Comparison of pregnancy specific protein - b in abortive and pregnant ewes

NEVZAT SAAT[1]*, YUSUF BILAL CETINKAYA[2], TUNAHAN OZTURK[2], BUSE OZTURK[1], AND ALI RISVANLI[3,4]

[1] Balikesir University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology Balikesir/Turkiye
[2] Balikesir University Institute of Health Science, Department of Veterinary Obstetrics and Gynecology Balikesir/Turkiye
[3] Firat University Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology Elazig/Turkiye
[4] Kyrgyz-Turkish Manas University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology Bishkek/Kyrgyz Republic

SUMMARY

This study aimed to investigate serum Pregnancy-Specific Protein B (PSPB) levels in pregnant and aborting ewes to evaluate its potential as a biomarker for pregnancy health and pregnancy loss in small ruminants. Additionally, the study examined whether PSPB concentrations differ in ewes that experienced abortion due to various bacterial agents (*Brucella* spp., *Salmonella* spp., and *Campylobacter* spp.). A total of 88 Akkaraman ewes were included in the study, comprising 29 pregnant and 59 aborting animals. The causes of abortion were confirmed microbiologically, and blood samples were collected from pregnant ewes on gestational days 28-30, and from aborting ewes approximately 10 days post-abortion. Serum PSPB levels were measured using a sheep-specific Enzyme-Linked ImmunoSorbent Assay (ELISA) assay. Chi-square test and one-way ANOVA test were used to evaluate the data. PSPB concentrations in pregnant ewes (19.13 ± 2.36 ng/mL) were significantly higher than those in aborting ewes (13.50 ± 0.66 ng/mL). Among aborting ewes, mean PSPB levels were 13.81 ± 1.40 ng/mL for *Salmonella* spp., 13.82 ± 2.17 ng/mL for *Campylobacter* spp., and 14.61 ± 1.60 ng/mL for *Brucella* spp.; however, no statistically significant differences were observed between the bacterial agents. The reduction in PSPB concentrations following abortion supports previous studies indicating that low PSPB or Pregnancy-Associated Glycoprotein (PAG) levels are associated with embryonic or fetal loss. These findings demonstrate that PSPB, which enters the maternal circulation around day 21 of gestation, is a reliable marker not only for pregnancy detection but also for predicting pregnancy loss. The lack of variation in PSPB levels according to the type of bacterial infection highlights its direct value in assessing abortion events. Overall, the results suggest that PSPB has potential as a standard biomarker for monitoring pregnancy health and the early detection of pregnancy loss in sheep. Advanced studies involving larger sample sizes and longitudinal monitoring are warranted to further validate the accuracy and reliability of PSPB in clinical applications.

KEY WORDS

Abortion; Bacterial agent; Pregnancy specific protein b; Ewe.

INTRODUCTION

Small ruminants play a significant role not only in utilizing poor pastures, stubbles, and areas unsuitable for plant production but also in the production of meat, milk, wool, hair, mohair, and leather, which are essential for the textile industry (1). According to Food and Agriculture Organization (FAO) data from 2023, there are approximately 2.5 billion sheep and goats worldwide. This figure highlights the significant importance of small ruminants in the livestock sector (2).

Maintaining economic profitability in sheep farming enterprises is highly related to reproductive performance. The earliest possible diagnosis of pregnancy and pregnancy loss is crucial for improving efficiency in the sheep farming sector. In this regard, various methods are used for pregnancy diagnosis, including less practical approaches such as absence of estrus, abdominal palpation, and palpation of the caudal uterine artery, as well as more practical methods like radiography, rectal/abdominal palpation, hormonal tests (progesterone measurements, estrone sulfate determination, chorionic gonadotropin, or placental lactogen detection), pregnancy protein tests (Pregnancy Associated Glycoproteins, Pregnancy Specific Protein - B (PSPB)), and as a gold standard ultrasonography (3-5).

Among the tests commonly used in recent times, one of the most important is pregnancy protein tests. These proteins, introduced to the literature for the first time by Sasser et al. (1988), are a mixture of multiple protein forms and are referred to as PSPB (6). PSPB was first isolated from the bovine placenta (7). PSPBs are proteins produced in the binucleate cells of the fetal trophoblast (8).

