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Investigation of the Reliability of Pregnancy-Associated Glycoprotein Rapid Test in Pregnant Heifers and Non-pregnant Lactating Cows *, **

Nevzat SAAT [1, a]
Dilara BÜLBÜL [2, b]
Tunahan ÖZTÜRK [3, c]
Yusuf Bilal ÇETİNKAYA [3, d]

[1] Balikesir University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Balikesir, TÜRKIYE

[2] Balikesir University, Faculty of Veterinary Medicine, Balikesir, TÜRKIYE

[3] Balikesir University, Institute of Health Science, Department of Obstetrics and Gynecology, Balikesir, TÜRKIYE

[a] ORCID: 0000-0002-8135-6142
[b] ORCID: 0009-0002-7923-8558
[c] ORCID: 0009-0005-7838-4643
[d] ORCID: 0009-0009-8787-9591

In dairy cattle operations, the sustainability of reproductive efficiency depends on shortening the interval between parturition and subsequent conception, which is of great importance for farm profitability. Therefore, pregnancy diagnosis should involve not only the identification of pregnant animals but also the early and accurate detection of non-pregnant individuals. In this context, rapid test kits based on pregnancy-associated glycoproteins (PAG) provide a practical alternative diagnostic method. The aim of this study was to evaluate the diagnostic reliability of a PAG-based rapid test kit for early pregnancy detection in cattle under field conditions. The study included 10 heifers confirmed pregnant by ultrasonography at 30–40 days of gestation and 10 cows confirmed as non-pregnant at ≥110 days postpartum. Blood serum samples were analyzed using a commercial PAG rapid test kit. Test performance was assessed based on accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), and agreement with ultrasonographic findings was evaluated using the Kappa coefficient. In heifers tested at 30–40 days of pregnancy, accuracy, sensitivity, and PPV were all 100%. In non-pregnant postpartum cows, false-positive results were observed in 30% of cases, resulting in a specificity of 70%. Overall test performance was 85% accuracy, 100% sensitivity, 70% specificity, 77% PPV, and 100% NPV. The agreement between PAG test results and ultrasonography was good (Kappa = 0.70). Pearson correlation analysis demonstrated a moderate-to-strong positive correlation between PAG and ultrasonography results. In conclusion, PAG-based rapid test kits offer high sensitivity and practical advantages for early pregnancy diagnosis in cattle; however, their use is recommended after at least 100 days postpartum to reduce false-positive results.

Key Words: *Pregnancy diagnosis, pregnancy-associated glycoprotein, postpartum dairy cow, heifer*

Gebe Düvelerde ve Gebe Olmayan İneklerde Gebelikle İlişkili Glikoprotein Hızlı Testinin Güvenilirliğinin Araştırılması

