RESEARCH ARTICLE

Comparative Efficacy of PAG-based Rapid Strip Test Kit, Ultrasonography and Per Rectal Palpation for Early Pregnancy Diagnosis in Cattle and Buffaloes

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Abstract

The present study was conducted to evaluate and compare the accuracy and reliability of different methods of pregnancy diagnosis like PAG-based BovEasy rapid PD strip test kit at days 28, 32, 36 and transrectal ultrasonography on day 28 against per rectal palpation on day 45 post-AI for detection of early pregnancy in Gir cows and Jaffarabadi buffaloes. 22-days non-return postpartum healthy animals comprising 75 Gir cows and 75 Jaffarabadi buffaloes, (150,) aged 4 to 14 years, were selected from the Cattle Breeding Farm, KU, Junagadh. The BovEasy bovine rapid pregnancy detection test kits on Days 28, 32, and 36 post-AI showed high accuracy, sensitivity, and specificity across all time points in detecting early pregnancy as confirmed by USG on day 28 and per rectal palpation on day 45. For Gir cows, accuracy increased from 97.33% (on day 28) to 100% (on days 32 and 36), while Jaffarabadi buffaloes demonstrated 100% accuracy across all days. The combined data of cattle and buffaloes revealed accuracy improvement from 98.67% (on day 28) to 100% (on days 32 and 36), with sensitivity and specificity reaching 100%. The accuracy of transrectal ultrasonography on Day 28 post-AI for pregnancy was verified by presence of amniotic vesicle and fetal heartbeats against strip test on day 28 and per rectal palpation on day 45. It demonstrated 100% accuracy, sensitivity, and specificity in both breeds, with no false positive or negative result. The overall actual pregnancy rate was 36.7% (55/150). Overall, embryonic mortality was 4.7% (7/150). Reliability of early pregnancy detection methods when validated against per-rectal palpation results on Day 45 post-AI showed the similar results in both the species. Conception rate with diagnostic methods and embryonic mortality though higher in Gir cows did not differ from those in Jaffarabadi buffaloes.

Key words: Bovine, BovEasy rapid PD test kits, Conception rate, Embryonic loss, Rectal palpation, Ultrasonography.

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Introduction

Pregnancy diagnosis (cyesiognosis) plays a vital role in the livestock sector, helping to evaluate reproductive efficiency over time. Ensuring optimal reproductive performance is critical for sustainable and profitable livestock farming. Early pregnancy diagnosis helps identify reproductive issues like delayed puberty, anestrus, silent/unobserved estrus, duration of lactation and calving intervals, while providing a framework for effective reproductive management, treatment, and rebreeding. Accurate early pregnancy diagnosis is essential for detecting and minimizing early embryonic mortality and boosting farm productivity and profitability (Barik and Yadav, 2022). Estrus detection & successful conception are essential for achieving optimal pregnancy rates (Suthar and Dhami, 2010). Transrectal palpation is among the oldest and most widely practiced methods for diagnosing pregnancy in large animals from approximately 30 days to 50 days post-AI (Zemjanis, 1970). Typically, pregnancy is confirmed between 45 and 60 days through rectal palpation in bovines; however, this method cannot accurately assess fetal viability, leading to variability in its diagnostic precision.

The introduction of transrectal ultrasonography (USG) in the 1980s marked a significant advancement in early pregnancy detection. The real-time B-mode transrectal ultrasonography is now regarded as the "gold standard" for early pregnancy diagnosis in cows due to its capability to identify pregnancy from as early as 28 days of gestation. It allows visualization of early embryonic structures and assessment of embryo viability, including detection of the fetal heartbeat, as early as 19-24 days of gestation (Bagley

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et al., 2023). However, it has limitation of portability in the field, cost, skill and experience of its use with user. Hence, ELISA-based assays kits to measure pregnancy associated glycoprotein (PAG) levels in maternal blood have been developed recently for cattle and buffaloes. However, these tests are costly and not feasible for small farms. To address these issues, researchers in China/India have developed AniEasy/BovEasy on-farm PAG-based early pregnancy detection strip test kits with accuracy of 95% to 99%, and are made available in Indian markets. Therefore, this study was planned to validate, under controlled farm conditions, the effectiveness of this new diagnostic method with traditional pregnancy detection techniques like transrectal ultrasonography and rectal palpation for detection of early pregnancy and embryonic mortality in cattle and buffaloes.

Materials and Methods

The experiment was conducted at Cattle Breeding Farm, Kamdhenu University, Junagadh (Gujarat, India), during the December 2023 to October 2024 following approval of Institutional Animal Ethics Committee (IAEC) of the College (Protocol No.: KU-JVC-IAEC-LA-124-23).