Pregnancy Specific Protein B forms a large placental glycoprotein family belonging to the group of proteolytic enzymes. In ru-

*Corresponding Author:
Nevzat Saat (nevzatsaat@balikesir.edu.tr)

minants, it begins to be secreted by the superficial layer of the developing trophoblast from approximately day 21 of pregnancy and is released into the maternal circulation. Maternal blood concentrations increase until day 30. The physiological role of PSPB during pregnancy has not been determined; however, it is likely involved in maintaining the corpus luteum by stimulating prostaglandin E_2 production (9).

Pregnancy Specific Protein B is not only a good indicator of pregnancy but also of pregnancy loss (10). It has been observed that serum PSPB concentrations begin to decrease approximately 2.5 days after pregnancy loss (11). Therefore, serum PSPB can be used to detect embryonic loss (12,13).

In a study by Adeyeye et al. (2021), the weekly mean PSPB in pregnant ewes was reported to range from 0.27 to 133.90 ng/mL, while in non-pregnant ewes it ranged from 0.10 to 0.97 ng/ml. In the first trimester, PSPB levels peaked in the fifth week post-mating with an average of 100.60 ng/mL, while in the second trimester, they reached a peak in the tenth week post-mating with an average of 133.90 ng/mL. In the third trimester, there was a gradual increase in PSPB levels, reaching a peak a few days before parturition. A gradual and statistically significant ($p<0.05$) decrease in PSPB levels was observed from parturition to the third week postpartum when comparing postpartum and non-pregnant ewes. In the fourth week postpartum, serum levels showed no statistically significant difference ($p>0.05$) when compared to non-pregnant ewes (14).

The most significant problems in sheep are embryonic losses and abortions. Abortions in sheep can be classified as infectious or non-infectious. Among the causes of abortion, infectious diseases such as brucellosis, campylobacteriosis, salmonellosis, listeriosis, leptospirosis, and chlamydia infections are at the forefront. Non-infectious causes include genetic factors, heat stress, management and nutrition issues, and toxicities, among many other factors (15).

Brucella is a gram-negative intracellular bacterium. Most *Brucella* species cause a zoonotic and infectious disease known as brucellosis in their hosts; this disease manifests in animals with symptoms such as abortion, infertility, and lameness. Brucellosis can be transmitted through the inhalation of aerosolized bacteria, or via contact with, or ingestion of, contaminated tissues or tissue-derived products (16). The target cells and tissues include trophoblast cells, fetal lung, macrophages, and male and female reproductive organs. Subsequently, the fetal internal organs and placenta become heavily infected. In animals, *Brucella spp.* is characterized by abortion, retained placenta, orchitis, and epididymitis (17). In sheep and goats, late-term abortions and stillbirths occur during the later stages of pregnancy (15).

Salmonella species are facultative intracellular bacteria capable of invading and proliferating within various cell types under in vitro conditions (18). Salmonellosis is a zoonotic infectious disease caused by *Salmonella spp. Salmonella spp.* leads to mass abortions in sheep during the late stages of pregnancy; abortive lambs die within their first days of life (19). In sheep and goats, abortions and neonatal deaths occur during the late stages of pregnancy (15).

Campylobacter spp. are gram-negative, spiral bacteria. Infection with *Campylobacter spp.* is one of the most common causes of ovine abortion worldwide (20). In ewes infected with *Campylobacter*, abortion typically occurs in the late stages of pregnancy. Generally, weak lambs or stillborn lambs are born (21).

The aim of this study is to measure and compare Pregnancy-Specific Protein B (PSPB) levels in pregnant and aborting ewes. Additionally, it will investigate whether there is a difference in PSPB levels in ewes that abort due to bacterial abortion agents.

MATERIALS AND METHODS

Ewes

In this study, 29 pregnant and 59 from aborted Akkaraman ewes, similar breed, aged between 2 and 5 years and clinically healthy were used. The causes of abortion in the ewes were confirmed as *Brucella spp., Salmonella spp.,* and *Campylobacter spp.* through microbiological tests. Ewes were divided into two main groups as pregnant and aborted. The aborting ewes were further categorized into three subgroups based on the pathogen, forming a total of four groups. Samples were collected from four separate farms, ensuring each group included animals from different farms. All pregnant ewes were selected from a similar farm where they had access to pasture during the summer and were fed concentrate supplements under the same environmental and nutritional conditions. The pregnancy status of the animals selected for the pregnant group was confirmed with ultrasonography (Fujifilm, Sonosite Edge II Ultrasound, Japan). The animals were provided with hay and concentrate supplements and pasture feeding, while water was offered ad libitum. The welfare criteria were met with 2.5 m^2 per sheep.

