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Investigation of the relationship of apelin hormone response with some physiological parameters in Maedi-Visna infected sheep

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ABSTRACT: In this study, the objective was to assess the serological characteristics of sheep, during lactation and pregnancy periods, and rams belonging to the Red Karaman and White Karaman–Kangal race infected with maedi-visna infection and body condition score (BCS) of ≤2, 3- 3.5 and ≥4 (high) and to analyse the relationship between the level of apelin and its secretion. Apelin level in the blood serum samples obtained from the jugular veinof the sheeps was determined using ELISA method. As a result of the analyses, it was determined that the level of apelin was statistically different between the races; between lactating sheep and pregnant sheep; and sheep and rams. There was no difference between the BCS groups ($p > 0.05$). Race and sex interaction as well as race, sex and BCS triple interaction effects were found significant ($p < 0.05$). It was observed that the sheep belonging to the White Karaman–Kangal race were 18 times more likely to be infected with the virus. A decrease in apelin level was observed in the sheep with infection, and it was found that the risk of infection was 0.37 times higher in rams than in lactating sheep ($p < 0.05$).

Keywords: Maedi-Visna; ewe; Apelin; hormone; breed; gender; pregnancy; ELİSA

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INTRODUCTION

Maedi-visna (MV) is a viral infection that causes significant economic losses in the sheep breeding sector worldwide. Its causative agent, maedi-visna virus (MVV), belongs to the lentivirus class of Retroviridae family. It is in the small-ruminant lentivirus (SRLV) subgroup along with caprine arthritis encephalitis virus and classified under the same genus as the human lentivirus [human immunodeficiency virus (HIV)](Gayo et al. 2019;Thormar et al.2005).

MVV infection causes progressive weight loss as well as non-purulent chronic inflammation development in lungs, mammary glands, joints and central nervous system in adult sheep and goats(Russo et al., 1988; Phelps and Smith,1993). It is transmitted through ingestion of infected colostrum and milk and inhalation of respiratory secretions(Gomez-Lucia et al.2018;Peterhans et al.2004; Blacklaws et al. 2004). Primary targets of MVV are monocytes/macrophages and dendritic cells (Slingenbergh, 2019; Gendelman et al. 1986). These cells, which are affected by MVV, migrate to the regional lymph nodes through which the virus systemically spreads and dendritic cells (Slingenbergh, 2019) and persistently circulates throughout the body without getting affected by immune response. Thus, infection is classified as a slow virus infection owing to its long incubation period and the ability for continuous reservoir which is persistent without being affected by immune response and which can continue life-long (Gendelman et al. 1986;Stonos et al.2014;Singh et al., 2006).

Hormones are one of the important factors in the formation of suitable conditions for the reproduction and secretion of infected viruses. Cytokines such as interferon-gamma (IFN- γ) and granulocyte macrophage colony stimulating factor (GM-CSF) and hormones such as steroids provide the suitable cellular medium for virus reproduction (Zhang et al. 2002).

Hormones act as transcription factors by binding to regions called transcription binding sites in the lentivirus DNA (Puffer et al. 2000; Gomez-Lucia et al.2014;Gomez-Lucia et al.2018). Hypothalamic pituitary adrenal (HPA) axis plays an important immunomodulatory role in the development of viral infection (Bailey et al.2003).The apelin hormone which forms the basis of our study is excessively present in regions controlling the HPA axis activity such as the supraoptic nucleus, the paraventricular nucleus and the central nervous system (Newson et al.2009; O'Carroll et al. 2013).

Apelin is a hormone of the hormone–cytokine family; it is secreted from adipose tissue, has a 77 amino-acid precursor and has various isoforms such as apelin-12, 13, 17 and 36 (Tatemoto et al. 2001). Among its isoforms, apelin-13 has the highest biological activity owing to its pyroglutamate modification characteristics (Beltowski,2006; Kleinz and Davenport, 2005). Apelin–APJ (apelin receptor) is reported to have potential therapeutic effect in the prevention of HIV-1 infection (Fan et al. 2003; Kakizawa, 2016; Sing et al. 2006).

