DOI: 10.1002/vms3.1345

ORIGINAL ARTICLE

RUMINANTS

WILEY

Serum pregnancy–associated glycoprotein profiles during early gestation in Karya and Konya Merino sheep

Mehmet Akköse[1] | Elif Merve Çınar[2] | Murat Onur Yazlık[3] | Ufuk Kaya[4] | Yadigar Polat[5] | Çiğdem Çebi[6] | Ceyhan Özbeyaz[7] | Mehmet Rıfat Vural[3]

[1]Department of Livestock, Dalaman Agricultural Enterprise, General Directorate of Agricultural Enterprises, Muğla, Turkey

[2]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Turkey

[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

[4]Department of Biostatistics, Faculty of Veterinary Medicine, Hatay Mustafa Kemal University, Hatay, Turkey

[5]Department of Medical Services and Techniques, Vocational High School of Health Services, Kilis 7 Aralık University, Kilis, Turkey

[6]Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkey

[7]Department of Animal Sciences, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

Correspondence
Ceyhan Özbeyaz, Department of Animal Sciences, Faculty of Veterinary Medicine, Ankara University, Dışkapı, Ankara 06110, Turkey.
Email: ozbeyaz@ankara.edu.tr

Abstract

Background: Reported as being expressed by mono- and binucleate placental cells, pregnancy-associated glycoproteins (PAGs) are released into the blood circulation from the ruminant placenta. Circulating gestational PAGs levels may differ between sheep breeds.

Objective: This study was aimed at the close monitoring of the serum PAGs profiles of Karya and Konya Merino sheep during early pregnancy.

Methods: Fifteen Karya and 15 Konya Merino ewes were synchronized by a 12-day treatment with progesterone-impregnated intravaginal sponges. After the sponges were withdrawn, the ewes were administered 400 IU of equine chorionic gonadotropin. The ewes were allowed to mate naturally, and all animals were sampled for blood as of the day of mating (day 0) at weeks 1, 2, 3, 4 and 5. Pregnancy diagnoses were made by transabdominal ultrasonography at week 5. At weeks 6, 7, 8, 9 and 11, blood samples were collected only from the pregnant ewes. The blood samples were centrifuged at 3000 × *g*, and extracted sera were stored at −20°C until being used for laboratory analyses. Serum PAGs levels were determined with the aid of a commercial PAG-enzyme-linked immunosorbent assay test originally developed for pregnancy diagnosis in cattle. Differences in the between the PAGs levels throughout pregnancy and the group effect (Karya and Konya Merino) were determined with a two-way mixed analysis of variance. Pairwise comparisons were made using a Bonferroni adjustment.

Results: PAGs levels showed a linear increase with the advance of pregnancy in both Karya and Konya Merino sheep. No difference was detected between the breeds for serum PAGs levels. The serum PAGs levels of the pregnant and non-pregnant ewes differed as of the fourth week.

Conclusion: The serum PAGs levels of the Karya and Konya Merino ewes were similar during the first 11 weeks of gestation, and pregnancy diagnosis could be made based on serum PAGs levels as of the 4th week in both breeds.

KEYWORDS
ewe, gestation, management, pregnancy diagnosis, serum

1 | INTRODUCTION

Today, the need to optimize profits in sheep farming encourages breeders to adopt various management strategies, including the limiting of the mating period and the diagnosis of pregnancy at an early stage (De Carolis et al., 2020). Although ewes show, on average, six–nine oestrus cycles during a single breeding season, restricting their mating period to two or three cycles both facilitates flock management during the lambing period and enables the uniform production of mutton lambs. On the other hand, an unlimited and more extended mating period leads to a longer lambing period and results in an increase in associated problems (Sargison et al., 2018). The accurate diagnosis of the pregnancy status of ewes enables non-pregnant ewes to be prepared for re-mating, the appropriate formulation of the rations of pregnant ewes in view of their energy requirements, the prevention of dystocia-associated lamb mortalities, the optimization of the birthweight of lambs and the reduction of feed costs by means of the culling of infertile ewes (Pickworth et al., 2020; Redden & Passavant, 2013).

Karya sheep, characterized by high fertility and high milk production, are known to have originated from the non-systematic introgressive crossbreeding of ewes belonging to sheep breeds local to the Aegean region, including, among others, the Dağlıç and Çine Çaparı, with rams of the breeds Sakız and Kıvırcık as well as Sakız X Kıvırcık hybrids (Karaca et al., 2018). Konya Merino sheep, characterized by high mutton and fleece production, on the other hand, are reported to have originated from the intra-selection of the G2 and G3 hybrids of the German Mutton Merino and Central Anatolian Akkaraman (Sertkaya & Öztürk, 2022; Ünal & Akçapınar, 2001).