Collecting samples

Blood samples from pregnant ewes were collected on days 28-30 of gestation. To confirm the abortion causes, sterile swab samples were collected from the ewes that were aborted and from the aborted fetuses. Blood samples from ewes that had been aborted during the 10^{th}-12^{th} week of pregnancy were collected approximately 10 days after the abortion date. Some studies have reported that PSPB levels decrease during the first 4 weeks postpartum (14). Blood samples were collected from the jugular vein of the sheep without anticoagulant, vacuumed tubes. The collected blood samples were brought to the laboratory within 1-2 hours, centrifuged at 3500 rpm for 10 minutes, and the serum was stored at -20°C until analysis (Fig. 1).

Microbiological Analysis

The samples collected were processed according to ISO 6579: 2002/ Amd 1: 2007 method for the detection of Salmonella. The samples was preenriched with peptone water (Oxoid) (24 h/37°C), enriched in Rappaport Vassiliadis Salmonella broth (Oxoid), and later incubated in xylose lysine desoxicholate agar (XLD, Oxoid) for 48 hour Suspected Salmonella colonies (completely black or pink to red with black centers; 3 colonies per sample) were transferred to lysine iron agar, triple sugar iron, and urea agar slants for further testing. A range of analytical procedures were employed for the identification of the sample, including urea, Triple Sugar Iron Agar medium tests, lysine decarboxylase, Voges-Proskauer, indole and ß-galactosidase tests (22).

Primary isolation of Brucella was achieved by culturing samples on Farrell's modified serum dextrose agar. This medium was prepared from blood agar supplemented with 5% horse serum, 1% dextrose, and pre-mixed antibiotics added per ml of medium as follows: Bacitracin (25 IU), Polymyxin B (5 IU), Cycloheximide (100 µg), Nalidixic acid (5 µg), Nystatin (100 IU), and Vancomycin (20 µg). The inoculated plates were incubated at 37°C in an aerobic environment containing 5% to