Experimental Animals and their Management

The present study involved 75 Gir cows and 75 Jaffarabadi buffaloes, aged between 4 and 14 years, which were housed at the Cattle Breeding Farm, KU, Junagadh. Only those animals showing normal cyclic activity and no detectable reproductive abnormalities were included in the study. These animals were selected on day 22 post-AI if they did not show any kind of signs of repeat estrus. Cattle and buffaloes were housed in different sheds having a loose housing system with unrestricted access to wholesome drinking water. They were fed according to the farm's standard schedule, which included seasonal fodder, hay, compounded concentrates and mineral mixture according to their production and reproduction status.

Blood Sampling and Assays Procedures

The mature animals with clinically sound reproductive tract and standing estrus were artificially inseminated using frozen-thawed semen during later half of estrus. Those animals that did not return to estrus by day 22 post-AI were checked for pregnancy from day 28 onwards by various methods. Blood samples (2 mL) were collected by Jugular veni-puncture in heparinized vacutainers on days 28, 32 and 36 post-AI for the PAG-based AniEasy/BovEasy bovine rapid pregnancy detection kits (Prompt Equipments Pvt. Ltd., Ahmedabad, India) on fresh blood as per the manufacturer's instructions (Fig. 1). The trans-rectal ultrasonography (TRUS) was done on day 28 post-AI using portable ultrasound scanner equipped with a probe of 5 MHz (Mindray Z5 Vet) and per rectal palpation was performed on day 45 post-AI.

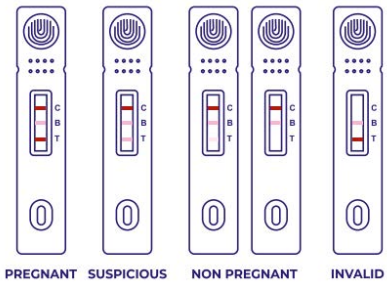

Fig. 1: Interpretation of rapid bovine pregnancy test strip kit result

Statistical Analysis

Descriptive statistics was used to analyse the data and compare the results of test days and methods of pregnancy detection. The chi-square test was applied to analyze conception rates and embryonic losses across different test days/groups. Sensitivity, specificity, accuracy and reliability of BovEasy/AniEasy rapid PD test kits in relation to USG and actual per rectal palpation findings were worked out using standard formulae.

Results and Discussion

Efficacy of AniEasy/BovEasy Bovine Rapid Pregnancy Detection Test Kits

The data outlined in Table 1 reflect pregnancy test results of 150 animals, comprised of 75 Gir cows and 75 Jaffarabadi buffaloes on days 28, 32 and 36 post-AI using AniEasy/BovEasy rapid bovine pregnancy detection test kits. The results were consistently similar with the use of AniEasy (China) and BovEasy (India) test kits on the same blood samples. In Gir cows, accuracy of these pregnancy detection strip test kits increased from 97.33% on Day 28 to 100% on Days 32 and 36 post-AI, and both sensitivity and specificity reached 100% after Day 28 post-AI, indicated highly reliable test results as time progressed. For Jaffarabadi buffaloes accuracy, sensitivity, and specificity was 100% across all three-time points, indicating that the test results were consistently accurate and reliable. The combined data for both species over three time points showed that an increase in accuracy from 98.67% on Day 28 to 100% by Day 32 and Day 36. Sensitivity also improved from 98.39% on Day 28 to 100% subsequently, and specificity reached 99% on Day 28, achieving 100% on the later days (Table 1). These data indicate the AniEasy/BovEasy rapid bovine pregnancy detection kit is a highly accurate, improving over time from earliest day 28 to day 32 post-AI.

Paré *et al.* (2008) obtained almost similar results on sensitivity, specificity, positive predictive value and negative predictive value of commercially available blood test kits by comparing with ultrasonography in cattle. The result of 100% sensitivity from day 32 for on-farm PAG-based rapid pregnancy detection was also confirmed by Giordano *et al.*

(2013). However, a slightly low result on day 24 noted, that was improved further with advancing days of pregnancy by Ricci *et al.* (2015). So for more accurate result with high sensitivity and specificity they suggested to test the animals by day 32, which is in accordance with our results.

The confusion matrix analysis of data in Table 1 show that on Day 28 post-AI, the kit correctly identified 61 pregnant animals (true positives) and 87 non-pregnant animals (true negatives), with only two suspicious cases of which 1 turnout to be false negative and 1 false positive. However, by Day 32 and Day 36 post-AI, the kit achieved perfect accuracy, correctly identifying all 62 pregnant animals (true positives) and 88 non-pregnant animals (true negatives) without any false positive or false negative. These findings indicate that this BovEasy rapid early bovine pregnancy detection kit of Prompt Equipments Pvt. Ltd., Ahmedabad, we tested, is highly reliable and accurate for early pregnancy detection in cattle and buffaloes, particularly from Day 32 post-AI onwards. The kit's exceptional performance and ease suggest its potential as a valuable tool for reproductive management in livestock production.

