Kocatepe Veterinary Journal

Kocatepe Vet J. (2024):17(3):192-202

DOI: 10.30607/kvj. 1495857

RESEARCH ARTICLE

Serum Progesterone and PAG Levels in Pregnant Karya Ewes

Çiğdem ÇEBİ[1*], Mehmet AKKÖSE[2]

[1]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Harran University, Sanliurfa, Türkiye*
[2] *Department of Livestock, Dalaman Agricultural Enterprise, General Directorate of Agricultural Enterprices, Dalaman, Muğla, Türkiye*

ABSTRACT

The present study was aimed the determination of serum PAG concentrations during pregnancy and the first month postpartum in Karya ewes and the investigation of correlations between the serum concentrations of the steroid hormone progesterone, upon which the continuation of pregnancy depends, and PAG levels throughout pregnancy. Blood samples were collected from each of the ewes at weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 18, 19, 20, and 21 after the day of mating (day 0), at the time of parturition, and at weeks 1, 2, 3 and 4 postpartum. PAG level and progesterone level during pregnancy and serum PAG level in the first postpartum month were determined. Plasma progesterone concentrations were lowest on the day of mating, but progressively increased as of week 9 of pregnancy and peaked at gestational week 18. Progesterone levels were observed to decrease after week 18. The mean PAG concentrations of the pregnant animals were observed to be at the basal level until the second week of pregnancy, but increased as of week 3 and peaked at week 19. PAG concentrations displayed a dramatic decrease during the first 4 weeks postpartum. Based on this finding, in the Karya ewes, the increase in the progesterone levels at gestational weeks 8 and 11 were associated with an increase in the PAG levels. Differences in the results reported for the correlation between PAG and progesterone levels could be due to less frequent sampling, species-specific differences in placental function and PAG cross-reactivity, as well as species- or subspecies-specific differences in PAG and progesterone dynamics during pregnancy.

Key Words: PAG, Pregnancy, Progesteron, Sheep

***

Gebe Karya Koyunlarında Serum Progesteron ve PAG Düzeyleri

ÖZ

Var olan çalışmanın amacı Karya koyunlarında gebelik süresince PAG düzeyi ve progesteron seviyesi ile postpartum ilk aydaki serum PAG düzeyini belirleyerek progesteron ile PAG arasındaki korelasyonların gebelik süresince saptanması amaçlandı. Tüm koyunlardan, koçların aşım yaptığı günden sonraki 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 18, 19, 20, 21'inci haftalar ile doğum sonrası 1, 2, 3 ve 4'üncü haftalarda kan örnekleri toplandı ve gebelik süresince PAG düzeyi ve progesteron seviyesi ile postpartum ilk aydaki serum PAG düzeyi belirlendi. Plazma progesteron konsantrasyonu gebeliğin 9. haftasından itibaren artış göstermiş olup, 18. haftada pik düzeye ulaşdı ve 18. haftadan sonra progesteron düzeylerinde düşüş gözlendi. Gebe hayvanlarda ortalama PAG konsantrasyonları ise gebeliğin ikinci haftasına kadar bazal düzeyde iken gebeliğin 3. haftasından itibaren yükselmeye başladı, gebeliğin 19. haftasında pik yaparak gebelik sonuna kadar da artış gösterdi. Doğumdan sonraki ilk 4 hafta süresince ise PAG konsantrasyonu dramatik bir şekilde bir düşüş gösterdi. Karya koyunlarında gebeliğin 8. ve 11. haftalarında progesteron seviyesi arttıkça PAG düzeyi de artış gösterdi. Bu bulgulara göre PAG ve progesteron arasında bildirilen korelasyonlardaki farklılıklar, daha az sıklıkta örnekleme yapılmasına, plasental fonksiyondaki türe özgü farklılıklara veya PAG çapraz reaktivitesindeki farklılıklara ve gebelik sırasında PAG ve progesteron dinamiklerindeki tür veya alt tür farklılıklarından kaynaklanıyor olabilir.

Anahtar Kelimeler: Gebelik, Koyun, PAG, Progesteron

To cite this article: Çebi Ç. Akköse M. Serum Progesterone And Pag Levels İn Pregnant Karya Ewes. Kocatepe Vet J. (2024):17(3):192-202

Submission: 04.06.2024 **Accepted**: 06.08.2024 **Published Online**: 26.08.2024

ORCID ID; *ÇÇ: 0000-0001-6876-2069 MA: 0000-0001-7517-4927*

*Corresponding author e-mail: cigdemcebi@harran.edu.tr

INTRODUCTION

The Karya breed is known to have emerged from the ungoverned crossbreeding performed by Turkish sheep breeders in Western Anatolia between Chios, Kıvırcık and Chios X Kıvırcık rams and ewes of several local fat-tailed breeds, including the Ödemiş, Çine Çaparı and Dağlıç. The Karya was named by the Breed Registration Commission of the Ministry of Agriculture and Forestry after the Carian civilization, known to have reigned in the region during antiquity, and was registered as an indigenous breed by the same Commission in 2013 (Karaca and Cemal 1998, Karaca and Cemal 2005; Yücel 2022). Elite flocks of the prolific Karya ewes show a twin pregnancy rate of almost 100% and sometimes present with triplet and quadruplet pregnancies. Early pregnancy diagnosis is very important for the economics of sheep production (Akdeniz 2022). The detection of pregnant and non-pregnant animals in the flock not only avoids feed waste on infertile animals, but also enables formulating rations according to the energy requirements of pregnant and nursing ewes, both of which contribute to preventing the development of metabolic diseases and reducing the occurrence of reproductive health problems. Moreover, distinguishing between singleton and multiple pregnancies by means of early pregnancy diagnosis enables producers to plan the lambing method in advance, such that the dependence on labor force is minimized and lambing efficiency is maximized (Akdeniz 2022; De Carolis et al. 2020; Kaplan Bilmez 2018). The timely and accurate diagnosis of pregnancy also contributes to the prevention of fertility losses (Kaplan Bilmez 2018). Despite the availability of a broad array of options for pregnancy diagnosis today (i.e. ultrasonography, measurement of pregnancy-associated glycoprotein levels, radiography, abdominal palpation, vaginal biopsy, the cervical mucus test, vaginal smear examination, serum progesterone analysis), the methods commonly used for early pregnancy diagnosis are ultrasonographic examination, measurement of blood plasma/serum progesterone levels and the detection of pregnancy-specific proteins (Akdeniz 2022; Kaplan Bilmez 2018). While ultrasonography offers a high level of diagnostic accuracy, its time-consuming and labour-intensive implementation, which requires the skills of an experienced practitioner and can be challenging for large flocks, is disadvantageous (Erdem and Sarıbay 2015; Lucy et al. 2013). One of the most popular methods used for pregnancy diagnosis on sheep farms is the measurement of the post-mating blood levels of the progesterone hormone, as proof of the presence of a functional corpus luteum (Anghel et al. 2011; Zamfirescu et al. 2011). In ewes, progesterone concentrations are detectable in the blood as of the 18th day after mating and progressively increase up to a level of 2-3 ng/ml on day 50 of pregnancy, displaying a rapid increase after the first three months of gestation to reach a peak level of 12-20 ng/ml (Kaplan Bilmez 2018). While a high level of success is achieved in the detection of pregnant ewes with progesterone measurement, cases of early embryonic death, corpus luteum persistence, the prolongation or shortening of the oestrus cycle, faulty insemination, and uterine and ovarian pathologies may cause misdiagnosis. Furthermore, the high progesterone levels observed during certain phases of the oestrus cycle may reduce the success of detecting non-pregnant ewes (Karen et al. 2001; Karen et al. 2004). An alternative to the mentioned pregnancy diagnosis methods is the detection of pregnancy-associated glycoproteins (PAGs) (Rovani et al. 2016; Steckeler et al. 2018). Pregancy-specific protein B (PSPB) and other PAGs are synthesized during pregnancy in ewes (and other female ruminants), and thus, are used as indicators of pregnancy. PSPB, which is a form of PAG, is also referred to as pregnancy-serum protein 60 kDa (PSP60) and SBU-3 antigen (Adeyeye et al. 2021; El Amiri et al. 2007). Given the role of PAGs as pregnancy indicators, in the past 30 years, commercial test kits have been developed for early pregnancy diagnosis based on the detection of PAGs belonging to the aspartic proteinase family (Akköse 2020; Szenci 2021). These PAG ELISA kits, originally developed for use in cattle, also produce successful results for early pregnancy diagnosis in sheep and goats (Akdeniz 2022; Akköse 2020).