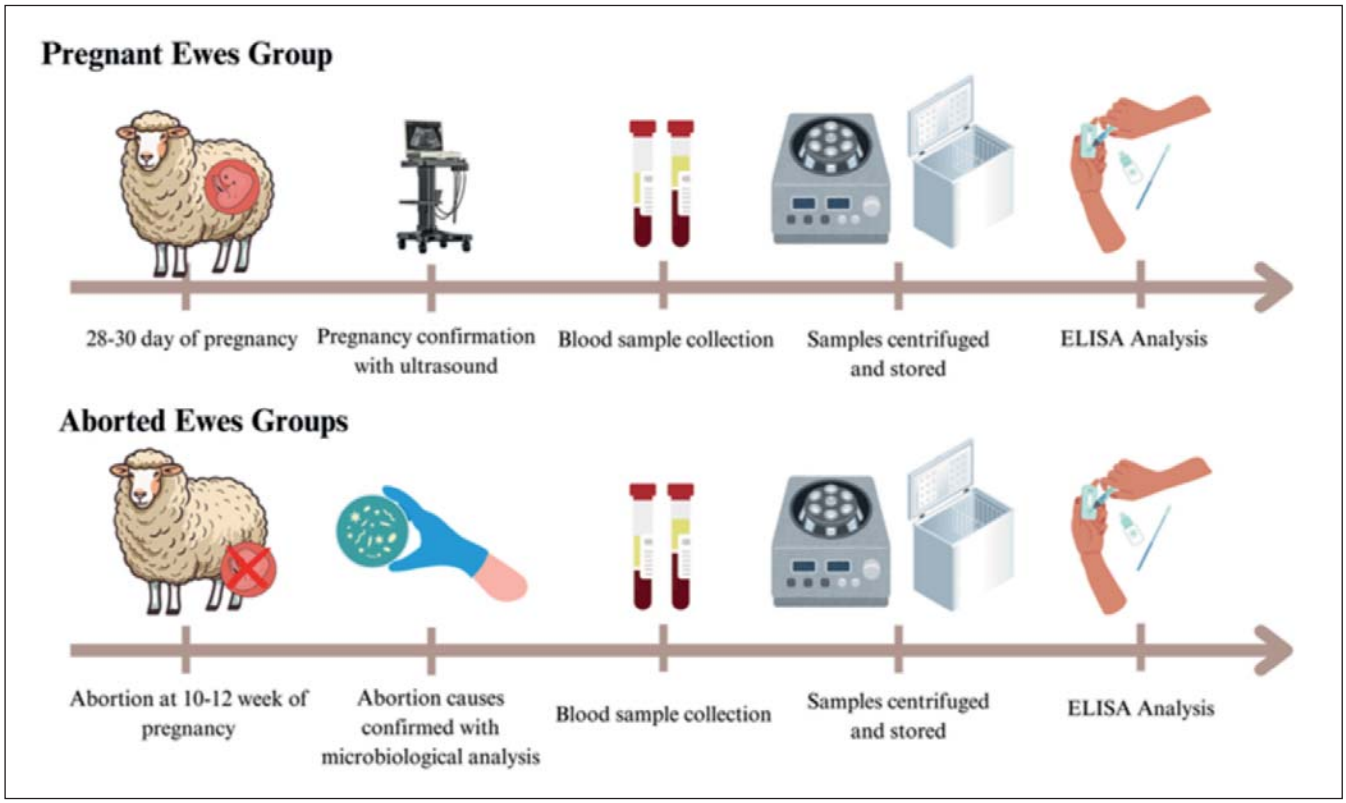

Figure 1 - Experimental design.

10% CO , and examined for Brucella-like colonies after three to five days. Plates with no visible growth after seven to ten days of incubation were appropriately discarded. The identification of the isolates at the Brucella genus level was facilitated by a comprehensive consideration of the colony morphology, Gram staining and growth characteristics, as well as the catalase, oxidase and urease test results. The subcultures were subjected to a series of phenotypic tests, encompassing catalase, oxidase, urea hydrolysis, motility, growth in 20 and 40 µg/mL of basic fuchsine and thionin, CO_2 requirement, H_2S production, and hemolysis tests. These tests were performed in accordance with standard protocols for each individual test (23).

To culture *Campylobacter* spp. from necropsy samples, including placenta, uterus, and combined fetal liver and lung tissues, tissue samples were finely chopped using sterile scissors or scalpels, and then inoculated onto an appropriate culture medium. Mueller-Hinton (MH) agar was used as the culture medium, supplemented with Preston Campylobacter selective supplement (trimethoprim, rifampicin, polymyxin B, cycloheximide; SR0117E, Thermo Fisher, USA) and Campylobacter growth supplement (sodium metabisulfite, sodium pyruvate, ferrous sulfate; SR0232E, Thermo Fisher, USA). One series of plates was subjected to an incubation process for a period of three days at a temperature of 37°C. In contrast, another series of plates was exposed to an incubation process at a temperature of 42°C within a microaerophilic atmosphere (Thermo Scientific, CN0035A, USA). The suspected colonies were examined using both macroscopic and microscopic morphology. A series of biochemical tests were carried out on the colonies. These included assessments of catalase, oxidase, and hydrogen sulfide production. The identification of Campylobacter isolates was conducted in accordance with their capacity for growth at temperatures of 25°C and 42°C, sensitivity to nalidixic acid and cephalothin, and hippurate hydrolysis (24).

PSPB Analysis

The sheep-specific PSPB Enzyme-Linked ImmunoSorbent Assay (ELISA) test kit has been observed to be an effective method for early pregnancy diagnosis in sheep. The same ELISA kit was used for both aborting and non-aborting ewes. Blood samples collected on day 30 of gestation and from ewes that aborted between the 10^{th} and 12^{th} weeks of pregnancy were centrifuged to separate the serum. PSPB levels in the serum samples were measured using ELISA kits (PSPB ELISA kit, Catalog No: E0094Sh, BT Lab, China) and determined with an ELISA reader (ELx800, BioTek, USA) (25).

Statistical Analysis

The results are presented as values ± standard error of the mean (SEM). Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics 30 software. To examine the relationship between the PSPB values measured from pregnant ewes and those from ewes that aborted, the Chi-Square test was applied. A one-way ANOVA test was conducted to compare PSPB levels in ewes that aborted due to three different agents*: Brucella spp., Salmonella spp.,* and *Campylobacter spp.* A significance threshold of $p < 0.05$ was used to indicate statistical significance.

RESULTS

In ELISA results from blood samples collected from the pregnant ewes at gestational days 28-30, the mean PSPB value for 29 ewes was measured as 19.13±2.36 ng/mL.