Süt sığırı işletmelerinde reprodüktif verimliliğin sürdürülebilmesi, doğum ile yeniden gebe kalma arasındaki sürenin kısaltılmasına bağlıdır ve bu durum işletme kârlılığı açısından önemlidir. Bu nedenle gebelik muayenesinde yalnızca gebe hayvanların değil, gebe olmayan bireylerin de erken ve doğru şekilde tespit edilmesi gerekmektedir. Bu bağlamda, gebelikle ilişkili glikoproteinlere (PAG) dayalı hızlı test kitleri pratik bir alternatif tanı yöntemi sunmaktadır. Bu çalışmanın amacı, sığırlarda erken gebelik tanısında kullanılan PAG tabanlı hızlı test kitinin saha koşullarındaki tanısal güvenilirliğini değerlendirmektir. Çalışmaya, gebeliğin 30–40. günlerinde ultrasonografi ile gebe olduğu doğrulanan 10 düve ile postpartum 110. gün ve sonrasında gebe olmadığı belirlenen 10 inek dahil edilmiştir. Tüm hayvanlardan alınan kan serum örnekleri ticari bir PAG hızlı test kiti kullanılarak analiz edilmiştir. Test performansı; doğruluk, duyarlılık, özgüllük, pozitif tahmin değeri (PPV) ve negatif tahmin değeri (NPV) parametreleri üzerinden değerlendirilmiş, test sonuçları ile ultrasonografi bulguları arasındaki uyum Kappa katsayısı ile analiz edilmiştir. Elde edilen sonuçlara göre, gebeliğin 30–40. günlerinde test edilen düvelerde doğruluk, duyarlılık ve PPV %100 olarak belirlenmiştir. Postpartum dönemde test edilen gebe olmayan ineklerde %30 oranında yanlış pozitiflik saptanmış ve özgüllük %70 olarak hesaplanmıştır. Genel test performansı doğruluk %85, duyarlılık %100, özgüllük %70, PPV %77 ve NPV %100 olarak bulunmuştur. PAG hızlı test sonuçları ile ultrasonografi arasındaki uyum iyi düzeyde olup Kappa katsayısı 0.70 olarak belirlenmiştir. Pearson korelasyon analizi, PAG ve ultrasonografi sonuçları arasında orta–yüksek düzeyde pozitif bir korelasyon olduğunu göstermiştir Sonuç olarak, PAG tabanlı hızlı test kitleri sığırlarda erken gebelik tanısında yüksek duyarlılık ve pratik kullanım avantajı sunmakla birlikte, yanlış pozitif sonuç riskini azaltmak amacıyla postpartum dönemde doğumdan en az 100 gün sonra kullanılması önerilmektedir.

Anahtar Kelimeler: *Gebelik teşhisi, gebelikle ilgili glikoprotein, sağmal inek, düve*

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Correspondence

Nevzat SAAT
Balikesir University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology
Balikesir – TÜRKIYE

nevzatsaat@balikesir.edu.tr

Introduction

The modern dairy cattle industry is of critical importance for both global food security and rural welfare, as high-yield milk production provides employment in rural areas and ensures a continuous supply of animal protein (1, 2). Sustaining economic

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profitability in dairy operations is directly related to herd milk production and reproductive performance. The continuity of productivity depends on the herd's ability to regularly re-enter lactation cycles. Therefore, the economic success of a dairy enterprise is closely associated not only with high milk yield but also with the maintenance of reproductive efficiency (3).

Early and accurate pregnancy diagnosis in dairy cattle is a key component of effective reproductive herd management (4). The primary objective of pregnancy monitoring is not only to identify pregnant animals but also to accurately detect non-pregnant individuals (5). Early pregnancy diagnosis shortens the open days period, thereby improving the financial efficiency of dairy farms. Timely identification of non-pregnant cows after artificial insemination facilitates faster rebreeding and reduces feeding and management costs (1, 5).

In Türkiye, the most commonly used pregnancy diagnostic methods under field conditions in dairy cattle are rectal palpation and transrectal ultrasonography. However, the use of biochemical-based diagnostic approaches for early pregnancy detection has been increasing (6). In addition to rectal palpation and ultrasonography, several biochemical parameters measurable in maternal blood—such as pregnancy-associated glycoproteins (PAG), pregnancy-specific protein B (PSPB), interferon-tau (IFN-τ), and early pregnancy factor (EPF)—are also employed for pregnancy detection (1, 7–10). In particular, commercially available rapid test kits based on the detection of PAG in milk, serum, plasma, and whole blood are gaining increasing attention in field veterinary practice due to their practical applicability, rapid results, user-friendly nature, and cost-effectiveness. The literature indicates that PAG-based lateral flow tests offer significant advantages in terms of reliability, speed, and suitability for field conditions in early pregnancy diagnosis (6, 11, 12).