Pregnancy, which is defined as the period in-between fertile mating and parturition, starts immediately after the fertilization of the ovum and lasts for 147-152 days in ewes. During this period, several biomarkers, including among others, enzymes, growth factors, inhibitors, hormones and proteins, either appear or increase in the maternal blood circulation (Adeyeye et al. 2021). The majority of the biomarker proteins are of fetoplacental origin and are produced by mono- and binucleate trophoblast-derived cells in the ruminant placenta, from which binucleate giant cells migrate into the maternal circulation through the foetal chorion (Adeyeye et al. 2021; Ranilla et al. 1994). Various cDNAs encoding different PAG molecules during different phases of pregnancy have been identified in the ovine placenta, similar to the placenta of other ruminant species, and it has been reported that more than 100 PAG genes are involved in the production of glycoproteins in large and small ruminants (De Carolis et al. 2020; Ledezma-Torres 2009). The production of PAG molecules starts with the implantation of the embryo, and during placentation, these molecules enter the maternal circulation, such that their detection in the ovine maternal blood plasma is possible as early as 18 to 20 days after conception with the radioimmunoassay (RIA) and enzyme immunoassay (EIA) techniques (De Carolis et al. 2020). While the detection of placental proteins in the maternal blood is a tool most commonly used for pregnancy diagnosis (El Amiri et al. 2007), the plasma and milk levels of PAG/PSPB molecules can also be used for the assessment of the fetal number, and the determination of pregnancy losses and obstetric diseases (Adeyeye et al. 2021; Roberts et al. 2017). Furthermore, the measurement of the levels of PAG/PSPB molecules in ovine peripheral blood samples may provide valuable input for developing feeding strategies for pregnant animals as well as for implementing measures to prevent the occurrence of metabolic disorders in the dam and enable the growth of the fetus (El Amiri et al. 2007). However, PSPB concentrations may vary in ruminants during pregnancy, in association with several parameters, including the genus or subspecies, number of previous births of the dam (parity), fetal number, fetal sex, birth weight, nutritional status of the dam, environmental conditions and method used for the measurement of PSPB concentrations (Adeyeye et al. 2021; De Carolis et al. 2020, Roberts et al. 2017). The present study was aimed at the determination of serum PAG concentrations during pregnancy and the first month postpartum in Karya ewes, one of the major local sheep breeds of Türkiye, and at the investigation of any correlation between the serum concentrations of the steroid hormone progesterone, upon which the continuation of pregnancy depends, and PAG levels throughout pregnancy.

MATERIALS and METHODS

Animals

This study was conducted pursuant to the approval of the Local Ethics Board for Animal Experiments of Pamukkale University. The study was carried out between December 2023 and February 2024 at a sheep farm located in the Serinhisar (Kızılhisar) district of the Denizli province in Türkiye. The study animals were comprised of ten 2- to 5-year-old Karya ewes, which weighed 40-60 kg and were confirmed to have no health problem. Firstly, the ewes were synchronized for oestrus by the insertion of progesterone-impregnated intravaginal sponges (Esponjavet, Hipra, Spain) for a period of 12 days. Immediately after the removal of the sponges, the ewes were administered with 400 IU of equine chorionic gonadotropin (eCG) (Folligon, MSD, Spain). All ewes were allowed to mate naturally. The ewes that had mated were recognized by the paint on their dorsum after the introduction of teaser rams with marking harnesses. Pregnancy diagnosis was made by abdominal ultrasonography (Siui 838, China) on day 35 post-mating (Tekin and Köse, 2022), such that the empty ewes were synchronized and prepared for mating for a second time. Pregnancy was confirmed based on the observation of fetal fluids, placentomes and fetal structures at ultrasonographic examination. Blood samples were collected from each of the ewes into gel-coated vacutainers (Hematube, Ankara, Türkiye) at weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 15, 17, 18, 19, 20, and 21 after the day of mating (day 0), at the time of parturition, and at weeks 1, 2, 3 and 4 postpartum. Pregnancy was diagnosed by transabdominal ultrasonography on day 35 post-mating. Samples taken during the first 5 weeks from the non-pregnant ewes were excluded from the study, and after the animals were examined for pregnancy, only those confirmed to be pregnant were sampled for blood. The blood samples were firstly allowed to coagulate at room temperature, and were later centrifuged at 3000 rpm for 5 min for the extraction of sera. Until being used for laboratory analyses, the blood serum samples were stored at -20 0C. PAG level and progesterone level during pregnancy and serum PAG level in the first postpartum month were determined. Serum PAG levels were measured using a commercial ELISA kit originally developed for pregnancy diagnosis in cattle (IDEXX Laboratories, Westbrook, ME). The ELISA test was performed by experienced technical staff in accordance with the manufacturer's instructions. The test results were read on a spectrophotometer at a wavelength of 450 nm. The S-N (sample minus negative) values of the samples were calculated by subtracting the optical density (OD) of the negative control from the OD of the sample. Based on the correlation of the PAG concentrations of the samples with the OD values measured, the S-N values were used as the serum PAG levels of the samples. The results of the plasma samples of the ewes were assessed in accordance with the manufacturer's instructions such that values, which were ≥ 0.3, were considered positive and indicated pregnancy, whilst those, which were <0.3, were considered negative and indicated the absence of pregnancy. At the end of the study, the stored serum samples were quantitatively analyzed for progesterone concentrations at the MG Veterinary Diagnosis Laboratory using chemiluminescence assay (CLIA) kits. The CLIA method is based on the measurement of the light emission resulting from the antigen-antibody reaction with the aid of a calibrated luminometer. The sensitivity and specificity of the CLIA method were 100% and 95.5%, respectively. The specificity of the assay for progesterone and the assay range have been reported as 0.21-60 ng/mL (0.67-190.8 nmol/L). Based on the results of the analyses, the ewes, which were determined to have serum progesterone concentrations of >1.0 ng/ml, were considered to be pregnant.