Microbiological analysis of blood samples and aborted fetus-

es collected from ewes that aborted in gestational weeks 10-12 indicated that 9 ewes experienced abortions due to *Salmonella spp.,* 10 due to *Campylobacter spp.*, and 40 due to *Brucella spp*. ELISA results from blood samples taken approximately 10 days post-abortion showed a mean PSPB value of 13.50±0.66 ng/mL across 59 ewes. In this study, a statistically significant difference was found between the PSPB values in pregnant ewes and aborted ewes. (Fig. 2).

The study investigated whether PSPB levels differed in abortions caused by the bacterial agents *Salmonella spp., Campylobacter spp.,* and *Brucella spp.* The mean PSPB level in ewes aborting due to *Salmonella pp*. was found to be 13.81±1.40 ng/mL, for those aborting due to *Campylobacter spp*. 13.82±2.17 ng/mL, and for *Brucella spp*. 14.61±1.60 ng/mL. Statistical analysis revealed no significant difference when the three agents were compared collectively or individually ($p < 0.01$) (Fig. 3).

DISCUSSION

In this study, a statistically significant difference was found between the PSPB values in pregnant ewes (n=29) with a mean of 19.13±2.36 ng/mL and those in aborted ewes (n=59) with a mean of 13.50±0.66 ng/mL.

In the study by Adeyeye et al. (2021) conducted on 30 Yankasa ewes, PSPB values ranged from 0.27 to 133.90 ng/mL from the first to the last week of pregnancy. Postpartum measurements indicated a decrease from 114.82 ng/mL to as low as 2.38 ng/mL within the first 4 weeks after birth. Similarly, in this study, there was also a statistically significant decrease in PSPB values between pregnant and non-pregnant (aborted) ewes. This similarity is thought to be due to the fact that the molecule measured and the animals studied are of the same species. However, the PSPB values for both pregnant and non-pregnant ewes in these two studies do not show similarity. The reason for this lack of similarity is the use of different ELISA kits (14).

In another study in 2007, 710 cows experiencing late embryonic death, Gabor et al. suggested that low PSPB concentrations could be associated with late embryonic loss, revealing that PSPB measured at low levels on days 30-36 post-insemination statistically significantly indicated late embryonic death. Similarly, in this study, PSPB levels in aborted ewes were found to be significantly lower than in pregnant ewes. This similarity between studies, despite the different animal species, may be due to both being taxonomically from the same family and measuring the same molecule (26).

In another study by Mosaad et al. in 2024, serum Pregnancy Associated Glycoprotein (PAG) and progesterone levels were measured in goats that experienced lead (Pb) induced abortions. PAG levels in aborted goats (2.963±0.350 ng/mL) were found to be significantly lower than those in singleton (9.492±0.286 ng/mL) and twin pregnancies (27.231±0.638 ng/mL). Similarly, in this study, PSPB levels in ewes that aborted (13.50±0.66 ng/mL) were statistically significantly lower than those in pregnant ewes (19.13±2.36 ng/mL). Since PAG and PSPB are proteins of a similar molecular structure belonging to the same family and used for pregnancy diagnosis, the similarity in results between the two studies is thought to be due to this relationship (27).

CONCLUSIONS

In conclusion, this study demonstrates that PSPB, which is used

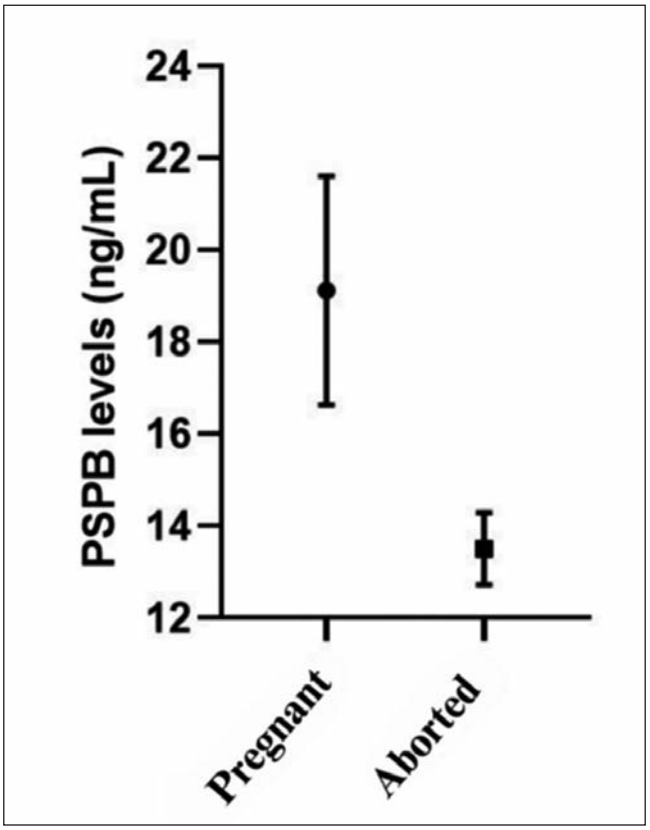

Figure 2 - Mean value of Pregnancy Specific Protein - B (PSPB) in pregnant and aborted ewes.