In the last 30 years, research has been conducted on pregnancy diagnosis by means of the measurement of the levels of pregnancy-associated glycoproteins (PAGs) secreted by the mono- and binucleate cells of the trophectoderm of the ruminant placenta. PAGs, including pregnancy-specific protein B (PSPB), pregnancy-specific protein 60 and the SBU-3 antigen, were initially described as placental antigens also found in the maternal serum immediately after implantation (Sousa et al., 2006). To date, 11 PAGs cDNA codes have been identified from the ovine placenta using the radioimmunoassay (RIA) method (De Carolis et al., 2020). The detection of PAGs in the maternal circulation is considered a reliable indicator of pregnancy in ruminants (Akkaya Doğan & Köse, 2022; El Amiri et al., 2015; Karakuş et al., 2021; Savela et al., 2009; Silva et al., 2007). Using the RIA and enzyme-linked immunosorbent assay (ELISA) methods, these molecules have been demonstrated to reach detectable levels in the ovine plasma as of the 18th–20th days of pregnancy (Alabart et al., 2010; Barbato et al., 2009; De Carolis et al., 2020; El Amiri et al., 2003, 2007, 2014, 2015). Depending on the breed, plasma PAGs levels have been reported to alter during pregnancy (Milisits-Németh et al., 2018; Redden & Passavant, 2013; Ranilla et al., 1994). In previous research conducted in Türkiye, the early pregnancy serum PAGs levels of ewes of the Konya Merino (Kaplan et al., 2018; Uçar et al., 2018) and Awassi (Akköse et al., 2021) breeds, both of which are commonly raised in the country, have been monitored. This study was aimed at the comparative assessment of the early pregnancy serum PAGs profiles of Karya ewes, characterized by high milk production and fertility, and Konya Merino ewes, bred for meat and wool production.

2 | MATERIALS AND METHODS

2.1 | Animals

This study was carried out between June and August in 2021 at a sheep farm located in the Serinhisar (Kızılhisar) town of the Denizli province in Türkiye. The study material comprised 15 Karya and 15 Konya Merino ewes, all of which were aged 2–5 years, weighed 40–60 kg and did not present with any health problems. All animals were maintained under the same environmental and nutritional conditions. All ewes were fed with barley straw, oat hay and additional concentrate and had access to fresh water and mineral blocks.

The sheep raised at the farm were synchronized using 60 mg of medroxyprogesterone acetate-soaked intravaginal sponges (Esponjavet, Hipra) for a period of 12 days. At the time of the removal of the sponges, each ewe was administered 400 IU of equine chorionic gonadotropin (Folligon, MSD). We did not use any PGF2α analogues for the synchronization of the ewes. All ewes were allowed to mate naturally. Rams equipped with marking harnesses were introduced at sponge removal to identify the mated ewes (and the mating date), and the ewes were checked twice daily. The day on which mating marks were observed was recorded as the day of mating (day 0). On day 35 after mating, a pregnancy examination was performed by transabdominal ultrasonography (Siui 838). Ultrasonographic pregnancy diagnosis was based on the observation of the foetal fluids, placentomes and foetal structures. The ewes identified as being non-pregnant were re-synchronized and prepared for re-mating. However, the non-pregnant/re-mated ewes were not included in the study, and the results pertain to ewes that conceived on the first mating.

Jugular blood samples were collected into gel-coated vacutainers (Hematube) from all the ewes at weeks 1, 2, 3, 4 and 5 after mating (day 0), and from the pregnant ewes only at weeks 6, 7, 8, 9 and 11 post-mating. After being allowed to coagulate at room temperature, the blood samples were centrifuged at 3000 rpm for 5 min to extract the sera. The serum samples were stored at −20°C until being used for laboratory analyses.

Serum PAGs levels were determined using a commercial ELISA kit originally developed for bovine pregnancy diagnosis (Idexx Laboratories). Experienced technical staff performed the tests according to the manufacturer's instructions. Briefly, 96-well microtitre plates were coated with an anti-PAGs monoclonal antibody produced against the PAG-55 protein fraction, which is composed of PAG-4, PAG-6, PAG-9, PAG-16, PAG-18 and PAG-19. The samples and controls were incubated in the anti-PAGs monoclonal antibody-coated microtitre plates. After 2 h of incubation, the test samples were washed, and captured PAGs were detected with a PAGs-specific antibody (detector solution) and horseradish peroxidase conjugate. Next, the unbound conjugate was washed away, and 3,3',5,5'-tetramethylbenzidine substrate was added to the wells. Optical density (OD), which varies depending on the PAGs concentration, was measured at a wavelength of 450 nm using a spectrophotometer. The sample minus negative ($S - N$) value of each sample was calculated by subtracting the mean OD value of two negative controls from the OD value of the samples. As the colour intensity obtained with the test is proportional to the amount of PAGs present in the samples, the $S - N$ values were used as the serum PAGs levels of the samples.