Statistics

The study data were statistically evaluated using the 'IBM SPSS Statistics 26' software package. Prior to data analysis, the normality distribution of the data was evaluated with the Shapiro-Wilk test. The results are given as mean ± standard deviation. The differences between the two groups were assessed with the Mann-Whitney U test. The correlation between the variables was evaluated with Spearman's rho correlation coefficient. Statistical significance was set at $p<0.05$.

RESULTS

Progesterone concentrations of the pregnant animals

As can be seen in Figure 1, the plasma P4 concentrations were lowest on the day of mating, but progressively increased as of week 9 of pregnancy and peaked at gestational week (GW) 18. Progesterone levels were observed to decrease after GW 18.

PAG concentrations of the pregnant animals

The mean PAG concentrations of the pregnant animals were observed to be at the basal level until the second week of pregnancy, but increased as of GW 3 and peaked at week 19. PAG concentrations displayed a dramatic decrease during the first 4 weeks postpartum. The PAG levels of the Karya ewes during pregnancy and during the postpartum period are shown in Figure 1 and Figure 2.

The correlation between PAG and P4 concentrations

As can be seen in Table 1, the PAG and progesterone concentrations were determined to be positively correlated with each other at GW 8 (r: 0.699) and GW 11 (r: 0.736) ($p<0.05$). Based on this finding, in the Karya ewes, the increase in the progesterone levels at gestational weeks 8 and 11 were associated with an increase in the PAG levels.

PAG and P4 concentrations in single and multiple pregnancies

As shown in Table 2, the mean PAG level at GW 7 was 0.50 ± 0.11 in the ewes with singleton pregnancy and 0.67 ± 0.09 in the ewes with twin pregnancy, and the Mann-Whitney U test revealed that the difference between the two groups was statistically significant (Z: -2.009; $p<0.05$). Furthermore, the mean PAG level at GW 11 was 0.93 ± 0.21 in the ewes with singleton pregnancy and 1.62 ± 0.20 in the ewes with twin pregnancy, and statistical analysis showed that the two groups significantly differed from each other (Z: -2.514; $p<0.05$). At week 13 of pregnancy, the mean PAG level was 1.04 ± 0.37 in the singleton-pregnant ewes and $1.81\pm.28$ in the twin-pregnant ewes, and it was ascertained that the two groups significantly differed from each other (Z: -2.402; $p<.005$). The mean PAG levels determined at GW 15 were $1.16\pm.26$ and $2.04\pm.15$ in the singleton-pregnant and twin-pregnant ewes, respectively, and the difference between the two groups was found to be statistically significant (Z: -2.611; $p<0.05$). The mean PAG levels of the ewes with singleton and twin pregnancies at GW 17 were $1.38\pm.31$ and $2.22\pm.24$, respectively, and statistical analysis revealed that the difference between the two groups was significant (Z: -2.611; $p<0.05$). During the remaining weeks of pregnancy, namely, at weeks 1, 2, 3, 4, 5, 6, 8, 9, 18, 19, 20 and 21, no statistically significant difference was observed between the mean PAG levels of the ewes with singleton and twin pregnancies ($p>0.05$). Based on the findings obtained in the present study, it was determined that the PAG levels of the twin-pregnant ewes were higher than those of the singleton-pregnant ewes at GWs 7, 11, 13, 15 and 17.

As shown in Table 3, the mean progesterone levels of the ewes with singleton and twin pregnancies at week 11 of pregnancy were 3.22 ± 1.11 and 6.65 ± 2.90, respectively, and the Mann-Whitney U test revealed that the two groups significantly differed from each other (Z: -2.193; $p<0.05$). During the remaining weeks of pregnancy, no such statistically significant difference was determined between the progesterone levels of the singleton-pregnant and twin-pregnant ewes ($p>0.05$). Based on the findings of the present study, it was ascertained that at GW 11, the progesterone levels of the twin-pregnant ewes were higher than those of the singleton-pregnant ewes. No significant difference was determined during the remaining weeks of pregnancy.