Pregnancy-associated glycoproteins are secreted by trophoblastic binucleate cells of the placenta and enter the maternal circulation, making them reliable biomarkers for early pregnancy diagnosis. PAG can be detected in serum or milk samples and, when analyzed using ELISA-based methods, allow pregnancy detection with approximately 95–98% accuracy as early as days 28–30 of gestation (2, 5, 9). Variations in PAG concentrations during early gestation not only assist in pregnancy diagnosis but may also serve as potential indicators for predicting the risk of embryonic loss (13). In cases of non-pregnancy, PAG measurements provide highly reliable results, with accuracy exceeding 95%. Negative PAG results obtained after day 40 post-insemination indicate non-pregnancy with very high confidence (>95%) (11). However, PAG consist of multiple isoforms with differing biochemical structures and half-lives, which may allow their persistence in maternal circulation during the late postpartum period, thereby increasing the risk of false-positive results (14).

The aim of this study was to provide insight into the reliability of serum PAG measurements for early pregnancy diagnosis, to evaluate the advantages and limitations of PAG testing, and to promote its use as a tool to enhance the economic efficiency of dairy farming. Ultimately, the study seeks to contribute to shortening the calving-to-conception interval, increasing national livestock numbers, and supporting the development of future research initiatives in the livestock sector.

Materials and Methods

Research and Publication Ethics: All procedures performed in this study were conducted in accordance with the approval granted by the Local Ethics Committee of the Experimental Animals Breeding, Care, Application, and Research Center (Approval no: 2025/5-22, Approval date: 22.05.2025). The owner of the farm from which blood samples were collected was informed about the study, and written informed consent was obtained.

Animals: In this study, clinically healthy Holstein cows (n = 20) were used. The animals ranged in age from 1 to 6 years and had an average body weight of approximately 650 kg. Throughout the experimental period, all animals were maintained under identical environmental and management conditions. The cows were housed in group pens equipped with adequate resting areas, proper ventilation, and dry bedding. In accordance with the recommendations of the National Research Council (15), the animals were fed a total mixed ration (TMR) formulated based on their milk yield. Clean and fresh drinking water was provided ad libitum. At the beginning of the study, the body condition scores (BCS) of all animals were approximately 3 on a 1–5 scale.

Ultrasonographic Pregnancy Diagnosis: All animals received an estrus synchronization protocol followed by artificial insemination. All gynecological examinations were performed using transrectal ultrasonography with a portable B-mode ultrasound device equipped with a 5–10 MHz probe (SIUI CTS-800 Veterinary Ultrasound, Shantou, China). Based on the ultrasonographic findings, a total of 20 cattle were included in the study, comprising 10 heifers confirmed to be at 30–40 days of gestation and 10 cows determined to be non-pregnant at ≥110 days postpartum.

Collecting Samples: Blood samples (8 mL) were collected from all animals (n= 20) via the coccygeal vein into anticoagulant-free tubes and kept on ice for less than 2 hours until serum separation. Serum was obtained by centrifugation at +4°C (4000 × g for 5 minutes), after which the samples were labeled and stored at −20 °C until analysis.

Sample Analysis: The PAG-based rapid pregnancy test kit used in this study is a commercially available lateral flow immunoassay, with an approximate unit cost of 9 USD per test under local market conditions in Türkiye. The test was selected not only for its diagnostic performance but also for its suitability for routine field use and its cost-effectiveness as a practical diagnostic tool. According to the instructions provided in the test kit insert (Anieasy Bovine Pregnancy Rapid Test

Kit, colloidal gold, Shenzhen, China) (16), 120 µL (three drops) of blood, milk, or urine samples were applied to the sample well of the Pregnancy-Associated Glycoprotein rapid (lateral flow) test, followed by the addition of two drops of diluent. After sample application, a 20-minute period was allowed for result development, and the outcomes were documented within the first 10 minutes. The rapid test yields four possible result patterns. A positive reaction was defined by the distinct appearance of both the control and test lines. When the control line was clearly visible but the test line appeared faint, the result was classified as suspected positive. A negative result was indicated by the presence of the control line only. Tests in which the control line failed to appear were considered invalid, regardless of the visibility of the test line.