2.2 | Statistical analyses

The normality distribution of the study data was determined using skewness and kurtosis coefficients. Descriptive statistics were calculated for the study groups. Results were expressed in mean ± standard error of the mean. Changes that occurred in the serum PAGs levels during pregnancy and the group effect (Karya and Konya Merino) were determined by a two-way mixed analysis of variance. Pairwise comparisons were made using a Bonferroni adjustment. Statistical calculations were made using the Statistical Package for Social Sciences (SPSS) version 24.0 software. Statistical significance was set at $p < 0.05$.

3 | RESULTS

Pregnancy examinations performed by abdominal ultrasonography on day 35 post-mating demonstrated that, out of the 15 Karya ewes, 4 were non-pregnant and 11 were pregnant, whereas out of the 15 Konya Merino ewes, 3 were non-pregnant and 12 were pregnant. Both breeds displayed similar PAGs profiles during the first 11 weeks of gestation (Table 1).

During the first 3 weeks, no difference was observed between the serum PAGs levels of the pregnant and non-pregnant ewes of both breeds. The serum PAGs levels of the pregnant ewes were observed to differ significantly from those of the non-pregnant ewes as of the fourth week. The serum PAGs levels of the pregnant Karya and Konya Merino ewes were observed to display a steady increase during the first 11 weeks of gestation (Figure 1).

4 | DISCUSSION

Early pregnancy diagnosis has become an essential tool for reproductive management and profitability in sheep production (Karen et al., 2001). PAGs are specific proteins that aid in pregnancy diagnosis and small ruminant management. However, different results have been reported for PAGs levels, which show volatility during the early gestation period (El Amiri et al., 2014, 2015; Kaplan et al., 2018; Ranilla et al., 1994). Previous studies also suggest that differences in PAGs or PSPB levels could be associated with the sheep breed (Redden & Passavant, 2013; Ranilla et al., 1994). The present study demonstrated that PAGs levels change as pregnancy advances. However, these changes were similar in the two breeds. Furthermore, it was also determined that the measurement of PAGs levels aided in the determination of the pregnancy status as early as the fourth week of gestation.

Serum PAGs levels displayed a steady increase with gestational age in both the Konya Merino and Karya ewes. This result confirms early pregnancy profiles previously reported for the serum PAGs levels of the Konya Merino (Uçar et al., 2018), Awassi (Akköse et al., 2021; Akkuş & Yaprakcı, 2022), Awassi X Merino crossbreed (Karen et al., 2003), German Blackheaded Mutton (GBM), Rhoen X GBM X Dorper crossbreed (Ledezma-Torres et al., 2006), Sarda X Lacaune crossbreed (De Carolis et al., 2020) and Corriedale and Ile de France X Texel crossbreed (Rovani et al., 2016), the serum PAG1 levels of the Polypay X Dorset crossbreed (Roberts et al., 2017) and the serum PSPB levels of the Awassi (Akkuş & Yaprakcı, 2022) and Yankasa (Adeyeye et al., 2021) with minor differences. The steady increase in the level of PAGs as pregnancy advances appears to be associated with increased PAGs production in the binucleate cells of the placenta. On the other hand, minor differences between the PAGs expression patterns reported in different studies could indicate differences in the foetal growth rates of breeds. Dissimilarities with previous studies (El Amiri et al., 2014, 2015; Kaplan et al., 2018; Ranilla et al., 1994) for results pertaining to early gestation are attributed to the genetic variations of breeds.

The measurement of serum PAGs levels by the RIA method enables distinguishing between pregnant and non-pregnant ewes as of day 18 (Barbato et al., 2009; De Carolis et al., 2020; El Amiri et al., 2003, 2015; Karen et al., 2003) or as of week 4 (Ledezma-Torres et al., 2006). Pregnant and non-pregnant ewes can be identified with a high accuracy as of day 24 of gestation, based on the measurement of serum PAGs levels by the ELISA test as a more reliable alternative to the RIA method (El Amiri et al., 2007; Redden & Passavant, 2013). Today, commercial PAG-ELISA pregnancy diagnosis test kits are available for use in cattle (Akkose et al., 2019; Akköse, 2023; Szenci, 2021) but have not been validated for specific use in sheep yet. However, the high level of similarity between the molecular structure of bovine and ovine PAGs (Xie et al., 1991) allows the safe use of bovine PAG-ELISA tests in sheep (Akköse, 2020; Chaves et al., 2020; de Miranda e Silva Chaves et al., 2017; Roberts et al., 2019; Steckeler et al., 2018). Precisely, highly accurate pregnancy diagnoses can be performed on day 33 of

TABLE 1 Serum pregnancy-associated glycoproteins (PAGs) $S-N$ values of the pregnant Karya ($n = 11$) and Konya Merino ($n = 12$) ewes during the first 11 weeks of gestation.