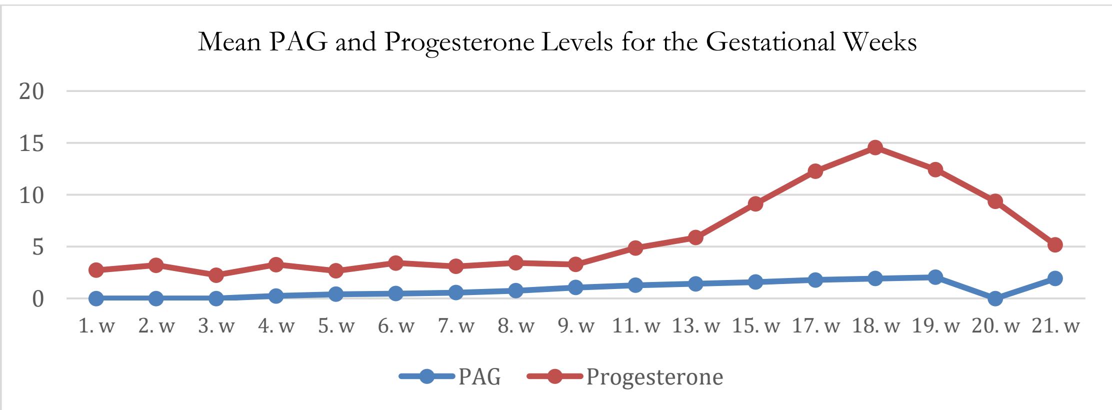

Figure 1. Mean PAG and Progesterone Levels for the Gestational Weeks

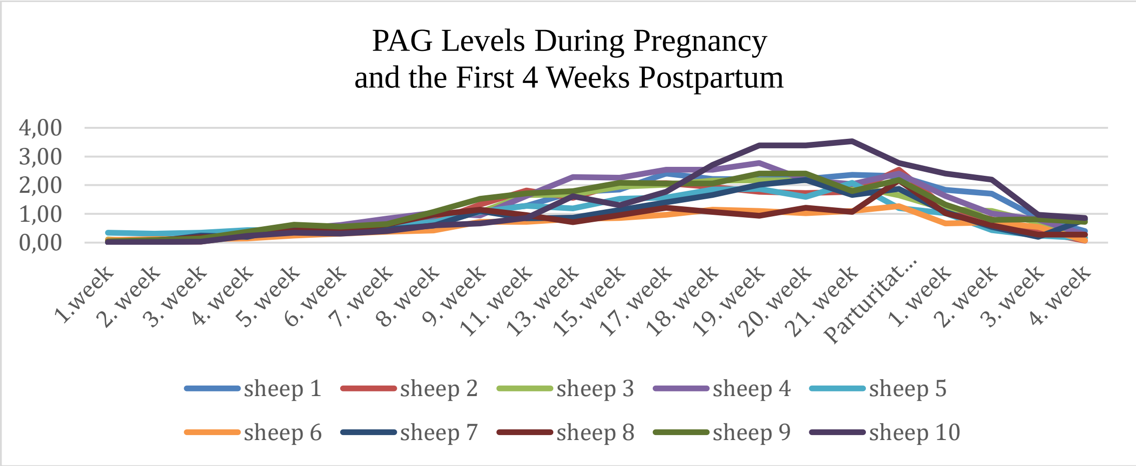

Figure 2. PAG levels during pregnancy and the first 4 weeks postpartum

Table 1. The correlation between PAG and progsterone levels for the gestational weeks (n:10)

Gestational Week	r	p
Week 1	0.228	0.527
Week 2	0.350	0.321
Week 3	-0.463	0.178
Week 4	-0.177	0.625
Week 5	-0.213	0.554
Week 6	0.469	0.172
Week 7	0.278	0.437
Week 8	**0.699**	**0.024***
Week 9	0.576	0.082
Week 11	**0.736**	**0.015***
Week 13	0.261	0.467
Week 15	0.321	0.365
Week 17	0.511	0.132
Week 18	0.285	0.425
Week 19	0.073	0.841
Week 20	-0.103	0.776
Week 21	0.188	0.603

Table 2. PAG differences for singleton and twin pregnancies

Gestational Week	Singleton (n:5) x±ss	Twin (n:5) x±ss	Test	p
Week 1	0.1±0.15	0.04±0.04	Z: -0.106	p: 0.916
Week 2	0.1±0.12	0.04±0.03	Z: -1.178	p: 0.239
Week 3	0.19±0.12	0.06±0.04	Z: -1.471	p: 0.141
Week 4	0.25±0.11	0.26±0.07	Z: -0.420	p: 0.674
Week 5	0.40±0.11	0.44±0.11	Z: -0.525	p: 0.599
Week 6	0.43±0.11	0.55±0.06	Z: -1.946	p: 0.052
Week 7	0.50±0.11	0.67±0.09	**Z: -2.009**	**p: 0.045**
Week 8	0.66±0.20	0.87±0.17	Z: -1.776	p: 0.076
Week 9	0.94±0.23	1.19±0.23	Z: -1.149	p: 0.251
Week 11	0.93±0.21	1.62±0.20	**Z: -2.514**	**p: 0.012**
Week 13	1.04±0.37	1.81±0.28	**Z: -2.402**	**p: 0.016**
Week 15	1.16±0.26	2.04±0.15	**Z: -2.611**	**p: 0.009**
Week 17	1.38±0.31	2.22±0.24	**Z: -2.611**	**p: 0.009**
Week 18	1.69±0.66	2.18±0.23	Z: -1.567	p: 0.117
Week 19	1.86±0.97	2.26±0.37	Z: -1.149	p: 0.251
Week 20	1.88±0.95	2.14±0.25	Z: -0.943	p: 0.346
Week 21	1.89±1	1.99±0.24	Z: -0.731	p: 0.465

Table 3. Differences between the progesterone levels of the ewes with singleton and twin pregnancies for the gestational weeks

Gestational week	Singleton (n:5) x±ss	Twin (n:5) x±ss	Test	p
Week 1	2.60±1.24	2.88±0.82	Z: -0.522	p: 0.602
Week 2	2.52±1	3.92±1.69	Z: -1.576	p: 0.115
Week 3	2±.96	2.50±1.21	Z: -0.736	p: 0.462
Week 4	2.28±1.54	4.28±3.38	Z: -1.149	p: 0.251
Week 5	1.96±0.70	3.40±2.16	Z: -1.358	p: 0.175
Week 6	2.22±0.68	4.64±3.48	Z: -1.781	p: 0.075
Week 7	2.34±0.31	3.86±1.95	Z: -1.681	p: 0.093
Week 8	2.48±0.69	4.40±2.92	Z: -1.467	p: 0.142
Week 9	2.70±0.74	3.88±2.68	Z: -0.940	p: 0.347
Week 11	3.22±1.11	6.65±2.90	**Z: -2.193**	**p: 0.028**
Week 13	4.60±1.73	7.16±4.20	Z: -1.567	p: 0.117
Week 15	7.36±1.24	10.92±4.26	Z: -1.567	p: 0.117
Week 17	8.02±2.05	16.51±7.93	Z: -1.991	p: 0.052
Week 18	8.18±2.78	20.92±14.1	Z: -1.567	p: 0.117
Week 19	8.32±3.97	16.54±10.33	Z: -1.257	p: 0.209
Week 20	6.72±3.14	12.02±9.88	Z: -0.522	p: 0.602
Week 21	3.88±0.98	6.46±4.95	Z: -0.522	p: 0.602

