



Research Article

Preliminary Trial on The Use of Sodium Hypochlorite for Pregnancy Diagnosis in Bali Cattle

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Abstract | This study evaluated the potential of using 16% sodium hypochlorite as a rapid and low-cost method for pregnancy diagnosis in Bali cattle based on urine samples. 160 urine samples were collected from Bali cows, comprising 80 samples from confirmed pregnant animals and 80 from non-pregnant animals. Each sample was tested with 16% sodium hypochlorite solution, and the resulting reactions were visually assessed and classified as positive or negative. Out of the 80 pregnant samples, 72 were correctly identified as positive (true positives), while 8 were false negatives. Among the 80 non-pregnant samples, 71 were correctly identified as negative (true negatives), and 9 were false positives. This yielded a sensitivity of 90.0%, a specificity of 88.75%, and an overall diagnostic accuracy of 89.38%. The findings indicate that sodium hypochlorite has promising potential as a simple and accessible screening tool for pregnancy diagnosis in Bali cattle. Although not entirely free from diagnostic error, its affordability and ease of use make it suitable for preliminary field applications. Further studies are recommended to refine the method and validate its effectiveness on a broader scale.

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Introduction

Bali cattle (*Bos javanicus*) are a vital genetic resource native to Indonesia, particularly valued for their adaptability, disease resistance, and role in traditional agriculture. Predominantly raised by smallholder farmers in Bali and surrounding regions,

these cattle contribute significantly to food security, draught power, and rural livelihoods. To maintain and improve their productivity, effective reproductive management is essential (Sima *et al.*, 2023), and early pregnancy diagnosis plays a crucial role in achieving this goal (Çiplak, 2024; Puja *et al.*, 2024; Ott *et al.*, 2025). Early and precise pregnancy diagnosis can

reduce the calving interval by minimizing postpartum period (Sarangi *et al.*, 2022).

Conventional methods for pregnancy diagnosis in cattle include rectal palpation and ultrasonography (Vindrola and Mellisho, 2023). Ultrasonography examination nowadays is commonly used in livestock for health examination (Gunawan *et al.*, 2024). Ultrasound examination and transrectal palpation are currently the most commonly used methods for pregnancy diagnosis (Jaśkowski *et al.*, 2024). While accurate, these techniques require skilled personnel, and specialized equipment, and can be stressful for the animal, limiting their application in rural or resource-limited areas.

In response to these challenges, attention has turned to alternative, non-invasive, and cost-effective diagnostic approaches, particularly those involving the chemical analysis of urine, which offers a simple way to detect hormonal and metabolic changes associated with pregnancy (Ferlizza *et al.*, 2020). Among these emerging methods is the sodium hypochlorite test, a novel field-based technique that has shown promising results in recent studies.

The sodium hypochlorite (NaOCl) test functions by reacting with specific compounds in the urine of pregnant cows, producing a color change or foam reaction indicative of gestation. A recent study reported high sensitivity (97.39%) and overall accuracy of 93.33%, with the ability to detect pregnancy as early as 16 to 25 days post-insemination (Siad *et al.*, 2023). This method is particularly appealing in low-resource settings due to the widespread availability of sodium hypochlorite and the simplicity of test administration.

Despite its potential, the application of the sodium hypochlorite test in Bali cattle remains under-explored. Breed-specific physiological differences may affect test outcomes, and local environmental and dietary factors can influence urine composition. Therefore, there is a clear need to evaluate the effectiveness of this test specifically in Bali cattle under field conditions.

This research aims to assess the reliability, practicality, and diagnostic performance of the sodium hypochlorite urine test in detecting early pregnancy in Bali cattle. The findings of this study are expected to contribute to improved reproductive efficiency and

support more sustainable cattle farming practices among smallholder communities in Indonesia.

Materials and Methods

Study location and duration

The study was conducted at Bali Cattle Breeding Center, located in Badung, Gianyar, Klungkung, Bangli, and Tabanan, Bali, Indonesia. Procedures does not require ethical clarification because there is no direct intervention to the sample.

Animals

A total of 160 female Bali cattle of reproductive age (2.5–9 years), with known breeding histories, were selected for the study. The cows were maintained under similar feeding and management conditions. Animals were grouped as follows Pregnant group: Cows confirmed pregnant via ultrasonography at 30–60 days post-insemination. Non-pregnant group: Cows confirmed open (non-pregnant).