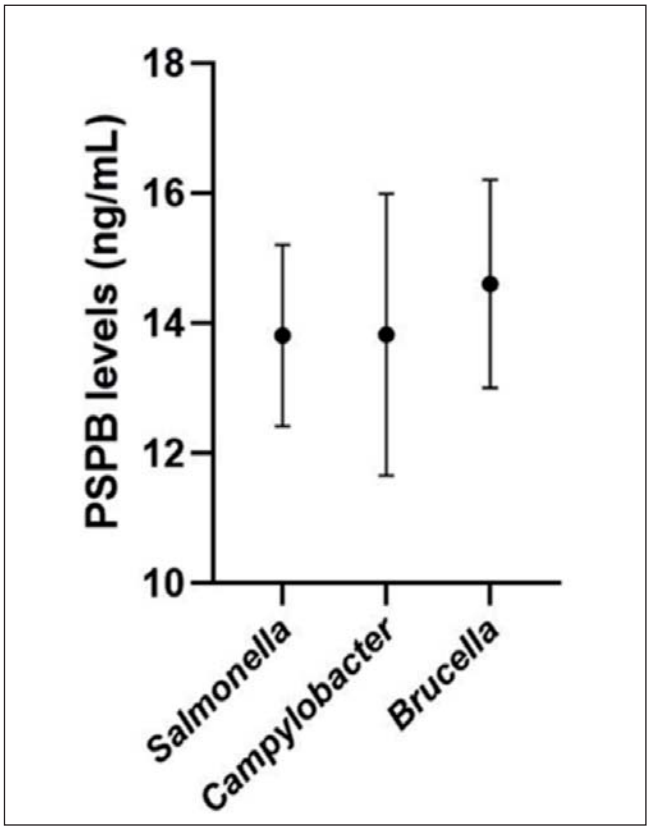

Figure 3 - Pregnancy Specific Protein - B (PSPB) levels in sheep with abortion caused by bacterial agent.

with high reliability for pregnancy diagnosis in ewes from days 28-30 onwards, exhibits significantly lower concentrations in ewes that experienced abortion compared to pregnant ewes. Furthermore, it is suggested that PSPB could serve as an indicator of pregnancy loss and may aid in predicting such losses in advance. Additionally, multiple bacterial agents are known to cause abortion in ewes. In this study, the serum PSPB concentrations of animals that experienced abortion due to *Salmonella spp., Campylobacter spp.,* and *Brucella spp.* were measured, and it was found that the causative agent of abortion had no effect on PSPB levels. Further scientific studies are necessary to confirm these findings.

Ethical Approval

The authors confirm that the journal's ethical policies, as specified on the author guidelines page, have been adhered to, and that approval from the appropriate ethics review committee has been obtained. The guidelines of the "Balikesir University Local Ethics Committee for Animal Experiments" (reference number: 2022/2-2) were followed.

Acknowledgements

We extend our gratitude to Assoc. Prof. Dr. Ayse Ebru BORUM for her contributions to the microbiological analyses and to the farm personnel for their assistance in sample collection.

Author Contributions

Yusuf Bilal CETINKAYA, Tunahan OZTURK and Buse OZTURK conceived the presented idea. Nevzat SAAT developed the theory and performed the calculations. Yusuf Bilal CETINKAYA, Tunahan OZTURK, Buse OZTURK and Nevzat SAAT conducted the experiment. All authors contributed to the collection and preservation of the samples. Yusuf Bilal CETINKAYA and Tunahan OZTURK wrote the manuscript. All authors discussed the results and contributed to the final manuscript. Nevzat SAAT and Ali RISVANLI supervised the project.

Conflict of Interest Statement

All authors declare that there is no financial conflict of interest with any institution, organization, or individual related to the article titled "Comparison of Pregnancy Specific Protein - B in Abortive and Pregnant Ewes" and there is no conflict of interest among the authors.

