet]. 2021; 17:359. doi: https://doi.org/g9dzxd

[17] Uztimür M, Ünal CN. Evaluation of pregnancy toxemia in goats: Metabolic profile, hormonal findings, and redox balance. Small Rumin. Res. [Internet]. 2024; 241:107385. doi: https://doi.org/pzw6

[18] Shimizu H, Oh–i S, Hashimoto K, Nakata M, Yamamoto S, Yoshida N, Eguchi H, Kato I, Inoue K, Satoh T, Okada S, Yamada M, Yada T, Mori M. Peripheral administration of nesfatin–1 reduces food intake in mice: the leptin–independent mechanism. Endocrinol. [Internet]. 2009; 150(2):662-671. doi: https://doi.org/d9tt7g

[19] Su Y, Zhang J, Tang Y, Bi F, Liu JN. The novel function of nesfatin–1: anti–hyperglycemia. Biochem. Biophys. Res. Commun. [Internet]. 2010; 391(1):1039-1042. doi: https://doi.org/b363pz

[20] Ogiso K, Asakawa A, Amitani H, Nakahara T, Ushikai M, Haruta I, Koyama KI, Amitani M, Harada T, Yasuhara D, Inui A. Plasma nesfatin–1 concentrations in restricting–type anorexia nervosa. Peptides [Internet]. 2011; 32(1):150-153. doi: https://doi.org/cc4xhs

[21] Zhang Z, Li L, Yang M, Liu H, Boden G, Yang G. Increased plasma levels of nesfatin–1 in patients with newly diagnosed type 2 diabetes mellitus. Exp. Clin. Endocrinol. Diabetes [Internet]. 2012; 120(2):91-95. doi: https://doi.org/fscsqc

[22] Tan BK, Hallschmid M, Kern W, Lehnert H, Randeva HS. Decreased cerebrospinal fluid/ plasma ratio of the novel satiety molecule, nesfatin–1/NUCB-2, in obese humans: Evidence of nesfatin–1/NUCB-2 resistance and implications for obesity treatment. J. Clin. Endocrinol. Metab. [Internet]. 2011; 96(4):669-673. doi: https://doi.org/fp35cx

[23] Yosten GL. Novel neuropeptides in the control of food intake: neuronostatin and nesfatin–1. Vitam. Horm. [Internet]. 2013; 92:1-25. doi: https://doi.org/pzxx

[24] Başar O, Akbal E, Köklü S, Koçak E, Tuna Y, Ekiz F, Gültuna S, Meriç Yılmaz F, Aydoğan T. A novel appetite peptide, nesfatin–1 in patients with non–alcoholic fatty liver disease. Scand. J. Clin. Lab. Invest. [Internet]. 2012; 72(6):479-483. doi: https://doi.org/g7rv2f

[25] Li Z, Gao L, Tang H, Yin Y, Xiang X, Li Y. Zhao J, Mulholland M, Zhang W. Peripheral effects of nesfatin–1 on glucose homeostasis. PloS One. [Internet]. 2013; 8(8):e71513. doi: https://doi.org/f5d2sk

[26] Yang M, Zhang Z, Wang C, Li K, Li S, Boden G, Li L, Yang G. Nesfatin–1 action in the brain increases insulin sensitivity through Akt/AMPK/TORC2 pathway in diet–induced insulin resistance. Diabetes. [Internet]. 2012; 61(8):1959-1968. doi: https://doi.org/f35rpk

[27] Nakata M, Manaka K, Yamamoto S, Mori M, Yada T. Nesfatin–1 enhances glucose–induced insulin secretion by promoting Ca^{2+} influx through L–type channels in mouse islet β–cells. Endocr. J. [Internet]. 2011; 58(4):305-313. doi: https://doi.org/dtrc74

[28] Aslan M, Celik O, Celik N, Turkcuoglu I, Yilmaz E, Karaer A, Simsek Y, Celik E, Aydin, S. Cord blood nesfatin–1 and apelin-36 levels in gestational diabetes mellitus. Endocrine [Internet]. 2012; 41:424-429. doi: https://doi.org/fzdrq9

[29] Bogardus C. Metabolic abnormalities in the development of non–insulin dependent diabetes mellitus. In: LeRoith D, Taylor SI, Olefsky JM, editors. Diabetes Mellitus: A fundamental and clinical text. 3rd ed. Philadelphia (USA): Lippincott–Raven. 1996; p. 459-467.

