COMPARISON OF A RAPID TEST WITH bPAG ELISA IN PREGNANCY DIAGNOSIS IN COWS

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ABSTRACT
Early pregnancy detection is vital for properly managing livestock farms by re-inseminating nonpregnant females and minimizing the calving intervals. The present investigation was executed to compare a rapid test (Dairy Cow Pregnancy Test from Span Biotech Ltd. Shenzhen, China) with a commercial bPAG ELISA test (IDEXX®) for pregnancy diagnosis in non-descriptive cows. The study also aimed to elucidate if the rapid test could be an alternative method to ELISA in the field. Blood and urine samples were collected from 43 cows to measure the concentrations of bovine pregnancy-associated glycoproteins (bPAGs) in the blood samples and detect progesterone via the rapid test in the urine samples. Examining the genital tracts was achieved after slaughtering the cows to determine the uterus' state, used as the reference standard for both tests. The results showed that the bPAG ELISA test was more accurate in pregnancy detection than the rapid test. However, there were no statistically significant differences (P > 0.05) between both methods' results. We conclude that the rapid test can be a suitable alternative method to the bPAG ELISA test for diagnosing pregnancy in cows in field conditions.

Keywords: bovine pregnancy-associated glycoprotein, early pregnancy detection

مقارنة بين اختبار سريع و bPAG ELISA في تشخيص الحمل في الأبقار

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المستخلص
يعد الكشف المبكر عن الحمل أمرًا حيويًا لإدارة مزارع الماشية بشكل صحيح عن طريق إعادة تلقيح الإناث غير الحوامل وتقليل فترات الولادة. تم إجراء التحقيق الحالي لمقارنة اختبار سريع (اختبار حمل بقرة الألبان من Shenzhen Biotech Ltd. China) مع اختبار bPAG ELISA التجاري (IDEXX®) لتشخيص الحمل في الأبقار غير الوصفية. هدفت الدراسة أيضًا إلى توضيح ما إذا كان الاختبار السريع يمكن أن يكون طريقة بديلة لـ ELISA في الحقل. تم جمع عينات الدم والبول من 43 بقرة لقياس تراكيز البروتينات السكرية المرتبطة بالحمل البقري (bPAGs) في عينات الدم والكشف عن هرمون البروجسترون عن طريق الاختبار السريع في عينات البول. تم فحص المسالك التناسلية بعد ذبح الأبقار لتحديد حالة الرحم، واستخدم كمعيار مرجعي لكلا الاختبارين. أظهرت النتائج أن اختبار bPAG ELISA كان أكثر دقة في الكشف عن الحمل من الاختبار السريع. ومع ذلك ، لم تكن هناك فروق ذات دلالة إحصائية (0.05 <P) بين نتائج كلتا الطريقتين. نستنتج أن الاختبار السريع يمكن أن يكون طريقة بديلة مناسبة لاختبار bPAG ELISA لتشخيص الحمل في الأبقار في الظروف الحقلية.

الكلمات الدالة: البروتين السكري المرتبط بالحمل البقري، الكشف المبكر عن الحمل، الاختبار السريع

INTRODUCTION

Low conception rates negatively impact the cattle industry by extending the calving intervals and, consequently, reducing the offspring number and milk production. The reproductive efficiency and profitability of dairy farms require early and accurate detection of pregnancy to eliminate a sterile cow and re-inseminate a nonpregnant cow as soon as possible (4). More significant economic benefits are obtained from the reduction of open days for nonpregnant cows. Hence, early pregnancy detection reduces the economic loss as a result of the treatment and re-insemination of a pregnant cow that is classified as nonpregnant. Accurate diagnosis of pregnancy also reduces the incidence of iatrogenic abortion, which is economically vital for the farmers (2). Hence, researchers try to find inexpensive, accurate, and practical methods of pregnancy detection that can provide accurate results in a short time (16). After insemination, adequate progesterone levels in the bloodstream are essential for a continuous and successful pregnancy (14). The corpus luteum and placenta are responsible for progesterone secretion (23). Progesterone concentration remains high during the gestation period due to corpus luteum graviditatis, which is present before the onset of parturition. After CL regression, the level of progesterone rapidly falls before the onset of parturition (26). High progesterone level prevents uterine contraction, provides a suitable environment for the conceptus, and influences endometrial secretions necessary for the embryo (11). Presently, several diagnostic techniques are frequently used in the farm. Traditional methods for detecting pregnancy in cattle, such as palpation per rectum or ultrasonography, are commonly used. With palpation per rectum, pregnancy diagnosis accuracy earlier than day 30 of gestation can be challenging (29) with abortion risk during the examination (20). Trans-rectal sonography is commonly used for pregnancy diagnosis in cows, but it needs to be conducted by specialists and has a lower accuracy rate before 33 days of gestation (3). After artificial insemination, nonpregnant cows' detection minimizes inter-insemination interval through bPAG detection in blood samples (12).