Funding

The supply of consumables used during the collection of materials was provided with the support of the Balıkesir University Scientific Research Projects Unit under project number 2020/116.

References

1. Costa, P. T., Vaz, R. Z., de Mendonça, G., Restle, J., Kroning, A. B., Ferreira, O. G. L., Farias, P. P. 2020. Consumer perception of products from the production chain of natural coloured sheep. *Small Rumin. Res.*, 192:106223. https://doi.org/10.1016/j.smallrumres.2020.106223
2. FAO. 2025. Crops and Livestock Products. *Food and Agriculture Organization of the United Nations*. https://www.fao.org/faostat/en/#data/QCL (accessed August 26, 2025)
3. Ali, A., Derar, D. R., Abdel-Razek, A.-R. Kh. 2024. Ultrasonography for the detection of pregnancy and study of embryonic and fetal development in camels, buffaloes, and sheep: Techniques, equations, and limitations. *Anim. Reprod. Sci.*, 268:107566. https://doi.org/10.1016/j.anireprosci.2024.107566
4. Safak, T., Tumer, K. C., Alp, Y. I., Ozen, T. Y., Yilmaz-Koc, O. 2025. Evaluation of the accuracy and performance of two commercial pregnancy-associated glycoprotein tests for early pregnancy detection in cows. *Vet. Med. Sci.*, 11. https://doi.org/10.1002/vms3.70226
5. Saat, N., Cetinkaya, Y. B. 2023. Sığırlarda doğum sonrası dönemde gebelikle ilişkili glikoprotein hızlı testinin güvenirliği. In: Goncagül, G., Günaydın, E. (eds.) *Veteriner Hekimlik Alanında Akademik Araştırma ve Derlemeler.* Platanus Publishing, Ankara, pp. 287-297. https://doi.org/10.5281/zenodo.10446730
6. Sasser, R. G., Williams, R. J., Bull, R. C., Ruder, C. A., Falk, D. G. 1988. Postpartum reproductive performance in crude protein-restricted beef cows: Return to estrus and conception. *J. Anim. Sci.*, 66:3033. https://doi.org/10.2527/jas1988.66123033x
7. Butler, J. E., Hamilton, W. C., Sasser, R. G., Ruder, C. A., Hass, G. M., Williams, R. J. 1982. Detection and partial characterization of two bovine pregnancy-specific proteins. *Biol. Reprod.*, 26:925-933. https://doi.org/10.1095/biolreprod26.5.925
8. Hameed, O. A., Mustafa, M., Madi, N., Tate, W. 2020. Evaluation of a rapid visual pregnancy (Bio-RPD) test for detection of pregnancy specific protein B (PSPB) in cow serum. *J. Anim. Sci. Livest. Prod.*, 4:1-4. https://www.primescholars.com/articles/evaluation-of-a-rapid-visual-pregnancy-bio-rpd-test-for-detection-of-pregnancy-specific-protein-b-pspb-in-cow-serum.pdf
9. 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 Vet. Hung.*, 66:329-336. https://doi.org/10.1556/004.2018.029
10. Gábor, G., Kastelic, J., Abonyi-Tóth, Z., Gábor, P., Endrődi, T., Balogh, O. 2016. Pregnancy loss in dairy cattle: Relationship of ultrasound, blood pregnancy-specific protein B, progesterone and production variables. *Reprod. Domest. Anim.*, 51:467-473. https://doi.org/10.1111/rda.12703
11. Pickworth, C. L., Sheffield, J., Poole, R. K. 2020. Pregnancy specific protein B concentration for the prediction of lambing rate in sheep. *Small Rumin. Res.*, 184:106052. https://doi.org/10.1016/j.smallrumres.2020.106052
12. Llanes, A., Whisnant, C. S., Knox, W. B., Farin, C. E. 2019. Assessment of ovulation synchronization protocols in goats and use of pregnancy specific protein B (PSPB) enzyme-linked immunosorbent assay (ELISA) to determine kid number at birth. *Domest. Anim. Endocrinol.*, 67:54-62. https://doi.org/10.1016/j.domaniend.2018.11.002
13. Humblot, P., Camous, S., Martal, J., Charlery, J., Jeanguyot, N., Thibier, M., Sasser R. G. 1988. Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows. *Reproduction*, 83:215-223. https://doi.org/10.1530/jrf.0.0830215
14. 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. *Maced. Vet. Rev.*, 44:55-62. https://doi.org/10.2478/macvetrev-2021-0010
15. Jainudeen, M. R., Hafez, E. S. E. 2000. Reproductive failure in females. In: Hafez, E. S. E. (ed.) *Reproduction in Farm Animals.*, 7th ed. Wiley, pp. 259-278. https://doi.org/10.1002/9781119265306.ch17
16. Celli, J. 2019. The intracellular life cycle of *Brucella* spp. *Microbiol. Spectr.*, 7:1-11. https://doi.org/10.1128/microbiolspec.bai-0006-2019
17. Adams, L. G. 2002. The pathology of brucellosis reflects the outcome of the battle between the host genome and the *Brucella* genome. *Vet. Microbiol.*, 90:553-561. https://doi.org/10.1016/S0378-1135(02)00235-3
18. Drevets, D. A., Leenen, P. J. M., Greenfield, R. A. 2004. Invasion of the central nervous system by intracellular bacteria. *Clin. Microbiol. Rev.*, 17:323-347. https://doi.org/10.1128/CMR.17.2.323-347.2004
19. Honchar, V., Iakubchak, O., Shevchenko, L., Midyk, S., Korniyenko, V., Kondratiuk, V., Rozbytska, T., Melnik, V., Kryzhova, Y. 2022. The effect of astaxanthin and lycopene on the content of fatty acids in the yolks of chicken eggs under different storage regimes. *Potravinarstvo Slovak J. Food Sci.*, 16:473-489.