The primary clinical symptoms of MV infection are primary interstitial pneumonia, encephalitis, lymphadenopathy, arthritis, mastitis (Anker and Coats, 1999) and most distinctly, chronic weight loss (Russo et al. 1988; Phelps and Smith, 1993). The reproduction and secretion of virus in MVV infection are affected by the reproductive cycle (lactation, pregnancy, etc.) (Gomez-Lucia et al. 2014; Ouzrout and Lerondelle,1990). In this study, the objective was to comparatively analyse through serological and molecular tests the effect of race, gender and lactation on serum apelin levels in fat-tailed sheep belonging to the Red Karaman and White Karaman–Kangal race having different body condition scores (BCS of ≤2, 3–3.5 or ≥4) in terms of MVV infection.

MATERIALSANDMETHODS

Ethical Approval

Ethical approval for this study was obtained from the Bayburt University Local Ethics Committee (27/03/2019/01; Approval Number: 2019-2).

Animal Selection and Creationof Groups

In this study, 180 sheep were used analyse the relationship between apelin level and secretion in terms of MVV infection through serological and molecular tests. The animals used in the study were divided into three groups as Group I (lactation group), ewes in the early lactation period (n=60); Group II (pregnancy group), ewes in the first period of pregnancy (on the 100th day of first pregnancy) (n=60); Group III (rams) by randomization so that the average total live weight of the groups was equal.

The study was conducted in 6 ewe farms in Bayburt Province center and two districts (Demirozu and Aydıntepe) (40.16N,39.89-K;40.22N,40.26-K;40.3N,40.14-K) which ewe production is performed under intensive conditions and records are regularly followed up. BCS controls and scoring were

performed by four referees prior to the inclusion of rams. A scale of 5 points with 0.5 intervals was used in BCS scoring. In the trial plan, sheep and rams were analysed under three different groups according to their BCS of ≤2, 3 and ≥4 (Sarı et al. 2013). During the study period, the environment and feed factor were taken into account.The content of feeds used in this study was analyzed (Table-1) according to the standard AOAC methods (AOAC, 2005).

Table 1. Nutrient composition of diets used in the study (%)

Ration Composition	Red Karaman	White Karaman-Kangal
Barley	65.0	65.0
Wheatbran	10.5	7.0
Soybeanmeal	22.0	20.5
Dicalciumphosphate	1.0	1.0
Salt	0,5	0,5
Premix	0.5	0.5
Chemical composition %		
Dry Matter	90.39	90.60
Crudeprotein	17.37	16.99
Crudeash	5.59	5.78
ADF	8.84	11.26
NDF	34.44	32.72
MEKcal/kg	2642	2620

1 kg vit.-min. Premix contains vitamin A, 7,000,000 IU; vitamin D3, 1,000,000 IU; vitamin E, 30,000 IU; Mn, 50,000 mg; Zn, 50,000 mg; Fe, 50,000; Cu, 10,000 mg; I, 8,000 mg; Co, 200 mg; Se, 150 mg; and Mg, 100 mg

Collection of Blood Samples

Blood sample of 10 ml was collected from the jugular vein of the ewe into the tubes with EDTA and without anticoagulant (VACUETTE® TUBE 9 ml Serum Clot Activator). The blood samples without anticoagulant were centrifuged at 3000 rpm/min for 10 minutes on a refrigerated centrifuge (NF 1200, NUVE, Ankara, TURKEY) in the laboratory, then blood serums were separated. The separated serums were transferred into sterile tubes and stored in deep freezers (-80°C) until the laboratory analyses were carried out. The blood samples with EDTA were stored in deep freezers (-80°C) until the DNA extraction.

Measurement of apelin hormone levels in serum

The basic principle of the ELISA method is based on the use of enzyme to determine the antigen-antibody combination in the sample. The enzyme used converts the colorless layer (chromogen) into a colored product, indicating the presence of antigen-antibody, and the intensity of the resulting color is read by the elisa plate reader at the recommended wavelength so the relevant concentration is determined (Marai et al. 2007).The minimum detectable concentration of the apelin hormone kit used in the measurement of apelin levels in the blood serums obtained as a result of the study is reported as <18.75 pg/ml. The race-specific ewe APLN ELISA Kit (Apelin, FineTest, Product code: ESH0081, CHINA) was studied in accordance with the procedure described in the manufacturer's catalog using the determination of 31.25-2000 pg/ml, the intra assay coefficient of 8.0% and the inter-assay coefficient of 10.0% (Tsiodras et al., 2010).