		Groups		
		Karya	Konya Merino	Overall means
Sampling time	Week 1	0.071 ± 0.022	0.032 ± 0.019	0.052 ± 0.015^{f}
	Week 2	0.075 ± 0.020	0.040 ± 0.017	0.058 ± 0.013^{f}
	Week 3	0.125 ± 0.026	0.092 ± 0.023	0.108 ± 0.017^{f}
	Week 4	0.257 ± 0.037	0.340 ± 0.032	0.298 ± 0.025^{e}
	Week 5	0.421 ± 0.048	0.492 ± 0.042	0.456 ± 0.032^{d}
	Week 6	0.488 ± 0.044	0.510 ± 0.038	0.499 ± 0.029^{d}
	Week 7	0.581 ± 0.056	0.602 ± 0.049	0.592 ± 0.037^{c}
	Week 8	0.763 ± 0.087	0.879 ± 0.076	0.821 ± 0.058^{b}
	Week 9	1.068 ± 0.097	1.037 ± 0.085	1.052 ± 0.064^{a}
	Week 11	1.277 ± 0.109	1.207 ± 0.096	1.242 ± 0.073^{a}
	Overall means	0.513 ± 0.041	0.523 ± 0.036	
p-Value	Group	0.850		
	Sampling time	<0.001		
	Group × sampling time	0.449		

Note: The superscripts letters (a–f) indicate differences between the PAGs concentrations in the same column. $S-N$: optical density levels of sample minus negative control.

gestation in ewes using bovine PAG-ELISA tests (Rovani et al., 2016) and as of day 26 or 30 using bovine visual pregnancy tests (Akköse, 2020; Xie et al., 1991). The present study strongly supports this argument. Serum PAGs levels were significantly different in pregnant ewes compared to non-pregnant ewes. This is detectable as early as the fourth week of gestation. The substantial increase observed in the serum PAGs levels of pregnant Karya and Konya Merino sheep from the third week to the fourth week of gestation could be indicative of the differentiation of binucleate cells in the trophoblast and their migration to the maternal endometrium, as well as of the onset of implantation starting from the third week. There may be several reasons for PAGs not being able to be detected in ovine serum samples with bovine PAG-ELISA tests before the fourth week of pregnancy, despite the similarity of the molecular structure of bovine and ovine PAGs. Although 11 PAGs cDNA gene families have been identified in sheep (De Carolis et al., 2020), at least 22 PAGs gene families have been determined in cattle (Telugu et al., 2009). The expression patterns of ovine PAGs vary with the gestational stage (El Amiri et al., 2004). PAGs classified as ancient and modern, based on phylogenetic analyses, differ in their expression patterns. Although some PAGs are expressed in all trophoblasts, some others are localized only to binucleate trophoblast giant cells (Wallace et al., 2015). Bovine PAG-ELISA test kits can detect only some PAGs (PAG-4, PAG-6, PAG-9, PAG-16, PAG-18 and PAG-19) of the large PAGs gene family, which are most common in the bovine placenta (Ricci et al., 2017). The abovementioned factors may also explain the differences observed among sheep breeds for the expression pattern of PAGs molecules reported in different studies.

Although the serum PAGs profiles of the ewes having not been assessed for singleton and multiple pregnancies constitute a limitation for the present study, the reason for such a classification having not been made was to mimic the probable results expected to be achieved with the use of the bovine PAG-ELISA test on the field for pregnancy diagnosis. Previous studies have reported that serum PAGs and PSPB levels determined with the RIA and sheep ELISA methods were higher in multiple pregnant ewes, compared to singleton pregnant ewes (Akkuş & Yaprakcı, 2022; El Amiri et al., 2014, 2015; Ranilla et al., 1997). However, the differences observed in the serum PAGs levels of singleton and multiple pregnant ewes measured with bovine PAG-ELISA pregnancy tests do not change the results of pregnancy diagnosis (Akköse et al., 2021; Alkan et al., 2020).

This study was restricted to the first 11 weeks of pregnancy and did not follow animals until parturition and into the post-partum period. As sheep display reproductive seasonality (Gündoğan et al., 2003) and their mating season is restricted, we focused on a framework in which early pregnancy diagnosis could be performed. Plasma PAGs levels have been shown to last for a long time post-partum (Haugejorden et al., 2006), and persistent levels from previous pregnancies could potentially confound the results, when using this test as the sole means of pregnancy diagnosis. As sheep have a long period of post-partum anoestrus until the onset of seasonal oestrus (Mallampati et al., 1971), we think that persistent levels of PAGs from previous pregnancies would not affect pregnancy diagnosis.