Serum samples were classified as positive when both the control and test lines were clearly visible. Negative and suspicious results were evaluated according to the instructions provided in the manufacturer's original reference manual. The reported percentage values are consistent with, and proportionally aligned to, the research and development validation data provided by the manufacturer.

It is acknowledged that retesting of serum samples classified as suspicious positive, through repeated examinations (e.g., one week later), could eliminate ambiguous and uncertain cases. However, this confirmatory approach was not applied in the present study, as the primary objective was to compare the diagnostic performance of the rapid test kit directly with ultrasonographic examination as the reference (gold standard) method.

Within this framework, the classifications were defined as follows: true positives referred to cows identified as pregnant by the rapid test kit and subsequently confirmed by ultrasonographic examination. True negatives denoted cows classified as non-pregnant by both the rapid test and ultrasonography. False positives referred to cows that tested positive for pregnancy using the rapid test but were confirmed as non-pregnant by ultrasonography. False negatives were defined as cows that yielded negative results in the rapid test but were diagnosed as pregnant by ultrasonographic evaluation.

Statistical Analysis: The following metrics were used for data analysis: accuracy ((true positive + true negative) / total number of samples), sensitivity (true positive / (true positive + false negative)), specificity (true negative / (true negative + false positive)), positive predictive value (true positive / total positive results), and negative predictive value (true negative / total negative results) (Table 1). The Kappa scoring scale applied in the study was as follows: 0.80–1.00 = very good, 0.60–0.80 = good, 0.40–0.60 = moderate, 0.20–0.40 = fair, and <0.20 = poor (Table 2). Additionally, the relationship between PAG and USG results was evaluated using Pearson correlation analysis. A correlation coefficient (r) was calculated to determine the strength and direction of the linear association between the two variables. Statistical analyses were performed using SPSS version 30.0 (IBM Inc. Chicago, USA) software, and the level of significance was set at $p<0.05$.

Confidence intervals could not be provided because the serum PAG concentrations (ng/mL) were not quantitatively measured. In addition, the detection thresholds of the PAG antibody bands impregnated on the lateral flow immunochromatographic test strip—specifically, the PAG concentration ranges (ng/mL) required to produce a positive or negative reaction—are not disclosed by the manufacturer. Consequently, quantitative interpretation of PAG levels and threshold-based confidence interval calculations were not feasible.

Table 1. Calculations od metrics

Metric Name	Formula	Description
Accuracy	(TP + TN) / Total	Proportion of all correctly classified cases
Sensitivity	TP / (TP + FN)	Ability to correctly identify true positives
Specificity	TN / (TN + FP)	Ability to correctly identify true negatives
Positive Predictive Value (PPV)	TP / (TP + FP)	Proportion of correct positive predictions
Negative Predictive Value (NPV)	TN / (TN + FN)	Proportion of correct negative predictions

TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative

Table 2. Kappa scoring scale

Kappa Value	Interpretation
0.80 – 1.00	Very Good
0.60 – 0.80	Good
0.40 – 0.60	Moderate
0.20 – 0.40	Fair
0.00 – 0.20	Poor

Results

In animals in Group 1 (n= 10), blood samples obtained from heifers whose first pregnancies were confirmed by ultrasonographic examination (30–40 days of gestation) yielded positive results in all cases when analyzed using the rapid test kit. Accordingly, the pregnancy rapid test kit demonstrated 100% accuracy from day 33 of gestation onward, and no false results were observed during this period.

In Group 2, consisting of non-pregnant animals at ≥110 days postpartum (n= 10), rapid testing of blood samples showed suspected positive results in 3 animals and negative results in 7 animals. Based on these findings, the PAG rapid test kit applied during the fourth postpartum month produced 30% false results and 70% correct results. These data are presented in Table 3. Overall evaluation of rapid test results from blood samples revealed an accuracy of 85%, sensitivity of 100%, specificity of 70%, positive predictive value (PPV) of 77%, and negative predictive value (NPV) of 100% (Table 3).