Table 1: Validation of BovEasy early pregnancy detection kits on days 28, 32 and 36 post-AI in 22 days non-return Gir cows and Jaffarabadi buffaloes against actual per rectal palpation on day 45

Sr. No.	Measure	Gir cows			Jaffarabadi buffaloes			Total		
		Day 28	Day 32	Day 36	Day 28	Day 32	Day 36	Day 28	Day 32	Day 36
1	Total animals tested	75	75	75	75	75	75	150	150	150
2	Total animals found positive	36	37	37	25	25	25	61	62	62
3	Total animals found negative	37	38	38	50	50	50	87	88	88
4	Suspicious result	2	0	0	0	0	0	2	0	0
5	Embryonic death	5 (between day 36th - 45th)			2 (between day 36th - 45th)			7 (between day 36th - 45th)		
		Per rectal examination (day 45th)								
6	Actual pregnant (day 45)	32			23			55		
7	Actual non-pregnant (day 45)	43			52			95		
8	True positive	36	37	37	25	25	25	61	62	62
9	True negative	37	38	38	50	50	50	87	88	88
10	False positive	1	0	0	0	0	0	1	0	0
11	False negative	1	0	0	0	0	0	1	0	0
12	Total	75	75	75	75	75	75	150	150	150
13	Accuracy (%)	97.33	100.00	100.00	100.00	100.00	100.00	98.67	100.00	100.00
14	Sensitivity (%)	97.30	100.00	100.00	100.00	100.00	100.00	98.39	100.00	100.00
15	Specificity (%)	97.36	100.00	100.00	100.00	100.00	100.00	99.00	100.00	100.00

Accuracy = (True positive + True negative) / (True positive + True negative + False positive + False negative)
Sensitivity = (True positive / (True positive + False negative)
Specificity = True negative / (True negative + False positive)

Ultrasonography versus Rectal Palpation for Early PD

A validation study was conducted to assess the accuracy of transrectal ultrasonography on Day 28 post-AI in detecting pregnancy in Gir cows and Jaffarabadi buffaloes (Table 2). The results were verified against early PD kits on Day 28 and actual pregnancy status determined by per rectal palpation on Day 45. A total of 150 animals (75 Gir cows and 75 Jaffarabadi buffaloes) were tested using ultrasonography on Day 28 post-AI. The results based on presence of amniotic vesicle and fetal heartbeats showed that 62 animals (41.3%) were pregnant, while 88 animals (58.7%) were non-pregnant, whereas with BovEasy early PD kit, the result on Day 28 in 2 animals particularly Gir cows, was confusing turning into one false positive and one false negative on Day 32/36 and even with per rectal confirmation on day 45. Per rectal palpation on day 45 confirmed 55 animals (36.7%) pregnant, while 95 animals (63.3%) were non-pregnant. Out of 75 Gir cows, 32 cows were pregnant and 43 cows were non-pregnant. Among 75 Jaffarabadi buffaloes, 23 were pregnant and 52 were non-pregnant (Table 2).

The results of transrectal ultrasonography on day 28 post-AI revealed 100% accuracy for actual pregnancy in both Gir cows and Jaffarabadi buffaloes, 100% sensitivity in detecting true positives (pregnant animals), 100% specificity in detecting true negatives (non-pregnant animals), with no false positives or false negatives were observed in either breed. These results on accuracy, sensitivity and specificity of transrectal ultrasonography technique on Day 28 were slightly more than those (100 vs 97%) found with BovEasy early PD kits on the same day post-AI. The present findings of USG agreed well with earlier reports by Patel (2005), Parikh *et al.* (2019) and Chaudhari *et al.* (2022) with regard to early accurate pregnancy diagnosis in bovines. Further, Szenci *et al.*

(1995) and Romano and Fahning (2013) reported their findings similar to current research around day 45 post-AI with 100% reliable results. Karen *et al.* (2011) and Borakhatariya *et al.* (2024) reported the same results on and around day 45 post-AI in buffaloes for the confirmation of pregnancy status by per rectal palpation. This method showed low reliability and accuracy earlier than day 35-40 post-AI.