5 | CONCLUSION

This study provides a comparative assessment of the early pregnancy serum PAGs levels of Karya and Konya Merino ewes for the first time,

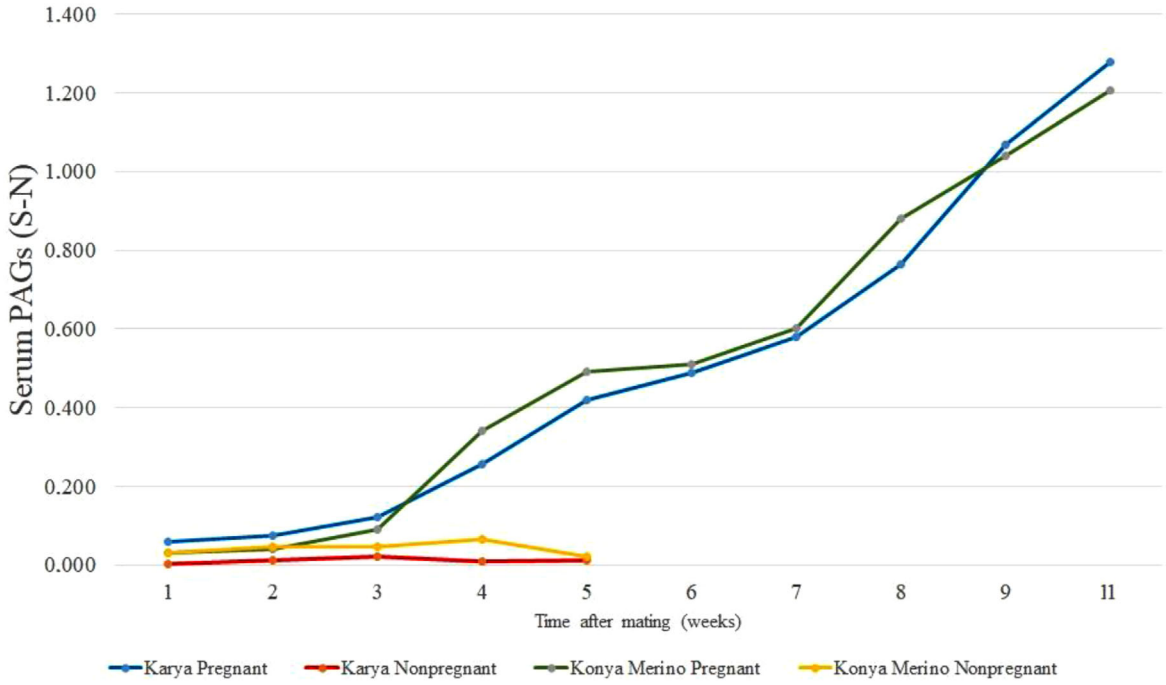

FIGURE 1 Pregnant Karya ($n = 11$) and Konya Merino ($n = 12$) ewes displayed similar pregnancy-associated glycoprotein (PAGs) patterns during the first 11 weeks of gestation. Pregnant and non-pregnant ewes can be identified as of the 4th week of gestation, based on serum PAGs levels. *S − N*: optical density levels of sample minus negative control.

and thereby, contributes to the knowledge available on PAGs in small ruminants. In conclusion, early pregnancy PAGs profiles were observed to be similar in these two breeds during the first 11 weeks of gestation, PAGs levels were determined to display a proportional increase with gestational age, and pregnant and non-pregnant ewes were able to be identified using a commercial bovine PAG-ELISA test as of the fourth week of gestation.

As sheep breeds are reared locally, further studies are needed to determine the PAGs concentrations of major native sheep breeds raised across the world that contribute to the rural economy.

AUTHOR CONTRIBUTIONS

Conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualization; writing — original draft; writing – review and editing: Mehmet Akköse. *Investigation; methodology; writing – original draft*: Elif Merve Çınar and Murat Onur Yazlık. *Data curation; formal analysis; writing – original draft; writing – review and editing*: Ufuk Kaya. *Data curation; formal analysis; writing – original draft*: Yadigar Polat. *Conceptualization; investigation; methodology; supervision; writing – original draft; writing – review and editing*: Çiğdem Çebi. *Conceptualization; methodology; writing – original draft; writing – review and editing*: Ceyhan Özbeyaz. *Methodology; writing – original draft; writing – review and editing*: Mehmet Rıfat Vural.

ETHICS STATEMENT

This study was conducted pursuant to the approval of the Local Ethics Board for Animal Experiments of Pamukkale University (PAUHDEK-2022/12).

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ORCID

Mehmet Akköse https://orcid.org/0000-0001-7517-4927
Elif Merve Çınar https://orcid.org/0000-0003-0684-7213
Murat Onur Yazlık https://orcid.org/0000-0002-0039-5597
Ufuk Kaya https://orcid.org/0000-0002-4805-0993
Yadigar Polat https://orcid.org/0000-0001-5603-2149
Çiğdem Çebi https://orcid.org/0000-0001-6876-2069
Ceyhan Özbeyaz https://orcid.org/0000-0002-3748-9992
Mehmet Rıfat Vural https://orcid.org/0000-0001-7252-7977

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.1345.