All animals were definitively classified as pregnant or non-pregnant by ultrasonographic examination. Animals confirmed as pregnant by ultrasonography were also identified as pregnant by the PAG lateral flow test kit. When the diagnostic accuracy of ultrasonography and the lateral flow test was compared in animals at 30–40 days of gestation, a concordance rate of 100/100 was observed. In contrast, this rate was calculated as 70/100 in animals evaluated during the fourth postpartum month (Table 4). The comparison between ultrasonography and the PAG test kit yielded a Kappa coefficient of 0.70, indicating a good level of agreement between the two methods. Pearson correlation analysis revealed a moderate-to-strong positive correlation between PAG and USG results ($r= 0.734$, $R^2= 0.538$, $p<0.01$, Figure 1). This indicates that approximately 53.8% of the variance in USG outcomes was explained by PAG results, suggesting a statistically significant and meaningful linear relationship between the two diagnostic methods. However, given the limited sample size (n= 20), this finding should be interpreted with caution and verified in larger cohort studies.

Table 3. Accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the glycoprotein test associated with pregnancy in dairy cows

Cow (n)	Ultrasonographic Examination (Gold Standard)	True Positive (A)	False Positive (B)	True Negative (C)	False Negative (D)	Accuracy %	Sensitivity %	Specificity %	Positive Predictive Value %	Negative Predictive Value %
Group 1 (n=10)	Pregnant	10	0	0	0	100	100	–	100	–
Group 2 (n=10)	Not Pregnant	0	3	7	0	70	–	70	–	70
Total Sample Size (n=20)	**100% Accurate Result**	**10**	**3**	**7**	**0**	**85**	**100**	**70**	**76.9% (~77%)**	**100%**

Rapid test results. Test data were organized as follows: true positive (A), false positive (B), true negative (C), and false negative (D). Accuracy = (A + C) / total number of samples, Sensitivity = A / (A + D), Specificity = C / (C + B), PPV = A / (A + B), NPV = C / (C + D).

Table 4. Accuracy results of the lateral flow test/ultrasonographic examination

Accuracy	Group 1 (n=10)	Group 2 (n=10)	Total Sample Size (n=20)
Lateral flow test / ultrasonographic examination	100/100	70/100	85/100

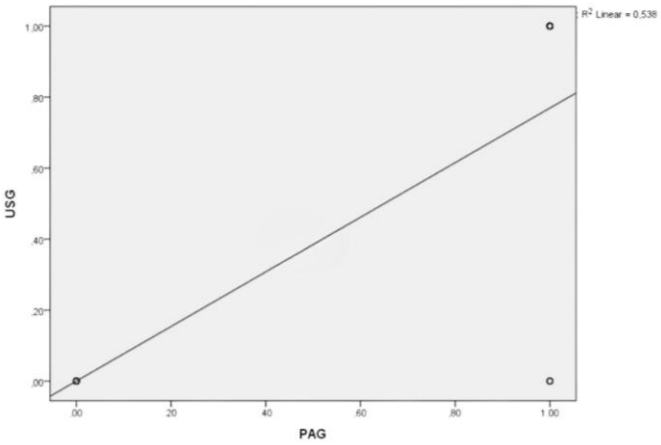


Figure 1. Linear correlation between pregnancy associated glycoprotein (PAG) rapid lateral flow test results and ultrasonographic (USG) findings

Discussion

In this study, the results of PAG rapid test kits were evaluated using blood samples collected from cattle at 30–40 days of first pregnancy and from cows that remained non-pregnant at ≥110 days postpartum. The findings demonstrated that PAG-based tests exhibit high reliability during early pregnancy. No false-positive results were observed in blood-based testing during the first month of gestation (0%), whereas a false-positive rate of 30% was detected during the fourth postpartum month. Accordingly, the sensitivity and specificity of the test were calculated as 100% and 70%, respectively. These results indicate that PAG rapid test kits represent an effective and practical method for early pregnancy diagnosis. However, the false-positive results observed during the postpartum period are thought to be associated with the prolonged half-life of PAG which can remain detectable in maternal circulation for up to 80–100 days after calving.