DISCUSSION

The present study was aimed at the investigation of the impact of pregnancy and the postpartum period on the serum PAG levels of ewes and the determination of any correlation between the levels of progesterone, a steroid hormone upon which the maintenance of pregnancy depends, and the levels of PAGs throughout the course of gestation. Previously, our research team had investigated the early pregnancy serum PAG profiles of ewes of the Karya breed, a major and highly prolific sheep breed indigenous to Türkiye (Akköse et al. 2024). To the best of our knowledge, there is no previous study on prepartum and postpartum plasma PAG profiles or the investigation of any possible correlation between these profiles and progesterone levels. In sheep production, pregnancy diagnosis is particularly significant and valuable during the early period, and early pregnancy diagnosis can be made by means of several methods, including among others, transrectal USG, the progesterone (P4) test and the measurement of PAG levels. Among the available clinical methods, USG offers a high level of accuracy for pregnancy diagnosis, but is labor-intensive and time-consuming, especially when performed in large flocks, and thus, efforts continue to develop more practical methods for use on the field. As a well-established alternative to ultrasonographic examination, the detection of high progesterone levels secreted from the ovarian corpus luteum throughout gestation is a reliable determinant for pregnancy diagnosis. While low progesterone concentrations are accurately indicative of oestrus or the absence of conception/pregnancy, the corpus luteum becoming a permanent structure, embryonic death, samples being collected either too early or too late, and some other conditions irrelevant to pregnancy may also lead to the detection of elevated blood progesterone levels and rule out the determinative quality of this parameter. Despite the progesterone hormone being required for the continuation of pregnancy in ewes, the high prevalence of false positive results hinders its use as a specific indicator for pregnancy diagnosis in sheep. On the other hand, PAGs, owing to the specific temporal detection they allow during the attachment of the conceptus, are considered as ideal bioimarkers for pregnancy and are commonly used for the diagnosis of pregnancy in ruminants (Akdeniz 2022; Roberts et al., 2017). In recent years, several studies have been conducted on the use of the measurement of the blood levels of pregnancy-associated glycoproteins (PAGs) as an alternative to ultrasonography. Although pregnancy diagnosis based on the detection of PAG levels requires laboratory equipment, the use of the right test kits have shortened the time required for the laboratory diagnosis of pregnancy. Furthermore, the most recently designed visual inspection kits have enabled both the clinical and on-the-field diagnosis of pregnancy (Chavez et al. 2017; Chavez et al. 2020). The molecular structure of PAGs being well-conserved across ruminant species allows for the use of the less costly and more easily accessible bovine test kits in sheep as well, and thus, a PAG kit originally developed for cattle was used for Karya ewes in the present study. While various research has been carried out to individually assess the aforementioned pregnancy diagnosis methods, only a limited number of previous studies provide a comparative assessment of the three referred methods for their accuracy in pregnancy diagnosis, based on their concurrent use in the same animals. This is the first study investigating the correlation between PAG and progesterone levels in the blood of pregnant Karya ewes throughout gestation, as well as PAG concentrations during the first month postpartum.

Placentation, which starts with implantation, is usually completed by days 50-60 of gestation in sheep and goats (Guillomot 1995). In sheep, corpus luteum-derived progesterone is required only until day 50 of gestation for the maintenance of pregnancy. Later, placental progesterone comes into play, and the placenta becomes the main source of progesterone (Özdemir Salcı 2015). The progesterone level being higher than the threshold value (>1 ng.ml-1) indicates either the presence of a pregnancy-associated functional corpus luteum, a natural oestrus cycle or a pathological condition of the ovaries or uterus (Karen et al. 2003). The findings of our study showed that the mean progesterone concentration was lowest on the day of mating, increased as of the 9th week of pregnancy, and reached its peak level at GW 18. Progesterone levels were observed to decrease after GW 18. In agreement with our study, it has been reported that in both Churra and Merino ewes, progesterone levels increased throughout pregnancy, peaked at weeks 19-20, and decreased two weeks prior to parturition (Ranilla et al.1994). In a previous study on pregnant Sarda and Lacaune ewes, the progesterone levels were determined as 5.26±0.7 ng/mL and 10.9±1 ng/mL, respectively, on gestational day 18, and as 6.05±0.5 ng/mL and 12.0±0.7 ng/mL, respectively, on gestational day 60, and thus, were reported at levels higher than those determined in the present study. In Barbari goats, progesterone concentrations, which were lowest on the day of mating were reported to significantly increase as of the second week of pregnancy, peak between gestational weeks 10-14, and decrease thereafter, reaching the basal level just before parturition (Ujjawala et al. 2013l). On the other hand, different from our study, Humblot et al. (1990) reported no significant increase or decrease in the mean progesterone concentrations of pregnant Alpine goats as of the 21st day of pregnancy.

Humblot et al. (1988) attributed the increase observed in progesterone levels during the first 3 weeks of gestation followed by relatively stabile levels throughout the remaining period of pregnancy to the possibility of progesterone production being regulated by factors other than those involved in antiluteolytic mechanisms. The luteotrophic factors found in the allantoic fluid may also show effect on the production of progesterone by the corpus luteum. As shown in Table 3, the mean progesterone concentrations of the ewes with singleton and twin pregnancies at week 11 of gestation were determined as 3.22±1.11 and 6.65±2.90, respectively, and the difference between the two groups was found to be statistically significant (Z: -2.193; $p<0.05$). During the other weeks of gestation, no statistically significant difference was observed between the progesterone levels of the ewes with singleton and twin pregnancies ($p>0.05$). Different from the present study, there are literature reports indicating a positive correlation between the mean progesterone concentrations of ewes with singleton pregnancy and the mean progesterone concentrations of ewes with twin pregnancy ($r=0.782$; $P<0.01$) (Hall et al. 1992; Ranilla et al. 1997; Ranilla et al. 1994). The mean progesterone concentration of twin-pregnant ewes has been reported to be higher than that of singleton-pregnant ewes between GWs 12-20 (Ranilla et al. 1997). Meshref et al. (2022) reported the level of progesterone measured on the day of mating in singleton- and multiple-pregnant Osseimi ewes as 0.23±0.01 ng/ml and 0.4±0.01 ng/ml, respectively. Progesterone levels measured in singleton- and multiple-pregnant Osseimi ewes have been reported as 2.9±0.01 ng/ml and 3.07±0.01 ng/ml, respectively, on day 10 of pregnancy and 8.35±0.22 and 9.52±0.175 ng/ml, respectively, on day 90 of pregnancy, and it has been indicated that the progesterone concentration increases with a greater number of fetuses (Meshref et al. 2022). Furthermore, Ranilla et al. (1994) and Hall et al. (1992) suggested that the level of progesterone required for the maintenance of pregnancy may vary with the size of the fetus. In parallel with our study, Mukasa-Mugerwa and Viviani (1992) reported that there was no consistent correlation between blood progesterone level and fetal size. The measurement of progesterone levels being costly, requiring a well-equipped laboratory, and depending on the collection of blood samples on a certain day, as well as on the availability of accurate knowledge on the day of mating or artificial insemination reduces the preference of this method for pregnancy diagnosis, when compared to ultrasonography (Medan et al., 2004).