Enzyme Linked Immunosorbent Assay (ELISA)

In the herd screening of MVV infection, the most sensitive and fastest screening test is performed by ELISA (Reina et al. 2009; Preziuso et al., 2010; Kurowska et al. 2018). Commercial kits (IDEXX MVV/CAEV p28 Ab screening, Lot:E891, IDEXX, USA) were used for the detection of Anti-MVV/CAEV antibodies (Arık et al. 2015; Çelik et al. 2018). The study was carried out with the ELISA method and in accordance with the suggestions of the producer company. The plates were measured at 450 nm to obtaining the optical density (OD) data, and the derived results were calculated in accordance with the procedures.

DNA extraction and PCR

Proviral DNA was extracted from whole blood (with EDTA) samples using High Pure Viral Nucleic Acid Kit (Roche, Germany) according to the manufacturers' instructions. Extracted MVV DNA was stored at -80°C until further use. LTR gene regions was amplified by PCR using specific primer pairs reported by

Extramania et al. (2002), producing fragments of approximately 300 nt. LTR-PCR was performed in a 20 uL reaction consisting of 10X reaction buffer, 2 mM MgCl2, 0.2 mM dNTP, 0.2 pmol each primer and 1.25 U Taq DNA polymerase (Fermentas, Lithuania) (Extramania et al. 2002; Yıldırım et al. 2011). The PCR cycling profile included a denaturation stage at 96 °C for 6 min, followed by 35 cycles of 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. PCR products were analyzed after electrophoresis in 1,5 % agarose gel containing ethidium bromide and visualized on a UV transilluminator (Kodak, 2019).

Sequencing and Phylogenetic Analysis

PCR fragments were purified from gel using a purification kit (High Pure PCR Product Purification Kit, Roche, Germany) and sequenced using a sequencing kit (BigDye Terminator v3.1 Cycle Sequencing kit, Applied Biosystems, USA) on a genetic analyzer (ABI 3130xl, Applied Biosystems, USA). After sequence assembly and editing using Bioedit (Version 7.0.5.3) and Clustal W, the samples were then compared with the GenBank nucleotide sequence database for sequence similarities via the basic length alignment search tool (BLAST) software of the National Centre for Biotechnology Information (NCBI) (Extramania et al. 2002; Altschul et al. 1997; Hall and BioEdit, 1999). Phylogenetic tree for the 276 bp fragment of Maedi Visna Virus was constructed using the Maximum Likelihood (ML) method of the MEGA X v.10.0.4 software, based on the evolutionary distances between different sequences calculated by the Kimura two-parameter model (Tamura et al. 2011). The confidence level of the ML tree was assessed by bootstrapping, using 1,000 replicates.The nucleotide sequence of TR-Bayburt-2019 from this study have been submitted to GenBank and assigned the accession number MN197477. A phylogenetic tree of the TR-Bayburt-2019 sequence comparing it to other reference sequences in the GenBank database formed a separate branch in which only the Turkish, American and Greek sequences were included, suggesting a common origin.