Estimating plasma progesterone level is considered one of the old methods used in pregnancy diagnosis 19–21 days post-insemination in cattle (5). Samples such as blood, milk, feces, and urine are used to monitor progesterone levels (8, 13, 22). Bovine pregnancy-associated glycoprotein (bPAG) belongs to the aspartic proteinase family of proteins. It is synthesized by the mononucleate and binucleate trophoblast cells of the trophectoderm (28). In many species, bPAGs are used as specific biomarkers for pregnancy detection (9, 19). For early detection of pregnancy, an ELISA is commonly used to measure bPAG in cows' plasma (21). One of the advantages of the ELISA technique is that many blood samples are tested simultaneously by colorimetric comparison (9). Nonetheless, this technique necessitates a laboratory to identify bPAG. Rapid pregnancy diagnosis tests can be easily applied in field circumstances without the involvement of instruments. However, the accuracy, specificity, and sensitivity of these tests need to be investigated before being used in livestock farms. Hence, the present research was conducted to compare a commercial bPAG ELISA test (IDEXX®) with a rapid test for cows' pregnancy diagnosis. We depended on the visual examination of the uterus as a gold standard to confirm both tests. We elucidated if the rapid test could be an alternative method to a commercial bPAG ELISA assay for early pregnancy detection.

MATERIALS AND METHODS

Study animals and sample collection

The study was accomplished from November 2017 to February 2018, and 43 cows of varying breeds, ages, and para were included. The cows were brought to the Sulaimani abattoir in the city of Sulaymaniyah, Iraq, for slaughter. Blood was taken from the coccygeal vein of the cows and put into test tubes with anticoagulants. Also, few milliliters of urine were directly taken from the urinary bladder after the animal was slaughtered, using a disposable syringe and needle. The blood samples were transported to the laboratory within six hours, centrifuged at 4500 RPM for 10 min to separate the plasma, and stored at -20°C until use. The urine samples were

utilized for pregnancy diagnosis immediately after collection.

Pregnancy diagnosis using the bPAG ELISA assay

The IDEXX Bovine Pregnancy Test Kit is an ELISA-based test designed to detect the early existence of pregnancy-associated glycoproteins (PAGs) in bovine plasma or serum. The bPAG plasma concentration in the collected plasma samples was determined by capture ELISA, using the IDEXX Bovine Pregnancy Test Kit (USA). The kit contains 96-well ELISA plates and essential reagents for plasma sample analysis in the cow. The analysis procedure was carried on according to the instruction from the manufacturer. A Microplate Reader (ELx800TM Absorbance Microplate Reader) was used to measure the absorbance at 450 nm.

Pregnancy diagnosis from urine samples

The Dairy Cow Pregnancy Test (Span Biotech Ltd. Shenzhen, China) is a rapid test that detects P4 in urine and is used for early pregnancy diagnosis in cows. The test strip contains antibodies to P4 that give two red lines; control and test, indicating pregnancy. A test strip is immersed in a urine sample, and the result is interpreted after five minutes. If there is not enough level of P4 in the urine, the test line does not appear as red, while two red lines at positions T and C indicate that the urine contains adequate amounts of P4.

Macroscopic examination

The genital organs of the slaughtered cows were examined to determine the status of the uterus in each slaughtered cow. The genital systems' visual examination was used as the gold standard to which both tests were compared. The stage of gestation in pregnant cows (in days) was determined by measuring the crown-rump length (CRL) of the fetuses and using the formula $(21 + CRL) \times 2.5$ (27).

Statistical analysis

The statistical comparisons were executed with IBM SPSS software package version 24.0. A Chi-squared test was used to analyze both techniques' accuracy in detecting pregnancy and corpus luteum in cows. A probability value < 0.05 was considered significant. The accuracy, specificity, and sensitivity of both techniques were compared to the uterus' visual examination. The results were categorized as correct negative (CN), correct positive (CP), false negative (FN), and false-positive (FP). Accuracy was measured by the formula $[(CP + CN) \div (CN + CP + FP + FN)] \times 100$. The sensitivity of each assay was calculated as $[CP \div (CP + FN)] \times 100$, while the specificity of each test was calculated as $[CN \div (CN + FP)] \times 100$. Also, positive predictive value was counted as $[CP \div (CP + FP)] \times 100$, and the negative predictive value was counted as $[CN \div (CN + FN)] \times 100$ (10).