PAG concentrations have been indicated to produce more reliable results for early pregnancy diagnosis than progesterone concentrations, as PAGs enable the differentiation of cases of pregnancy and prolonged oestrus. The earliest day of pregnancy on which PAGs have been detected in sheep in previous studies has been reported as day 20 post-mating (Karen et al. 2003). The present study demonstrated that the mean PAG concentration was at the basal level until the second week of pregnancy and progressively increased as of the third gestational week until reaching the peak at GW 19 (Figure 1 and Figure 2). Ledezma-Torres et al. (2006) and Sousa et al. (2006) described the determination of PAG levels as a reliable method for the on-farm early diagnosis of pregnancy in sheep as of the fourth gestational week. Ledezma-Torres et al. (2006) reported the PAG levels of non-pregnant ewes to be five-fold lower than the levels of pregnant ewes. Similarly, Anghel et al. (2011) confirmed that the PAG concentrations of non-pregnant Merino ewes were below 1.5 ng/ml. In previous studies by Rovani et al. (2016) and Uçar (2017), it was reported that increased PAG OD values were detectable as of days 33 and 35 post-mating. The findings of the present study agree with the reports of these researchers in that the pregnancy-positive values progressively increased as of the third week of pregnancy. Willard et al. (1995) suggested the primary mechanism underlying the passage of PAGs into the maternal circulation as the migration of binucleate cells from the placenta to the maternal endometrium as of the 18th day of pregnancy. Thus, the measurement of PAG levels for early pregnancy diagnosis in sheep before GW 3 may cause false negative results. The present study having demonstrated the increase of PAG concentrations as of the 3rd week post-mating is in agreement with previous reports made for the Karpat goat (Zamfirescu et al. 2011), Awassi and Merino sheep (Anghel et al. 2011; Karen et al. 2003), Sarda sheep (Barbato et al. 2009), a heterogenous group of sheep (Ledezma-Torres et al., 2006), Karya and Merino sheep (Akköse et al. 2024) and the Sirohi goat (Salve et al. 2016). In our study, we determined that PAG levels peaked at gestational week 19, progressively increased throughout pregnancy, and showed a dramatic decrease after parturition. In agreement with our study, Roberts et al. (2017) reported that PAG1 levels steadily increased from day 3 to day 120 of gestation. Moreover, De Carolis et al. (2020) and Gajewski et al. (1999) indicated that PAG concentrations increased until day 60 of pregnancy in sheep, and thereafter, decreased until day 120, reaching a peak level at the time of parturition. The initial tendency of increase followed by a decrease to the initial levels at mid-pregnancy reported for PAG levels in Sarda and Lacaune ewes (De Carolis et al. 2020), Berrichon ewes (Gajewski et al. 1999), Churra ewes (Ranilla et al. 1997), various sheep breeds (Ledezma-Torres et al. 2006), and Osseimi ewes (Meshref et al. 2022) was not observed in the present study in Karya ewes and has not been reported in Merino sheep either. While PAG concentrations between gestational weeks 9-17 vary among sheep breeds, the concentrations detected from GW 17 to lambing have been observed to increase in all breeds

(Karen et al. 2001). This difference could be related to the structure of the placenta and the decrease that occurs in the number of binucleate cells in sheep (De Carolis et al. 2020). Ovine PAG concentrations fluctuate throughout pregnancy in relation to breed, sex and fetal number (Gajewski et al. 1999; Ranilla et al. 1997). In the present study, the PAG levels of the twin-pregnant ewes at gestational weeks 7, 11, 13, 15 and 17 were found to be higher than those of the singleton-pregnant ewes. The demonstration of higher PAG concentrations in cases of multiple pregnancy, when compared to singleton-pregnancy, at the indicated time points, suggests a positive correlation between PAG concentration and fetal number. This finding is in agreement with a previous study reporting higher PAG concentrations in multiple pregnancies, compared to singleton pregnancies, in Sarda ewes (De Carolis et al. 2020). In a study by Ranilla et al. (1997), similarly, it was determined that the mean PAG concentrations of twin-pregnant ewes were higher than the levels of singleton-pregnant ewes, during the period from the GW 12 to lambing. However, this difference was statistically significant only at GW 21 (Karen et al. 2001). Furthermore, Roberts et al. (2017) reported that maternal serum PAG1 concentrations were higher during the last month of pregnancy in twin-pregnant cattle.. The higher PAG concentrations determined in cases of multiple pregnancy, when compared to singleton pregnancy, have been attributed to the larger placental mass, and therefore, the greater number of binucleate giant cells, which are the main source of PAGs, and the greater secretory activity of these cells (Ranilla et al. 1997). The prediction of the fetal number would be of benefit for sheep breeders as it would enable them to feed pregnant ewes accordingly in the prepartum period. Thus, the best option would be to use PAG concentrations as an indicator of multiple fetuses. However, it should be noted that multiple fetuses may not always be determined by measuring PAG concentrations (Roberts et al. 2017).

In the present study, PAG levels showed a rapid decrease after lambing and fell to the basal level by the 4th week postpartum. It was ascertained that the decrease in serum PAG levels occurred more rapidly, and thus, PAG clearance from the blood circulation occurred within a shorter time period in sheep, when compared to cattle (Mukasa-Mugerwa ve Viviani 1992). In parallel with our study, several literature reports have indicated that serum PAG levels, which show a continuous increase from week 17 of pregnancy to lambing, rapidly decrease after parturition and fall to the lowest level by week 4 postpartum (Ranilla et al. 1994; Ranilla et al. 1997; Szenci et al. 1998; Gonzalez et al. 1999; Gajewski et al. 1999; Roberts et al. 2017). It has been reported that PAG concentrations show a progressive decrease in the postpartum period and fall to a level of 0 ng/mL by day 28 postpartum (De Carolis et al. 2020). The decrease determined at the time of parturition could be related to the expulsion of the fetal membranes from ewes within 6 to 12 h after lambing or to a delayed postpartum sampling (Roberts et al. 2017). Differently, in cattle, PAG concentrations decrease slowly in the postpartum period, such that they are still detectable by day 100 after parturition. Therefore, contrary to the case in cattle, the rapid decrease of PAG levels in the postpartum period in ewes is critical to the use of PAGs for pregnancy diagnosis in sheep (De Carolis et al. 2020). However, the use of PAG1 for pregnancy diagnosis may cause false positive results in the event of remating after early pregnancy loss due to residual PAG1 levels remaining from the ended pregnancy (Roberts et al. 2017).