https://doi.org/10.5219/1774
20. Yaeger, M. J., Sahin, O., Plummer, P. J., Wu, Z., Stasko, J. A., Zhang, Q. 2021. The pathology of natural and experimentally induced *Campylobacter jejuni* abortion in sheep. *J. Vet. Diagn. Invest.*, 33:1096-1105. https://doi.org/10.1177/10406387211033293
21. Salihu, M. D., Junaidu, A. U., Oboegbulem, S. I., Egwu, G. O. 2009. Prevalence and biotypes of *Campylobacter* species isolated from sheep in Sokoto State, Nigeria. *Int. J. Anim. Vet. Adv.*, 1:6-9. https://maxwellsci.com/print/ijava/6-9.pdf
22. Obaidat, M. M., Al-Zyoud, A. A., Bani Salman, A. E., Davis, M. A. 2017. Antimicrobial use and resistance among commensal *Escherichia coli* and *Salmonella enterica* in rural Jordan small ruminant herds. *Small Rumin. Res.*, 149:99-104. https://doi.org/10.1016/j.smallrumres.2017.01.014
23. Çetin, C., Taci Cangül, İ., Akan, M., Özel, E., Akkoç, A. 2004. Meningitis associated with *Brucella abortus* in an aborted bovine foetus. *Turk. J. Vet. Anim. Sci.*, 28:451-453. https://journals.tubitak.gov.tr/veterinary/vol28/iss2/32
24. Elbehiry, A., Marzouk, E., Hamada, M., Al-Dubaib, M., Alyamani, E., Moussa, I. M., AlRowaidhan, A., Hemeg, H. A. 2017. Application of MALDI-TOF MS fingerprinting as a quick tool for identification and clustering of foodborne pathogens isolated from food products. *New Microbiol.*, 40:269-278. https://pubmed.ncbi.nlm.nih.gov/28825446/
25. Akkus, T., Yaprakci, O. 2021. Determination of pregnancy protein levels to distinguish between singleton and twin pregnancies in Awassi sheep. *Med. Weter.*, 77:6597-2021. https://doi.org/10.21521/mw.6597
26. Gábor, G., Tóth, F., Ózsvári, L., Abonyi-Tóth, Z., Sasser, R. 2007. Early detection of pregnancy and embryonic loss in dairy cattle by ELISA tests. *Reprod. Domest. Anim.*, 42:633-636. https://doi.org/10.1111/j.1439-0531.2006.00834.x
27. Mosaad, A. F., El-Nakhla, S. M., Abd El-Rasoul, F. H., Shehabeldin, A. M., Ali, A. A., Morsy, G. M. 2024. Effect of ambient lead on progesterone and pregnancy-associated glycoprotein 1 and their relationship with abortion in Zaraibi goats: A field study. *Trop. Anim. Health Prod.*, 56:40. https://doi.org/10.1007/s11250-023-03877-w