REFERENCES

Adeyeye, A. A., Leigh, O. O., Ate, I. U., Stephen, J., Raheem, K. A., & Ubah, S. A. (2021). Pregnancy-specific protein B in Yankasa ewes during pregnancy and postpartum periods. *Macedonian Veterinary Review*, *44*(1), 55–62.

Akkaya Doğan, A., & Köse, A. M. (2022). Evaluation of bovine visual ELISA test for detection of pregnancy-associated glycoproteins in early pregnancy diagnosis in goats. *Small Ruminant Research*, *212*, 106722.

Akköse, M. (2020). Evaluation of a bovine rapid visual PAG ELISA test and transabdominal ultrasonography for early pregnancy diagnosis in Awassi sheep. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *23*(5), 1366–1372.

Akköse, M. (2023). Comparative evaluation of two commercial pregnancy-associated glycoproteins tests for early detection of pregnancy in dairy cattle. *Theriogenology*, *200*, 11–17.

Akkose, M., Cebi Sen, C., Kirmit, A., Korkmaz, O., Yazlik, M. O., Gocen, M., Kuru, M., & Akçay, E. (2019). Pregnancy-associated glycoproteins (PAGs) and progesterone concentrations in Holstein heifers following two methods of estrus synchronization. *Veterinaria México*, *6*(2), 1–11.

Akköse, M., Çınar, E. M., Yazlık, M. O., Çebi, Ç., & Polat, Y. (2021). Pregnancy-associated glycoproteins concentrations during early gestation in pregnant Awassi sheep. *Medycyna Weterynaryjna*, *77*(11), 563–567.

Akkuş, T., & Yaprakcı, Ö. (2022). Determination of pregnancy protein levels to distinguish between singleton and twin pregnancies in Awassi sheep. *Medycyna Weterynaryjna*, *78*(1), 25–30.

Alabart, J. L., Lahoz, B., Folch, J., Martí, J. I., Sánchez, P., Delahaut, P., Colemonts, Y., Beckers, J. F., & de Sousa, N. M. (2010). Early pregnancy diagnosis in sheep by plasmatic pregnancy-associated glycoprotein (PAG) enzymoimmunoassay (EIA) Kit. In *XXXV Congreso de la Sociedad Espa&ñola de Ovinotecnia y Caprinotecnia (SEOC)* (pp. 199–202). SEOC.

Alkan, H., Kivrak, M. B., Satilmis, F., Tekindal, M. A., & Dinc, D. A. (2020). Detection of twin pregnancies in ewes by pregnancy-associated glycoprotein assay and transabdominal ultrasonography. *Domestic Animal Endocrinology*, *72*, 106399.

Barbato, O., Sousa, N. M., Debenedetti, A., Canali, C., Todini, L., & Beckers, J. F. (2009). Validation of a new pregnancy-associated glycoprotein radioimmunoassay method for the detection of early pregnancy in ewes. *Theriogenology*, *72*(7), 993–1000.

Chaves, C. M. S., da Costa, R. L. D., Duarte, K. M. R., Beltrame, R. T., & Quirino, C. R. (2020). Evaluation of a cattle rapid test for early pregnancy diagnosis in sheep. *Tropical Animal Health and Production*, *52*(3), 1345–1349.

De Carolis, M., Barbato, O., Acuti, G., Trabalza-Marinucci, M., Melo de Sousa, N., Canali, C., & Moscati, L. (2020). Plasmatic profile of pregnancy-associated glycoprotein (PAG) during gestation and postpartum in Sarda and Lacaune sheep determined with two radioimmunoassay systems. *Animals*, *10*(9), 1502.

de Miranda e Silva Chaves, C., Dias da Costa, R. L., Roncato Duarte, K. M., Machado, D. C., Paro de Paz, C. C., & Beltrame, R. T. (2017). Visual ELISA for detection of pregnancy-associated glycoproteins (PAGs) in ewe serum. *Theriogenology*, *97*, 78–82.

El Amiri, B., Delahaut, P., Colemonts, Y., Melo de Sousa, N., & Beckers, J. F. (2014). Investigation of pregnancy-associated glycoproteins (PAGs) by means of an enzymoimmunoassay (ELISA) sandwich kit for pregnancy monitoring in sheep. *Options Méditerranéennes: Série A. Séminaires Méditerranéens*, *108*, 299–303.

El Amiri, B., Karen, A., Sulon, J., Melo de Sousa, N., Alvarez-Oxiley, A., Cognié, Y., Szenci, O., & Beckers, J. (2007). Measurement of ovine pregnancy-associated glycoprotein (PAG) during early pregnancy in Lacaune sheep. *Reproduction in Domestic Animals*, *42*(3), 257–262.