In the present study, as shown in Table 1, a positive correlation was determined between PAG and progesterone levels at GW 8 (r: 0.699) and GW 11 (r: 0.736) ($p<0.05$). Based on this finding, at weeks 8 and 11 of pregnancy, increased progesterone levels were associated with increased PAG levels in the Karya ewes. The present study has determined, for the first time, the correlation between maternal serum progesterone levels and PAG levels throughout pregnancy in Karya ewes. Previous studies in cattle and goats differ in their results, such that while some researchers have reported a positive correlation between progesterone and PAG levels throughout pregnancy (Mercadante et al. 2013; Roberts et al. 2017; Tandiya et al. 2013), some other have reported no such correlation (Lobago et al. 2009). In parallel with our investigation, Roberts et al. (2017) reported a strong correlation between PAG1 and progesterone levels as of mid-pregnancy, and thus, suggested that PAG1 levels could be effectively used as an indicator of placental function throughout gestation in sheep. Furthermore, in a study on Sirohi goats, the correlation coefficient between PAG and progesterone concentrations was found to be statistically significant ($p<0.05$) and positive ($r=0.98$) from day 4 before oestrus to day 28 of pregnancy (Salve et al. 2016). Moreover, it has been reported that, in sheep, PAG and progesterone profiles differ, such that while PSPB displays a bimodal pattern of secretion and peaks twice during pregnancy on gestational days 60 and 120, progesterone concentrations show a steady increase throughout pregnancy (Roberts et al. 2017). Several studies have demonstrated that both progesterone and PAG levels are generally higher in cases of multiple pregnancy, in comparison to singleton pregnancy (Barbato et al. 2009), and that a strong positive correlation exists between progesterone and PAG levels (Roberts et al. 2017). These results differ from the report of Ranilla et al. (1997), which indicated an insignificant correlation between the progesterone and PAG concentrations of twin- and singleton-pregnant ewes. In fact, in the present study, excluding gestational weeks 8 and 11, no correlation was detected between the progesterone and PAG levels. Similar results have been previously reported for Churra and Merino ewes, suggesting no correlation to exist between progesterone and PAG concentrations throughout gestation (Ranilla et al. 1994).

CONCLUSION

Differences in the results reported for the correlation between PAG and progesterone levels could be due to less frequent sampling, species-specific differences in placental function and PAG cross-reactivity, as well as species- or subspecies-specific differences in PAG and progesterone dynamics during pregnancy. There is need for furthermore comprehensive studies on a greater number of ewes to more specifically determine the plasma PAG and progesterone profiles of Karya ewes throughout pregnancy. Based on our results, we conclude that the concurrent measurement of progesterone and PAG levels at a defined time point of pregnancy could serve as a predictive method for the diagnosis and differentiation of singleton and multiple pregnancies.

Conflict of interest: The authors have no conflicts of interest to report.

Authors' Contributions: CC and MA contributed to the project idea, design and execution of the study. CC and RF contributed to the acquisition of data. CC and MA analysed the data. CC and MA drafted and wrote the manuscript. CC and MA reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

Ethical approval: This research was conducted with the approval of Pamukkale University, Animal Research The Ethics Committee (**PAUHDEK-2023/38).**

Explanation: We have no presented as a oral, poster, abstract vs.

REFERENCES

Adeyeye, A.A., Abubakar, Y.U., 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. Mac Vet Rev, 44 (1), 55-62, doi.org/10.2478/macvetrev-2021-0010

Akdeniz E. (2022). Koyunlarda erken gebelik teşhisinde gebelikle ilişkili glikoproteinler (PAG), progesteron (P4) ve trabsrektral ultrasonografi (USG) yöntemlerinin karşılaştırılması. Balıkesir Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi.

Akköse, M. (2020). Evaluation of a bovine rapid visual PAG ELISA test and transabdominal ultrasonography for early pregnancy diagnosis in Awassi sheep. KSÜ Tarım ve Doğa Dergisi, 23(5):1366-1372.

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(1), e1345.

Anghel, A., Zamfirescu, S., Coprean, D., Elena, S., & Dobrın, N. (2011). Assessment of progesterone and pregnancy associated glycoprotein concentration for early pregnancy diagnosis in ewe. Annals of the Romanian Society for Cell Biology, 16(2), 133-136.

Barbato, O., Menchetti, L., Brecchia, G., & Barile, V.L. (2022). Using pregnancy-associated glycoproteins (PAGs) to improve reproductive management: From dairy cows to other dairy livestock. Animals, 12, 2033. https://doi.org/10.3390/ani12162033

Chaves, C.M.S., Costa, R.L.D., Duarte, K.M.R., Machado, D.C., & Paz, C.C.P. (2017). Visual ELISA for detection of pregnancy-associated glycoproteins (PAGs) in ewe serum. Theriogenology, 2017, 97, 78- 82.

Chaves, C.M.S., 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 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 (Basel), 25, 10(9), 1502, doi: 10.3390/ani10091502.

El Amiri, B., Karen, A., Sulon, J., Melo de Sousa, N., Alvarez-Oxiley, A.V., Cognié, Y., Szenci, O., & Beckers, J.F. (2007). Measurement of ovine pregnancy-associated glycoprotein (PAG) during early pregnancy in Lacaune sheep. Reprod Domest Anim, 42(3), 257-62. https://doi.org/10.1111/j.1439-0531.2006.00761.x.

Erdem, H.İ, & Sarıbay, M.K. (2015). Gebelik ve Tanı Yöntemleri. In: Semacan A, Kaymaz M, Fındık M, Rişvanlı A, Köker A. Çiftlik Hayvanlarında Doğum ve Jinekoloji. 2. Baskı. Malatya: Medipres, 507-522.

Gajewski, Z., Beckers, J.F., Sousa, N.M., Thun, R., Sulon, J., & Foundez, R. (1999). Determination of pregnancy associated glycoprotein concentration in sheep. A retrospective study. Adv Cell Biol, 2, 89–96.

Gonzalez, F., Sulon, J., Garbayo, J.M., Batista, M., Cabrera, F., Calero, P.O., Gracia, A., & Beckers, J.F. (1999). Early pregnancy diagnosis in goats of pregnancy associated glycoproteins in plasma samples. Theriogenology, 52, 717–725.

Guillomot, M. (1995). Cellular interactions during implantation in domestic ruminants. J Reprod Fertil, 49, 39–51

Hall, D.G., Hoist, P.J., & Shutt, D.A. (1992). The Effect of Nutritional Supplements in Late Pregnancy on Ewe Calostrum Production Plasma Progesterone and IGF- 1 Concentrations. Aust. J. Agric. Res, 43, 352-337.

Humblot, P., Camous, S., Martal, J., Jeanguyot, N., Thibier, M., & Sasser, R.G. (1988). Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows. J. Reprod. Fertil, 83, 215-223.**Humblot, P., De Montigny, G., Jeanguyot, N., Tetedoie, F., Payen, B., Thibier, M., & Sasser, R.G. (1990).** Pregnancy-specific protein B and progesterone concentrations in French Alpine goats throughout gestation. J Reprod Fertil, 89(1), 205-12. doi: 10.1530/jrf.0.0890205.

Karaca, O., & Cemal, G. (1998). Batı Anadolu koyunculuğunda genetik kaynakların korunma ve kullanımı. Ege Bölgesi I. Tarım Kongresi, 7-11 Eylül 1998, ADÜ, Ziraat Fakültesi, Aydın.

Karaca, O., & Cemal, G.(2005). Koyun genotiplerimizin ıslahı için örnek bir yapılanma: Adnan Menderes Üniversitesi - Grup Koyun Yetiştirme Programı (ADÜ-GKYP). HASAD Hayvancılık, 21, 241, 30-35.