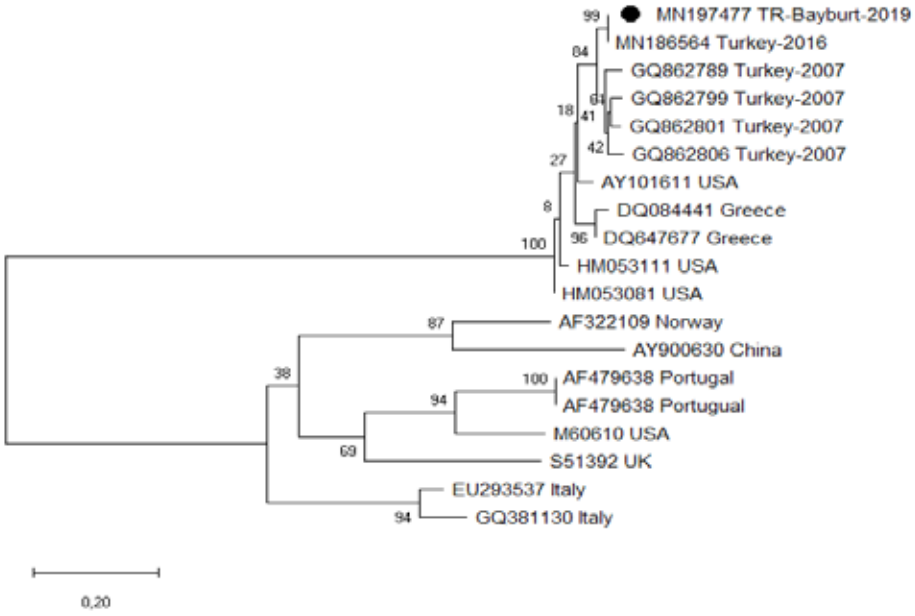

Figure 1. Molecular Phylogenetic analysis by Maximum Likelihood method

Maximum Likelihood tree constructed using the Kimura 2-parameter model, based on the LTR gene (276 bp). The numbers indicate bootstrap values (1,000 replicates) in the figure. Sequences characterized in this study are marked using black dots. The scale bar at the bottom represents genetic distances in nucleotide substitutions per site. Horizontal distances are proportional to sequence distances.

StatisticalAnalysis

In this study, the difference of serum apelin hormone levels in infected and non-infected animals was investigated by t-test. The effects of gender, body score and race factors on the serum apelin hormone levels for Red Karaman and White Karaman-Kangal ewe races in the lactation and pregnancy period were estimated. The gender, body score and race factors

were analyzed as fixed effects. The normality test was applied to the apelin measurements for the estimation of the effects of gender, body score and race on the serum apelin hormone levels of Red Karaman and White Karaman-Kangal ewe races at lactation and pregnancy period. Then, the univariate procedure was used in the generalized linear models (GLM) for the normally distributed apelin measurements. The analyse was performed in the full factorial setting with 3 factors (2x2x3) according to completely randomized design, and the interaction effects of the body score and race, body score and gender, race and gender, and body score, gender and race. The investigated effects and interactions with the statistical model as given below:

$$Y_{ijkl}=\mu+\alpha_i+\beta_j+\gamma_k+(\alpha\beta)_{ij}+(\alpha\gamma)_{ik}+(\beta\gamma)_{jk}+(\alpha\beta\gamma)_{ijk}+\varepsilon_{ijkl}$$

Where, Y is the apelin hormone, μ: Mean, α is the body score, β is the gender and γ is the race effect. Tukey's multiple comparison test was used to compare the differences for the means of apelin hormone. JMP 7 statistical package (JMP, 2019) was used in all analyses in this study. All significant differences were evaluated by testing at P<0.05 level.

Moreover, the gender, race and body score effects on Maedi Visna were investigated with binary logistic regression. Because the Maedi Visna was categorical variable and has just two categories (uninfected:0 and infected:1) the Wald test was used to verify that coefficient βi differs from 0. The applied binary logistic regression model was shown as follow: X_1, X_2, and X_3 are gender, race and body score independent effects and P(Y) probability of virus occurence or not.

$$P(Y)=\frac{1}{1+e^{-(\beta_o+\beta^1X_1+\beta_2X_2+\beta_3X_3)}}$$

RESULTS

Serum apelin values (pg/ml) measured in MVV-infected sheep and rams were presented in Table 2.It was determined that 47 of the 180 blood serums constituting the basis of the study were positive in terms of MVV antibody (Table 3). Accordingly, the apelin level was higher in non-infected animals than in infected animals, and this difference was statistically significant ($p < 0.01$). Moreover, serum apelin values (pg/ml) of the MVV-infected sheep measured during lactation and pregnancy, BCS periods and serum apelin values (pg/ml) of the rams were presented in Table 2.