Table 2: Validation of transrectal ultrasonography on day 28 post-AI in 22 days non-return Gir cows and Jaffarabadi buffaloes against actual per rectal palpation on day 45

Measure	Gir Cows	Jaffarabadi Buffaloes	Total
Total Animal Tested	75	75	150
Total Animal Found Positive	37	25	62
Total Animal found Negative	38	50	88
Embryonic Mortality	5	2	7
Actual Pregnant (day 45)	32	23	55
Actual non Pregnant	43	52	95
True Positive	37	25	62
True Negative	38	50	88
False Positive	0	0	0
False Negative	0	0	0
Total	75	75	150
Accuracy (%)	100.00	100.00	100.00
Sensitivity (%)	100.00	100.00	100.00
Specificity (%)	100.00	100.00	100.00

Conception Rate and Embryonic Mortality in Cows and Buffaloes

The present study evaluated conception rates and embryonic mortality (Table 3) in 75 Gir cows and 75 Jaffarabadi buffaloes. Conception rates were assessed on Days 28, 36, and 45 post-AI using different diagnostic methods, *viz.,* Rapid PD test kits, ultrasonography, and per rectal examination. The results showed that in Gir cows, the conception rates on Day 28 post-AI ranged from 48% to 49.33% by rapid test kit and USG, respectively. By Day 36 post-AI, conception rates with rapid test kit improved to 49.33%, but on Day 45 post-AI, the actual conception rate had decreased to 42.66% as confirmed by rectal palpation. The embryonic mortality rate was 6.67% (5/75) across three methods. In Jaffarabadi buffaloes, the conception rates on Day 28 post-AI were same 33.33% (25/75) across rapid test kit and USG, which also remained stable by Day 36 post-AI (33.33%), but on Day 45 post-AI as confirmed by rectal palpation, it decreased to 30.67% (23/75). The embryonic mortality rate was 2.67% (2/75). Present findings on pregnant-nonpregnant animals detected by BovEasy kit, USG and actual per rectal palpation confirmed that there were overall embryonic mortality in 7 animals between Day 28 and 45 examinations post-AI (Table 3). It was higher in Gir cows (6.67%, 5/75) than in Jaffrabadi buffaloes (2.66%, 2/75). However, statistically, no significant differences in conception rates or embryonic mortality rates were found between the methods of PD on Days 28, 36, and 45 post-AI within or between breeds studied, though the values were higher in cattle than in buffaloes. Certain degrees of early embryonic losses as we observed are inevitable by nature in different species of animals (Zemjani, 1970; Romano and Fahning, 2013; Roth *et al.,* 2022).

In Gir cows, overall conception rates found by Anzar *et al.* (2003) and Ramakrishnan *et al.* (2012) were nearly similar to our present findings. The overall conception rates obtained in Jaffarabadi buffaloes were comparable to those reported by Borakhatariya *et al.* (2024) and Pandey *et al.* (2011). The current results were in healthy cows and buffaloes without any hormonal treatments. Previous researchers however suggested that by hormonal interventions conception rate could be increased (Ramakrishnan *et al.,* 2012). In contrast to our findings, several researchers reported higher conception rates in cattle and buffaloes, ranging from 34.2% to 75% in cattle and/or buffaloes by day 45 to 60 post-insemination (Borakhatariya *et al.,* 2017; Abo-Farw *et al.,* 2021; Roth *et al.,* 2022; Sharma *et al.,* 2021).

Table 3: Conception rates (%) at different days post-AI with embryonic mortality (%) in cows and buffaloes as detected by different methods of pregnancy diagnosis

Breed/ Species	Groups/Methods of PD	No. of animals	Conception rate (%)			Embryonic losses (%)
			28 days post-AI	32/36 days post-AI	45 days post-AI	
Gir cattle	Rapid test kit	75	48.00% (36/75)	49.33% (37/75)	-	6.67% (05/75)
	Ultrasonography		49.33% (37/75)	-	-	
	P/R examination		-	-	42.66% (32/75)	
Jaffarabadi buffalo	Rapid test kit	75	33.33% (25/75)	33.33% (25/75)	-	2.67% (02/75)
	Ultrasonography		33.33% (25/75)	-	-	
	P/R examination		-	-	30.67% (23/75)	

No significant difference between the groups regarding the success or failure of conception.

Conclusions

The PAG based BovEasy rapid early bovine pregnancy detection kit demonstrated strong reliability and diagnostic accuracy from Day 28 (99%) and day 32 (100%) post-AI in Gir cows and Jaffarabadi buffaloes. These findings validate the kit as a useful tool for early pregnancy detection, offering a non-invasive and timely method for pregnancy diagnosis. Trans-rectal ultrasonography (TRUS) proved to be a highly reliable method for early pregnancy detection in Gir cows and Jaffarabadi buffaloes at 28 days post-AI. Its 100% accuracy and the early identification of embryonic loss can significantly enhance reproductive management practices in dairy farming with certain limitations. The pregnancy rate and inevitable embryonic losses were somewhat higher in cattle than in buffaloes. Diagnostic approaches like rapid test kits, ultrasonography and per rectal palpation are statistically equally effective in predicting pregnancy status. Thus, the early detection of pregnancy and embryonic mortality by simple, easy, cost-effective and farmers-friendly BovEasy test kit can be used as the potential tool for improving reproductive performance through timely interventions and monitoring.

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