Kline et al. (17) reported high false-positive rates in PAG testing during the postpartum period, particularly within the first 42 days, noting that false positivity reached 98.16% at day 42 postpartum using IDEXX lateral flow tests. When considered together with the current study, these results suggest that an adequate postpartum waiting period is essential for reliable PAG testing, as early application may lead to false-positive outcomes. Kline et al. (17) recommended the use of the IDEXX Alertys OnFarm Pregnancy Test from day 40 of gestation onward, and both studies demonstrated consistent results during early pregnancy. However, while PAG detectability in the postpartum period was limited to 42 days in a study by Kline et al. (17), the

present study identified detectable PAG levels extending up to 120 days postpartum. Agreement between ultrasonography and PAG testing was good in the present study (Kappa= 0.70), whereas Kline et al. (17) reported very good agreement (Kappa=0.84). These differences may be attributed to variations in analytical sensitivity and antibody characteristics of the test kits used.

In another study, Akköse et al. (18) reported 100% sensitivity and 97.3% accuracy for pregnancy diagnosis after day 28 of gestation. Both studies emphasize the critical role of early pregnancy detection in effective reproductive herd management. However, the 30% false-positive rate and 70% specificity observed in non-pregnant cows at ≥110 days postpartum in the present study highlight a significant limitation of PAG testing during the postpartum period due to the prolonged persistence of PAG. Although Akköse et al. (18) did not directly evaluate the postpartum period, the high specificity (94.9%) reported at day 28 of gestation supports the reliability of PAG testing in early pregnancy. Differences in test performance among studies may result from the use of different commercial kits, such as the Bioeasy rapid test used in the present study and the IDEXX ELISA kit used by Akköse et al. (18), as well as methodological differences. Additionally, while the present study demonstrated good agreement between ultrasonography and PAG testing (Kappa=0.70), Akköse et al. (18) reported no significant correlation between PAG concentrations and progesterone levels, suggesting variability in diagnostic concordance across methodologies.

Ricci et al. (19) reported that PAG may take up to 60 days postpartum to be completely cleared from maternal circulation and recommended performing pregnancy tests after this period to avoid false-positive results. In the present study, agreement between ultrasonography and serum-based PAG rapid testing was also classified as good (Kappa = 0.70). In contrast, Ricci et al. (19) reported Kappa values of 0.84 (very good) for plasma PAG ELISA and 0.77 (good) for milk PAG ELISA. These differences may be explained by methodological variations between rapid tests and laboratory-based ELISA assays, as well as differences in biological sample matrices. Ricci et al. (19) also reported that plasma PAG concentrations were approximately twice as high as milk PAG levels, highlighting the influence of sample type on PAG detection sensitivity.

Kaya et al. (20) compared the performance of ELISA-based bovine pregnancy tests and IDEXX rapid pregnancy tests using plasma samples. They emphasized the practical advantages of rapid test kits, particularly their ease of use under field conditions without the need for laboratory infrastructure. In the present study, the agreement between ultrasonography and PAG testing was again found to be good (Kappa = 0.70). Although Kaya et al. (20) did not report Kappa statistics, both studies accepted ultrasonography as the gold standard for pregnancy diagnosis.

In a study by Akköse et al. (21), the IDEXX rapid pregnancy test used in dairy heifers achieved 96.2% sensitivity and 96.6% accuracy at day 32 of gestation. These findings are consistent with the 100% accuracy and sensitivity observed in the present study during days 30–40 of pregnancy. Akköse et al. (21) reported that PAG concentrations increase earlier in heifers than in cows, allowing reliable identification of non-pregnant heifers from day 24 of gestation, although confirmation of early positive results was recommended. In contrast, the present study identified a 30% false-positive rate in cows tested at ≥110 days postpartum, which may be related to differences between the Bioeasy rapid test used in this study and IDEXX kits used in other investigations.