RESULTS AND DISCUSSION

Pregnancy was investigated in 43 cows using Dairy Cow Pregnancy Test and IDEXX Bovine Pregnancy Test. The data shown in Table 1 explicate the correlation between the correct positive, correct negative, false positive, and false negative groups. This grouping was based on pregnancy assessment results using the bPAGs ELISA and rapid tests for pregnancy diagnosis in the cows and comparison with the gross examination of the genital tract as a standard. Using the bPAGs ELISA test, the results indicated that 25 (58.1%) of 43 cows were diagnosed correctly as pregnant and 16 (37.2%) as nonpregnant. However, 21 (48.8%) cows were identified as pregnant using the rapid test, and 13 (30.3%) as nonpregnant. Two false-positive diagnoses (4.7%) were recorded with the bPAGs ELISA test, but all pregnant cows were diagnosed correctly. The rapid test gave five false-positive diagnoses (11.6%) and four false-negative diagnoses (9.3%). The accuracy, sensitivity, specificity, PPV, and NPV of the Dairy Cow Pregnancy Test and IDEXX Bovine Pregnancy Test are illustrated in Table 2. The bPAGs ELISA test's sensitivity for pregnancy detection reached 100%, while the rapid test's sensitivity reached 84%. The ELISA bPAGs test was 88.8% specific, while the rapid test was 72.2% specific in detecting pregnancy. The bPAGs ELISA test was more accurate for identifying pregnancy than the rapid test, as they were 95.3% and 79.0% accurate, respectively. However, the rapid test accuracy increased to 90.7% when used for the detection of corpus luteum.

Table 1. The values of correct positives, correct negatives, false positives, and false negatives of overall examined cows by bPAG ELISA and rapid test for pregnancy detection

	ELISA (bPAG)				Rapid test
Correct positive	21 (48.8%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	21 (48.8%)
Correct negative	0 (00.0%)	11 (25.6%)	2 (4.7%)	0 (00.0%)	13 (30.3%)
False-positive	0 (00.0%)	5 (11.6%)	0 (00.0%)	0 (00.0%)	5 (11.6%)
False-negative	4 (9.3%)	0 (00.0%)	0 (00.0%)	0 (00.0%)	4 (9.3%)
Total	25 (58.1%)	16 (37.2%)	2 (4.7%)	0 (00.0%)	43 (100%)

There were no significant differences (0.114) between and within both techniques. Test: Chi-squared

Table 2. The values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of the bPAG ELISA and rapid tests for pregnancy and corpus luteum detection

Method of pregnancy detection	Value (%)				
	Sensitivity	Specificity	PPV	NPV	Accuracy
ELISA-bPAG	100.0	88.8	92.5	100.0	95.3
Rapid test	84.0	72.2	80.7	76.4	79.0