Table 2: The Least square means (±SEM) and p values for simultaneous comparison of apelin in ewe and ramsinteraction of gender, races and body condition score (ng/ml)

Gender	N	Mean±SEM
Ewe in Lactation	60	2.806±0.160^{c}
Ewe in Pregnancy	60	5.820±0.160^{a}
Rams	60	4.497±0.160^{b}
Races	**N**	**Mean±SEM**
Red Karaman	90	4.69 ± 0.16^{a}
White Karaman-Kangal	90	4.17 ± 0.16^{b}
BCS	**N**	**Mean±SEM**
BCS ≤2	60	4.26±0.16
BCS = 3- 3.5	60	4.54±0.16
BCS≥4	60	4.32±0.16
Source of variation (P-values)		
Gender	0.00	
Races	0.04	
BCS	0.08	

Race*Gender (Mean±*SEM)*	**Ewe in Lactation**	**Ewe in Pregnancy**	**Rams**
Red Karaman	3.308±0.226^{c}	5.157±0.226^{b}	5.284±0.226^{b}
White Karaman-Kangal	2.304±0.226^{d}	6.482±0.226^{a}	3.709±0.226^{c}

Race*Gender*BCS	**Red Karaman(Mean±SEM*)***			**White Karaman-Kangal(Mean±SEM*)***		
BCS/Gender	**Ewe in Lactation**	**Ewe in Pregnancy**	**Rams**	**Ewe in Lactation**	**Ewe in Pregnancy**	**Rams**
BCS≤2	3.38±0.47^{defg}	5.29±0.47^{abcd}	5.05±0.47^{abcde}	2.25±0.47^{fg}	5.54±0.47^{abc}	4.05±0.47^{cdef}
BCS=3-3.5	3.38±0.47^{defg}	6.09±0.47^{abcde}	5.00±0.47$_{bcde}$	2.72±0.47^{fg}	6.90±0.47^{ab}	4.17±0.47^{cdef}
BCS≥4	3.17±0.47^{efg}	5.09±0.47^{abcde}	5.81±0.47^{abc}	1.95±0.47^{g}	7.01±0.47^{a}	2.90±0.47^{fg}

*Means within the same column showing different superscripts are significantly different (P < 0.05) * Significant at 0.05 level, ** Significant at 0.01 level, NS: Not significant (P > 0.05). SEM = standard error of the mean.* a, b, c, d, e, f, g: Means with different letters are statistically different (P<0.05).

As a result of the analyses of our study, it was determined that the apelin level showed a statistically significant difference between races ($p < 0.01$). Similarly, apelin levels showed statistically significant difference in rams and sheep in lactation and pregnancy ($p < 0.05$) (Table 2). It was detected that apelin levels did not change in BCS groups ($p > 0.05$). Moreover, it was detected that apelin levels in different sexes showed different trends according to races; in other words, it was found that the interaction effect of race and sex was statistically significant ($p < 0.01$). Conversely, race, BCS and race–BCS pairwise interaction effects were not significant, whereas triple interaction effect of race, sex and BCS was significant ($p < 0.05$).

Table 3. The Least square means standard errors of for serum apelin hormone level for virus infections

	The presence of the virus	N	Mean± Std. Error Mean	P
Apelin hormone level	0 (Negative)	133	5,22±0,121	0.00
	1 (Positive)	47	1,99± 0,073	

When the logistic regression analyses results are examined, it is obtained that the race effectis statistically significance on MVV enfection.The risk of MVV for White Karaman-Kangal race was18 times higher than Red Karaman race. In terms of gender, when the sheeps in lactation are accepted as indicators and pregnancy has no risk effect (P=0.996), but the risk of rams being infected is 0.37 times higher than sheeps in lactation. Moreover, when the BCS:3-3.5 was held as an indicator,the risk of MVV for BCS≤2 and BCS≥4 were 6.26 and 4.54 times higher risk than BCS:3-3.5, respectively (Table 4).