In another study conducted by Szelényi et al. (22) evaluated early pregnancy diagnosis in 637 Holstein cows. Ultrasonography identified 42.9% (n= 273) of the animals as pregnant and 57.1% (n= 364) as non-pregnant. Among ultrasonographically confirmed pregnant cows, 1.1% yielded negative results with the IDEXX Alertys OnFarm test, while 11.2% of ultrasonographically non-pregnant cows tested positive. Based on these results, overall accuracy, sensitivity, specificity, PPV, and NPV were reported as 93.1%, 98.9%, 88.7%, 86.8%, and 99.1%, respectively. While true-positive rates were comparable to those of the present study, differences in false-positive rates may be attributed to sample size and the use of different commercial test kits. Taken together, these findings support the high diagnostic value of PAG-based tests for early pregnancy detection while emphasizing that test timing is critical to minimizing false-positive results during the postpartum period.

In a study conducted by Aydın and Dinç (23), PAG levels were evaluated in milk samples using an ELISA kit. The test demonstrated a sensitivity of 92%, a specificity of 100%, a correct pregnancy detection rate of 100%, a correct non-pregnancy detection rate of 91.7%, and an overall accuracy of 95.8%. Moreover, a statistically excellent agreement was observed between ultrasonography and the ELISA test kit (kappa value >0.8), and the false-positive rate of the milk PAG test was reported as 8.03%. While the rates related to positive pregnancy diagnosis obtained from these two studies show similarity, differences are observed in the false-positive rates. These discrepancies are thought to primarily arise from variations in the number of animals, the measurement methods employed, and the types of samples analyzed.

Kaya et al. (24) evaluated the effectiveness of a commercial ELISA kit based on PAG for early pregnancy diagnosis in Holstein–Friesian heifers and lactating cows. In that study, the overall sensitivity, specificity, and accuracy of the PAG-ELISA test were reported as 95.9%, 94.7%, and 95.2%, respectively, and the test performance was emphasized to be higher in heifers compared to lactating cows. Furthermore, it was stated that false-positive results could be observed in some cows evaluated during the postpartum period, which might be associated with residual PAG levels from

previous pregnancies and individual variability. The findings obtained in the present study show partial similarity with the results reported by Kaya et al. (24). Both studies demonstrate that PAG-based tests are reliable during the early stages of pregnancy. However, differences were observed in the false-positive rates during the late postpartum period. These discrepancies in postpartum false-positive rates may be explained by differences in the diagnostic methods used (lateral flow rapid test vs. ELISA), the type of biological samples analyzed (plasma vs. serum), the limited number of animals included in the studies, and variations in the timing of postpartum evaluation.

In the present study, the detection of pregnancies in heifers on days 30–40, confirmed by ultrasonography (the gold standard), using a PAG-based rapid test kit, was conducted to evaluate the reliability, accuracy, and internationally recognized validity of the test. Considering the cost of the rapid test kit, ultrasonographic examination remains a more accurate and cost-effective approach for early pregnancy diagnosis and the detection of embryonic loss. In cattle, previous pregnancies may leave residual PAG molecules that can be detected by the rapid test kit, potentially leading to false-positive results. Therefore, ultrasonography continues to be the indispensable method for early pregnancy diagnosis and the detection of embryonic death in such cases.

In conclusion, PAG rapid test kits offer significant advantages for reproductive herd management due to their ease of application under field conditions, non-invasive nature, and practical usability by breeders. However, when used during the postpartum period, careful attention should be paid to test timing, and to minimize the risk of false-positive results, it is recommended that these tests not be applied before 100 days after calving. Overall, the findings indicate that PAG rapid test kits may represent an important alternative method for shortening the calving-to-conception interval in dairy herds, thereby improving productivity and economic efficiency. Furthermore, future studies comparing different commercial kits will be valuable in supporting the reliability and wider field application of these tests.

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