El Amiri, B., Remy, B., Melo de Sousa, N., & Beckers, J.-F. (2004). Isolation and characterization of eight pregnancy-associated glycoproteins present at high levels in the ovine placenta between day 60 and day 100 of gestation. *Reproduction Nutrition Development*, *44*(3), 169–181.

El Amiri, B., Remy, B., Sousa, N. M., Joris, B., Ottiers, N. G., Perenyi, Z., Mboko, H. B., & Beckers, J. (2003). Isolation and partial characterization of three pregnancy-associated glycoproteins from the ewe placenta. *Molecular Reproduction and Development*, *64*, 199–206.

El Amiri, B., Sousa, N. M., Alvarez Oxiley, A., Hadarbach, D., & Beckers, J. F. (2015). Pregnancy-associated glycoprotein (PAG) concentration in plasma and milk samples for early pregnancy diagnosis in Lacaune dairy sheep. *Research in Veterinary Science*, *99*, 30–36.

Gündoğan, M., Baki, D., & Yeni, D. (2003). Reproductive seasonality in sheep. *Acta Agriculturae Scandinavica*, *Section A*, *53*(4), 175–179.

Haugejorden, G., Waage, S., Dahl, E., Karlberg, K., Beckers, J. F., & Ropstad, E. (2006). Pregnancy associated glycoproteins (PAG) in postpartum cows, ewes, goats and their offspring. *Theriogenology*, *66*(8), 1976–1984.

Kaplan, Y., Özyurtlu, N., Köse, M., Atli, M. O., Küçükaslan, İ., & Kirbaş, M. (2018). Gebe Konya merinosu koyunlarında erken gebelikte gebelik ilişkili glikoproteinlerin plazma profilinin belirlenmesi. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, *14*, 307–314.

Karaca, O., Ata, N., Cemal, İ., & Yilmaz, O. (2018). Ewe live weight at birth and lamb birth weight in Karya sheep. In International Conference on Science and Technology, Prizren, Kosovo (pp. 879–884). ICONST.

Karakuş, O., Kaçar, C., Kuru, M., Kaya, S., Demir, M. C., Arı, U. Ç., Zonturlu, A. K., & Öztürkler, Y. (2021). Efficacy of a commercial test kit to determine early pregnancy in cows using whole blood and blood serum. *Tropical Animal Health and Production*, *53*(1), 1–6.

Karen, A., Beckers, J.-F., Sulon, J., de Sousa, N. M., Szabados, K., Reczigel, J., & Szenci, O. (2003). Early pregnancy diagnosis in sheep by progesterone and pregnancy-associated glycoprotein tests. *Theriogenology*, *59*(9), 1941–1948.

Karen, A., Kovács, P., Beckers, J. F., & Szenci, O. (2001). Pregnancy diagnosis in sheep: Review of the most practical methods. *Acta Veterinaria Brno*, *70*(2), 115–126.

Ledezma-Torres, R. A., Beckers, J. F., & Holtz, W. (2006). Assessment of plasma profile of pregnancy-associated glycoprotein (PAG) in sheep with a heterologous (anti-caPAG55þ59) RIA and its potential for diagnosing pregnancy. *Theriogenology*, *66*, 906–912.

Mallampati, R. S., Pope, A. L., & Casida, L. E. (1971). Effect of suckling on postpartum anestrus in ewes lambing in different seasons of the year. *Journal of Animal Science*, *32*(4), 673–677.

Milisits-Németh, T., Balogh, O. G., Egerszegi, I., Kern, L., Sasser, R. G., & Gábor, G. (2018). Detection of pregnancy in sheep using an ELISA for pregnancy-specific protein B. *Acta Veterinaria Hungarica*, *66*(2), 329–336.

Pickworth, C. L., Sheffield, J., & Poole, R. K. (2020). Pregnancy specific protein B concentration for the prediction of lambing rate in sheep. *Small Ruminant Research*, *184*, 106052.

Ranilla, M. J., Sulon, J., Carro, M. D., Mantecón, A. R., & Beckers, J. F. (1994). Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. *Theriogenology*, *42*(3), 537–545.

Ranilla, M. J., Sulon, J., Mantecón, A. R., Beckers, J. F., & Carro, M. D. (1997). Plasma pregnancy-associated glycoprotein and progesterone concentrations in pregnant Assaf ewes carrying single and twin lambs. *Small Ruminant Research*, *24*(2), 125–131.

Redden, R. R., & Passavant, C. W. (2013). Efficacy of pregnancy-specific protein B assay to detect pregnancy and lambing rates in sheep. *Sheep and Goat Research Journal*, *28*, 21–24.