Table 4. Coefficient of factors, standard errors, Wald statistics and odds ratios

						Odds Ratio
	N	B	S.E.	Wald	P	Exp(B)
Race	180	2.89	0.54	29.12	.000	18.02
BCS:3-3.5	60			9.56	.008	
BCS≤2	60	1.84	0.62	8.64	.003	6.26
BCS≥4	60	1.51	0.61	6.13	.013	4.54
Ewe in Lactation	60			4.21	.122	
Ewe in Pregnancy	60	-21.81	4613.26	0.00	.996	0.00
Ram	60	-1.01	0.49	4.21	.040	0.37
Constant		-2.78	0.63	19.57	.000	0.06

Likelihoodratio(omnibüs,p<0.001)andHosmer-Lemeshowtest(p=0.099)

DISCUSSION AND CONCLUSION

MVV is an infection of adult sheep; it leads to symptoms associated with progressive interstitial pneumonia in its maedi form and symptoms associated with meningoencephalitis in its visna form. The development of infection usually takes 3–4 years for the maedi form and approximately 2 years for the visna form, and a large portion of the sheep can carry the virus through their lifetime or complete their economic lifetime without manifesting symptoms (Tan and Alkan, 2002;Lopez and Martinson, 2017).Seroprevalence studies are important because they affect the success of eradication and control programmes.

The first clinical symptom of MVV infection manifests as the disruption of body condition and with the progression of the disease, death occurs owing to respiratory problems(Karaoğlu et al. 2003). In our current study, although the apelin level was higher and statistically different in the non-infected animals compared with the infected ones ($p < 0.01$), the effect of BCS on apelin level was not different at a statistically significant level ($p \geq 0.05$). We believe that this may be caused by the duration for which the sheep were infected by the virus and the latent course of infection. It is consistent with the results of study where the relationship between MVV infection and various BCSs are analysed and the difference is shown in the results (Tefera et al. 2016; Özkan et al. 2014). Because our study is the first study analysing the change intervals and effects of the apelin level in various BCSs, literature data is limited in terms of discussion of the results. Conversely, although BCS levels are not significant in terms of apelin, according to the logistic regression analysis results, it was determined that the risk of infection by MVV was 6.26 and 4.54 times higher in the BCS ≤ 2 and BCS ≥ 4 groups compared with the BCS = 3–3.5 group. This is consistent

with the literature data stating that the first symptom of infection is the disruption of body condition.

Genetic factor is an important component in infection control. Breeding of races known to have genetic resistance (Wachendörfer et al.1995)is an important strategy in infection control. Several studies report that some races are extremely sensitive to MVV infection and that some show high resistance (Wachendörfer et al.1995; Simard and Briscoe, 1990; Houwers et al. 1984; Cutlip et al. 1992). The seropositivity in the sheep of White Karaman–Kangal race with MVV infection was lower than that in those without the infection. When the effect of race on apelin level was analysed, it was found statistically significant ($p < 0.05$). Indeed, according to the logistic regression analysis, the White Karaman–Kangal race sheep were 18 times more likely to be infected by the virus. Red Karaman race sheep were more resistant to infection compared with the White Karaman Kangal race sheep in the study sample, and this is consistent with results of previous study [42]and the studies reporting race resistance Simard and Briscoe, 1990; Burgu et al. 1990; Kandil et al.1997). We propose breeding of sheep belonging to the Red Karaman race in the regions with high infection seroprevalence owing to its resistance to MVV infection. However, there is a need for a larger number of studies to support our recommendation regarding the effect of the race.

Epidemiologic studies have reported that sex, particularly female sex, plays a primary role in infection transmission owing to transfer of MVV by infected sheep to their lambs through milk and colostrum (Slingenbergh, 2019; Simard and Briscoe, 1990; Burgu et al. 1990; Kandil et al. 1997; Muz et al. 2013). Sex difference and correlation of infection incidence have been reported in previous studies (Simard and Briscoe, 1990; Burgu et al.1990). According to the infection status of the rams in our study sample, the rate was 56% in the rams belonging to the White Karaman–Kangal race and 6% in those belonging to the Red Karaman race. It was 73% in the non-pregnant sheep belonging to the White Karaman–Kangal race and 20% in those belonging to the Red Karaman race. The results of our study are consistent with the studies evaluating the effect of gender(Simard and Briscoe, 1990; Burgu et al.1990). Burgu et al. (1990) found the effect of sex statistically insignificant (Burgu et al.1990). We believe that this may be caused by the increase in seropositivity due to close contact with infected female sheep during the mating period, although the rams are separately housed.