Kaplan Bilmez Y. (2018). Koyunlarda erken gebelik teşhisinde sığır gebelik teşhisinde sığır gebelik ilişkili glikoprotein kitlerinin kullanılabilirliğinin araştırılması. Dicle Üniversitesi Sağlık Bilimleri Enstitüsü, Yüksek Lisans Tezi.

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

Karen, A., Beckers, J. F., Sulon, J., Amiri, B., Szabados, K., Ismail, S., Reiczigel, J., & Szenci, O. (2003). Evaluation of false transrectal ultrasonographic pregnancy diagnoses in sheep by measuring the plasma level of pregnancy-associated glycoproteins. Reproduction Nutrition Development, 43(6), 577-586.

Karen, A., Szabados, K., Reiczigel, J., Beckers, J.F., & Szenci, O. (2004). Accuracy of transrectal ultrasonography for determination of pregnancy in sheep: effect of fasting and handling of the animals. Theriogenology, 61 (7-8), 1291-1298.

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-caPAG55+59) RIA and its potential for diagnosing pregnancy. Theriogenology, 66(4), 90612.doi:10.1016/j.theriogenology.2006.02.031.

Lobago, F., Bekana, M., Gustafsson, H., Beckers, J.F., Yohannes, G., Aster, Y., & Kindahl, H. (2009). Serum profiles of pregnancy-associated glycoprotein, oestrone sulphate and progesterone during gestation and some factors influencing the profiles in Ethiopian Borana and crossbred cattle. Reprod Domest Anim, 44, 685-692.

Lucy, M., Green, J., & Pooc, S. (2013). Pregnancy determination in cattle: A review of available alternatives. Proceedings Applied Reproductive Strategies in Beef Cattle. Staunton VA, 15(16), 165-176.

Medan, M., Watanabe, G., Absy, G., Sasaki, K., Sharawy, S., & Taya, K. (2004). Early pregnancy diagnosis by means of ultrasonography as a method of improving reproductive efficiency in goats. J Reprod Dev 50: 391-97.

Mercadante, P.M., Waters, K.M., Mercadante, V.R., Lamb, G.C., Elzo, M.A., Johnson, S.E., Rae, D.O., Yelich, J.V., & Ealy, A.D. (2013). Subspecies differences in early fetal development and plasma pregnancy-associated glycoprotein concentrations in cattle. J Anim Sci, 91: 3693-3701.

Meshref, A.E., Moawad, A.A., Helmy, S.M., Kasem, H.H., & Abouzed, T.K. (2022). Assessment of blood and urine pregnancy associated glycoprotein 1 in pregnant and aborted Osseimi ewes. Kafrelsheikh Veterinary Medical Journal. 20(2): 7-12. https://kvmj.journals.ekb.eg/article_259495.html

Mukasa-Mugerwa, E., & Viviani, P. (1992). Progesterone concentrations in peripheral plasma of Menz sheep during gestation and parturition. Small Rumin Res, 8: 47-53.

Özdemir Salcı E.S. (2015). Koyunlarda farklı doğum indüksiyon yöntemlerinin hormonal ve immünolojik yönden karşılaştırılması. Uludağ Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi.

Ranilla, M.J., Sulon, J., & Carro, M.D. (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., Mantecon, 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 Rum Res, 24, 125-31.

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. doi: 10.1016/j.theriogenology.2016.10.029. Epub 2016 Nov 9.

Rovani, M.T., Cezar, A.S., Rigo, M.L., Gasperin, B.Z., & Nóbrega Júnior, J.E. (2016). Evaluation of a bovine pregnancy-associated glycoprotein enzyme-linked immunosorbent assay kit for serological diagnosis of pregnancy in sheep. Cienc Rural, 46, 362-367.

Salve, R.R., Ingole, S.D., Nagvekar, A.S., Bharucha, S.V., & Dagli, N.R. (2016). Pregnancy associated protein and progesterone concentrations during early pregnancy in Sirohi goats. Small Ruminant Research, 141, 45-47. doi.org/10.1016/j.smallrumres.2016.07.003.

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 Domestics Animals, 54(2), 280-288.

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

Szenci, O. (2021). Recent Possibilities for the Diagnosis of Early Pregnancy and Embryonic Mortality in Dairy Cows. Animals (Basel), 3:11(6), 1666. doi: 10.3390/ani11061666.

Szenci, O., Beckers, J.F., Humblot, P., Sulon, J., Sasser, G., Taverne, M.A., Varga, J., Baltusen, R., & Schekk, G. (1998). Comparison of ultrasonography, bovine pregnancy-specific protein B, and bovine pregnancy-associated glycoprotein 1 tests for pregnancy detection in dairy cows. Theriogenology. 1;50(1):77-88. doi: 10.1016/s0093-691x(98)00115-0.

Tandiya, U., Nagar, V., Yadav, V.P., Ali, I., Gupta, M., Dangi, S.S., Hyder, I., Yadav, B., Bhakat, M., Chouhan, V.S., Khan, F.A., Maurya, V.P., & Sarkar, M. (2016). Temporal changes in pregnancy-associated glycoproteins across different stages of gestation in the Barbari goat. Anim Reprod Sci, 142, 141-148.

Tekin, T.C., & Köse, A.M. (2022). The Effectiveness of Transabdominal Ultrasonography on the 35th Day of Pregnancy in Sheep for Determining Pregnancy and Number of Fetuses. J VetBio Sci Tech, 7(2), 143-152.

Uçar U. (2017). Koyunlarda gebelik ile ilişkili glikoproteinler (PAGs) ile gebelik teşhisi, Dicle Üniversitesi Sağlık Bilimleri Enstitüsü Yüksek Lisans Tezi.

Ujjawala, Tandiya., Nagar, V., Yadav, V.P., Ali, I., Gupta, M., Dangi, S.S., Hyder, I., Yadav, Brijesh., Bhakat, M., Chouhan, V.S., Khan, F.A., Maurya, V.P., & Sarkar, M. (2013). Temporal changes in pregnancy-associated glycoproteins across different stages of gestation in the Barbari goat. Animal Reproduction Science, 142(3–4), 141-148.

Willard, J.M., White, D.R., Wesson, C.A., Stellflug, J., & Sasser, R.G. (1995). Detection of fetal twins in sheep using a radioimmunoassay for pregnancy-specific protein B. Journal of Animal Science, 73(4), 960-966. DOI:10.2527/1995.734960x

Yücel İ. (2022). Göçer Karya Koyunu Yetiştiriciliğinin Sürdürülebilirlik Değerlendirmesi. Aydın Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi.

Zamfirescu, S., Anhhel, A., Nadolu, D., & Dobrin, N. (2011). Plasmatic profiles of pregnancyassociated glycoprotein and progesterone levels during early pregnancy in carpathian goat Annals ofthe Romanian Society for Cell Biology, 16(2), 50-53.