[30] Herpertz S, Wagener R, Albus C, Kocnar M, Wagner R, Best F, Schleppinghoff BS, Filz HP, Förster K, Thomas W, Mann K, Köhle K, Senf W. Diabetes mellitus and eating disorders: a multicenter study on the comorbidity of the two diseases. J. Psychosom. Res. [Internet]. 1998; 44(3-4):503-515. doi: https://doi.org/dtdd2q

[31] Duehlmeier R, Fluegge I, Schwert B, Ganter M. Insulin sensitivity during late gestation in ewes affected by pregnancy toxaemia and in ewes with high and low susceptibility to this disorder. J. Vet. Intern. Med. [Internet]. 2013; 27:359–366. doi: https://doi.org/f4rb2w

[32] Oh–I S, Shimizu H, Satoh T, Okada S, Adachi S, Inoue K, Eguchi H, Yamamoto M, Imaki T, Hashimoto K, Tsuchiya T, Monden T, Horiguchi K, Yamada M, Mori M. Identification of nesfatin–1 as a satiety molecule in the hypothalamus. Nature [Internet]. 2006; 443(7112):709-712. doi: https://doi.org/bzkh96

[33] Liu Y, Chen X, Qu Y, Song L, Lin Q, Li M, Su K, Li Y, Dong, J. Central nesfatin–1 activates lipid mobilization in adipose tissue and fatty acid oxidation in muscle via the sympathetic nervous system. BioFactors. [Internet]. 2020; 46(3):454-464. doi: https://doi.org/pzz6

[34] Dong J, Xu H, Xu H, Wang PF, Cai GJ, Song HF, Wang CC, Dong ZT, Ju YJ, Jiang ZY. Nesfatin–1 stimulates fatty–acid oxidation by activating AMP–activated protein kinase in STZ–induced type 2 diabetic mice. PLoS One. [Internet]. 2013; 8(12):83397. doi: https://doi.org/pzz7

[35] Pietrocola F, Galluzzi L, Bravo–San Pedro JM, Madeo F, Kroemer G. Acetyl coenzyme A: A central metabolite and second messenger. Cell. Metab. [Internet]. 2015; 21(6):805–821. doi: https://doi.org/f7d239

[36] Watanabe M, Tozzi R, Risi R, Tuccinardi D, Mariani S, Basciani S, Spera G, Lubrano C, Gnessi L. Beneficial effects of the ketogenic diet on nonalcoholic fatty liver disease: a comprehensive review of the literature. Obes. Rev. [Internet]. 2020; 21(8):e13024. doi: https://doi.org/gpnmrs

[37] Bradshaw PC. Acetyl–CoA metabolism and histone acetylation in the regulation of aging and lifespan. Antioxidants [Internet]. 2021; 10(4):572. doi: https://doi.org/gnh2xt

[38] Tagaya Y, Osaki A, Miura A, Okada S, Ohshima K, Hashimoto K, Yamada M, Satoh T, Shimizu H, Mori M. Secreted nucleobindin-2 inhibits 3T3-L1 adipocyte differentiation. Protein Pept. Lett. [Internet]. 2012; 19(9):997-1004. doi: https://doi.org/f35tz6

[39] Kolodziejski PA, Pruszynska Oszmalek E, Micker M, Skrzypski M, Wojciechowicz T, Szwarckopf P, Skieresz Szewczyk K, Nowak KW, Strowski MZ. Spexin: A novel regulator of adipogenesis and fat tissue metabolism. Biochim. Biophys. Acta, Mol. Cell. Biol. Lipids [Internet]. 2018; 1863(10):1228-1236. doi: https://doi.org/gfdwwt