Identifying nonpregnant cows is essential to decrease calving intervals and improve milk production, thereby improving the livestock industry's productivity. This study compared the accuracy of two pregnancy diagnostic methods using the uterus' visual examination, after the cows' culling, as a reference (gold) standard. Other researchers also used this method as a standard to compare different diagnostic methods of pregnancy (30), since the probability of falsely diagnosing pregnancy is 0.0%. Other methods of pregnancy detection might not be as accurate. For example, ultrasound might be 93.7 to 97.8% accurate in pregnancy diagnosis on day 27 (24). Many studies relied on determining progesterone levels via blood, milk, and fecal samples using ELISA and radioimmunoassay techniques to detect pregnancy (8, 22). Scientists discovered specific antigens related to pregnancy during the last two decades, called pregnancy-associated glycoproteins (PAGs) in different species (18). Even though the methods mentioned above can detect pregnancy with high accuracy, they require radioisotopes or readable laboratory support. On the other hand, the rapid test can examine many samples simultaneously in a short time under field conditions, since it is easy to carry and inoffensive to maternal and fetal safety. The bPAG ELISA test was 100.0% sensitive in pregnancy diagnosis. This outcome is consistent with that recorded by Ghaidan et al. (4), who applied the same ELISA test kit. A similar result has also been achieved using different ELISA kits, such as Bovine Preg Test DG29®. In contrast, other researchers (7, 25) recorded lower sensitivity rates than the outcomes obtained in the current study, which ranged between 93.9% and 98.8%). The rapid test in the current investigation was 84.0% sensitive. This record was lower than the previously documented 89.4% by Moussafir et al. (16) using Ubio quickVet rapid test and 97.0% by Northrop et al. (17) using the Visual Pregnancy Test. Both tests were based on the detection of bPAG. This variation in both rapid commercial tests' results may be due to the use of different antibodies to detect specific PAG because there are more than 22 encoded genes of bPAG (6). The study's rapid test was based on progesterone metabolites in the urine in low progesterone producing cows (15). There was not enough progesterone to be detected by the rapid test, which increased false negatives and decreased sensitivity (4). The bPAG ELISA test's specificity for identifying nonpregnant cattle in this study was 88.8%, almost equivalent to the 88.0% reported by Northrop et al. (17) using the same ELISA test. A lower specificity (81.3%) was documented by Moussafir et al. (16) using Bovine Preg Test DG29®. The rapid test's lower specificity can be explained by the fact that five cows were in the diestrus phase with corpora lutea, increasing the number of cows falsely diagnosed as pregnant. The rapid test specificity in the current study (72.2%) was lower than the bPAG ELISA test's specificity. The rapid test in this study is dependent on the detection of progesterone metabolites in urine, which may increase incorrect diagnoses of nonpregnant cows, especially in the diestrus phase, leading to decreased specificity (1, 4). The bPAG ELISA test was 95.3% accurate in pregnancy detection. Nearly equivalent

accuracy (94.0%) was reported by Moussafir *et al.* (16) using Bovine Preg Test DG29®. Five cows were in the diestrus phase in this study, which led to inaccurate diagnoses of pregnancy in these animals and reduced the rapid test accuracy. If the rapid test were used to detect corpus luteum, the accuracy would reach 90.7%. In other words, the rapid test is more accurate when the history of insemination is known. However, there were no significant differences (p-value = 0.114) between the bPAG ELISA test results and the rapid test in this investigation. This study's outcomes imply that the rapid test is a cheaper and faster alternative to the more expensive and time-consuming ELISA-based assay.

CONCLUSION

The bPAG ELISA and rapid tests were reliable and convenient in pregnancy diagnosis using urine and plasma samples, provided they are collected from those cows with recorded data of insemination. This research's outcomes affirm that the bPAG ELISA test is a more precise method than the rapid test to detect pregnancy. However, the precision of the rapid test increases when the time of insemination is known. In these circumstances, the rapid test is an excellent choice to detect pregnancy in the field.
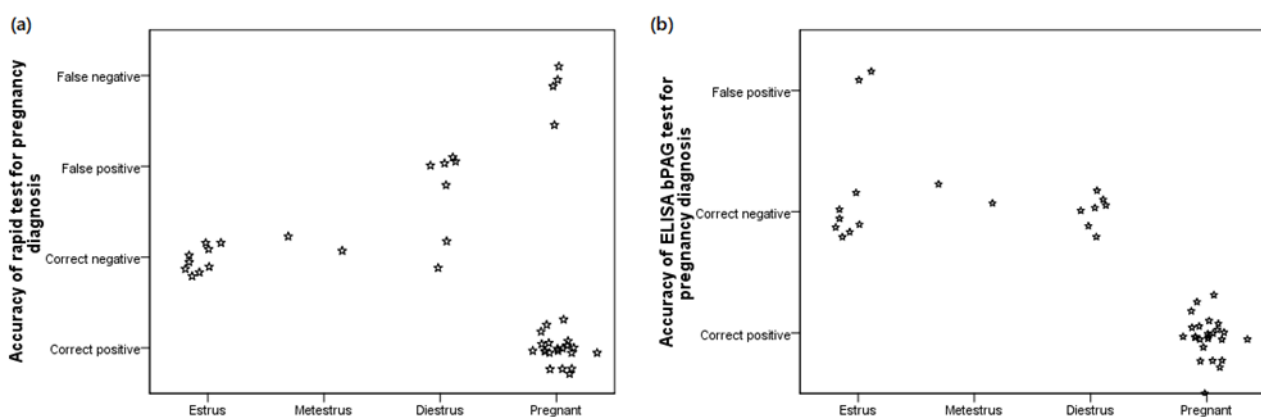

Figure 1. Scatter plots representing the number of cows. Part (a) shows the accuracy of the rapid test in pregnancy detection. Part (b) shows the accuracy of the bPAG ELISA test in the detection of pregnancy

Conflict of interest

The authors declare no conflict of interest.

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