Ricci, A., Carvalho, P. D., Amundson, M. C., & Fricke, P. M. (2017). Characterization of luteal dynamics in lactating Holstein cows for 32 days after synchronization of ovulation and timed artificial insemination. *Journal of Dairy Science*, *100*(12), 9851–9860.

Roberts, J., May, K., Ajani, O., Kaneene, J., Lansing, E., Biosystems, A., Mi, & Lansing, M. I. (2019). A comparison of pregnancy diagnosis methods in commercial sheep using lambing as a gold standard. *Clinical Theriogenology*, *11*(2), 107–113.

Roberts, J. N., May, K. J., & Veiga-Lopez, A. (2017). Time-dependent changes in pregnancy-associated glycoproteins and progesterone in commercial crossbred sheep. *Theriogenology*, *89*, 271–279.

Rovani, M. T., Cezar, A. S., Rigo, M. L., Gasperin, B. G., Nóbrega Júnior, J. E., da Torres, F. D., Gonçalves, P. B. D., & Ferreira, R. (2016). Evaluation of a bovine pregnancy-associated glycoprotein enzyme-linked immunosorbent assay kit for serological diagnosis of pregnancy in sheep. *Ciencia Rural*, *46*(2), 362–367.

Sargison, N., Criylly, J. P., & Hopker, A. (2018). Practical lambing and lamb care. *A veterinary guide* (4th ed., p. 83). Wiley Blackwell.

Savela, H., Vahtiala, S., Lindeberg, H., Dahl, E., Ropstad, E., Beckers, J.-F., & Saarela, S. (2009). Comparison of accuracy of ultrasonography, progesterone, and pregnancy-associated glycoprotein tests for pregnancy diagnosis in semidomesticated reindeer. *Theriogenology*, *72*(9), 1229–1236.

Sertkaya, Ş., & Öztürk, A. (2022). Fleece yield and some characteristics of wool in Anatolian Merino sheep. *Selcuk Journal of Agriculture and Food Sciences*, *36*(1), 8–12.

Silva, E., Sterry, R. A., Kolb, D., Mathialagan, N., McGrath, M. F., Ballam, J. M., & Fricke, P. M. (2007). Accuracy of a pregnancy-associated glycoprotein ELISA to determine pregnancy status of lactating dairy cows twenty-seven days after timed artificial insemination. *Journal of Dairy Science*, *90*, 4612–4622.

Sousa, N. M., Ayad, A., Beckers, J. F., & Gajewski, Z. (2006). Pregnancy-associated glycoproteins (PAG) as pregnancy markers in the ruminants. *Journal of Physiology and Pharmacology*, *57*, 153–171.

Steckeler, P., Weber, F., Zerbe, H., Rieger, A., & Voigt, K. (2018). Evaluation of a bovine visual pregnancy test for the detection of pregnancy-associated glycoproteins in sheep. *Reproduction in Domestic Animals*, *54*(2), 280–288.

Szenci, O. (2021). Recent possibilities for the diagnosis of early pregnancy and embryonic mortality in dairy cows. *Animals*, *11*(6), 1666.

Telugu, B. P. V., Walker, A. M., & Green, J. A. (2009). Characterization of the bovine pregnancy-associated glycoprotein gene family–analysis of gene sequences, regulatory regions within the promoter and expression of selected genes. *BMC Genomics*, *10*(1), 1–17.

Uçar, U., Köse, M., & Atlı, M. O. (2018). Konya Merinosu koyunlarda gebelik ilişkili glikoproteinlerin gebelikteki plazma profili ve erken gebelik tanısında kullanılabilirliği. *Dicle Üniversitesi Veteriner Fakültesi Dergisi*, *11*(2), 77–82.

Ünal, N., & Akçapınar, H. (2001). Important yield traits and selection opportunities in Central Anatolian Merino, 1. Important yield traits. *Journal of Lalahan Livestock Research Institute*, *41*(1), 45–58.

Wallace, R. M., Pohler, K. G., Smith, M. F., & Green, J. A. (2015). Placental PAGs: Gene origins, expression patterns, and use as markers of pregnancy. *Reproduction*, *149*(3), R115–R126.

Xie, S. C., Low, B. G., Nagel, R. J., Kramer, K. K., Anthony, R. V., Zoli, A. P., Beckers, J. F., & Roberts, R. M. (1991). Identification of the major pregnancy-specific antigens of cattle and sheep as inactive members of the aspartic proteinase family. *PNAS*, *88*(22), 10247–10251.

How to cite this article: Akköse, M., Çınar, E. M., Yazlık, M. O., Kaya, U., Polat, Y., Çebi, Ç., Özbeyaz, C., & Vural, M. R. (2024). Serum pregnancy–associated glycoprotein profiles during early gestation in Karya and Konya Merino sheep. *Veterinary Medicine and Science*, *10*, e1345.
https://doi.org/10.1002/vms3.1345