In pregnant animals, research and detection of infected pregnant sheep prevalence has a strategic importance in abortion events or the occurrence of smaller than normal and weak offspring births (Burgu et al.1990;Kandil et al. 1997; Oguma et al.2013) and the understanding of prevalence rate and ratio of the infection in the lambs to be born, development of disease prevention and eradication programmes. It should be emphasised that in our study sample, all sheep in the early pregnancy period had negative results for MVV infection because during pregnancy highly complicated physiological processes occur (Ouzrout and Lerondelle, 1990).

In early pregnancy, apelin levels increase to regulate normal placentation(Van Mieghem et al. 2010) thus, we concluded that the increased hormone concentration may have an inhibitory effect on virus expression. Due to current literature information being limited in comparison to our pregnancy period study results, when studies similar to our studies are examined, they show compatibility (Slingenbergh, 2019; Ouzrout and Lerondelle, 1990). Respiratory fluids, milk and colostrum containing infective monocytes and macrophages are one of the primary transmission paths. Thus, lactation is one of the important factors in the spread of infection(Gomez-Lucia et al.2018; Legastelois et al. 1998). It is reported that infection spreads at a rate of 28% at the end of 10 hours via milk and colostrum. Moreover, because of the lactation period providing the opportunity for the sheep to be isolated from milk within the 5 months (Simard and Briscoe, 1990) isolation during the lactation period may present a strategic importance in eradication programmes. Similarly, apelin levels are reported to show a significant increase in apelin expression during mammary gland, pregnancy and lactation periods (Habata et al. 1990).A decrease was observed in apelin levels in infected sheep; compared with lactating sheep with infection, rams were found to have 0.37 times higher risk of being infected ($p < 0.05$). There are no studies evaluating serum apelin levels in MVV-infected sheep during lactation; however, the results of our study are consistent with the results of the study reporting that there are severe increases in apelin expression during the lactation period (Habata et al. 1990).

MVV infection is also called ovine lentivirus-induced lymphoid interstitial pneumonia (Mingujion et al.2015). It is reported that sheep may acquire infec-

tion at any age by droplet inhalation via respiration. In pathogenesis, hyperplasia and fibrosis in muscle fibres develop as a result of infiltration and thickening in the intraalveolar regions in lungs (Luján et al.2019).It has been reported that apelin which forms the basis of our study is important in the prevention of fibrosis in lungs(Pchejetski et al2011; Wang et al. 2014) and regulation of pulmonary vascular homeostasis (Kim, 2014).

Hormones play an important role in viral infections owing to providing an appropriate medium for the reproduction and excretion of infectious viruses as a result of host cell reactivation (Ouzrout and Lerondelle, 1990). Thus, we hypothesise that hormonal treatments based on therapeutic molecules form an alternative and important strategic approach in viral therapies.

Apelin is a promising therapeutic agent in many pathologies such as inflammatory (Zhou et al.2016; Tsiodras et al. 2010) and cardiovascular diseases induced by oxidative stressZhou et al. 2016; Tsiodras et al. 2010),obesity (Wysocka,2018) and cancer (Rayalam et al.2011İ Uribesalgo et al. 2019). Moreover, apelin has the ability to prevent infection owing to its inhibitory effect on HIV and human lentivirus (Esposito et al. 2002;Gayo et al. 2019; Thormar, 2005), both of which have homolog similarity with MVV (Kleinz, 2005; Zou et al. 2000).Because there is no treatment or vaccination for MVV infection (Polledo et al. 2013). We hypothesise that apelin is a potential therapeutic agent owing to its physiological role. However, it is necessary to conduct studies on a larger number of animals and different sheep races regarding apelin treatment to determine the physiological mechanisms and to support with prospective studies which can be developed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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