Urine sample collection

Fresh midstream urine samples were collected from each animal during the morning hours using sterile containers. Samples were obtained by spontaneous urination. Urine were immediately labeled and tested within 1 hour of collection to ensure accuracy.

Sodium hypochlorite test procedure

After being collected, 2/3 of the Balinese cow urine is mixed with 1/3 of sodium hypochlorite (16%), and then left for 5 minutes. Positive test results (pregnant) are indicated by the presence of foam and the development of a white ring with a chalky texture. Negative (nonpregnant) test showed little to no foam, and no significant color change.

Data analysis

The diagnostic performance of the sodium hypochlorite test was evaluated. The test results are classified into one of the following categories: True positive (TP): positive test and pregnant cow on ultrasound. True negative (TN): Negative test and non-pregnant cow at ultrasound scan. False positive (FP): Test positive but cow not pregnant on ultrasound. False negative (FN): Test negative but cow pregnant on ultrasound. The sensitivity of each test was measured as $100 \times (TP \div (TP + FN))$, and specificity as $100 \times (TN \div (TN + FP))$. Accuracy was calculated as $100 \times (TP + TN / TP + TN + FP + FN)$. Accuracy for pregnant cows was

derived as $100 \times (TP/TP + FP)$, whilst accuracy for non-pregnant subjects was determined as $100 \times (TN/TN + FN)$ (Pandit and Adhikari, 2023). Data were analyzed using SPSS for Windows V.22.0.

Result

A total of 160 urine samples were collected, consisting of 80 samples from confirmed pregnant Bali cows and 80 from confirmed non-pregnant cows. The pregnancy diagnosis test using 16% sodium hypochlorite solution yielded the following results: 72 samples were regarded as true positive (72 pregnant cows correctly identified as pregnant) (Figure 1), while 71 were classified as true negative (non-pregnant cows correctly identified as non-pregnant). There were 9 false positive data (non-pregnant cows incorrectly identified as pregnant), and 8 were regarded as false negative (pregnant cows incorrectly identified as non-pregnant) (Table 1).

Table 1: Confirmation of pregnancy in Bali cattle through urine examination using sodium hypochlorite.

Variable	Pregnant	Non-Pregnant
Positive	72 (TP)	9 (FP)
Negative	8 (FN)	71 (TN)

*Note: TP: True Positive, FP: False Positive, FN: False Positive, TN: True Negative.

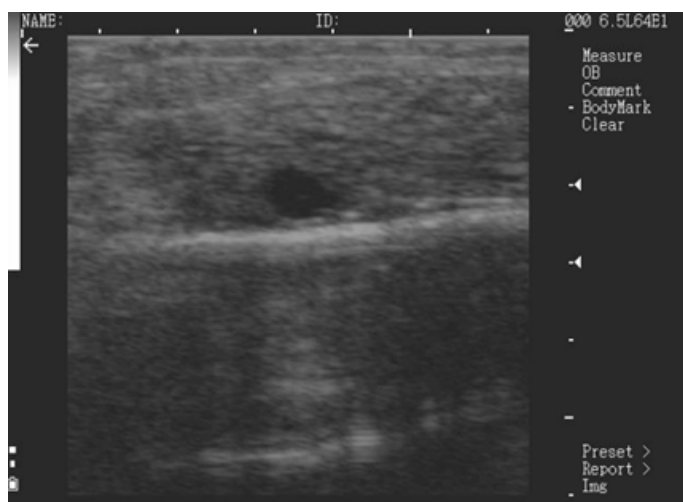


Figure 1: Pregnancy confirmation using ultrasound (30 days postartificial insemination) reveals the gestational sac, characterized by an anechoic echogenicity.

From these results, the diagnostic performance of the test can be summarized as follows: Sensitivity (ability to detect pregnancy) was 90.0%, Specificity (ability to detect non-pregnancy): Was 88.75%, and Overall accuracy was 89.375%. These findings

suggest that the sodium hypochlorite-based test shows promising potential as a simple, preliminary method for pregnancy diagnosis in Bali cattle, with high sensitivity and specificity.

Discussion

The findings from this preliminary trial demonstrate that 16% sodium hypochlorite has a potential as a simple and cost-effective diagnostic tool for early pregnancy detection in Bali cattle. The test achieved a sensitivity of 90.0% and a specificity of 88.75%, which are relatively high for a field-based, chemical-based test. These values indicate that the test was generally reliable in differentiating between pregnant and non-pregnant animals based on a simple urine reaction, but further validation is required (Rawat *et al.*, 2016).

The observed high sensitivity (72 out of 80 pregnant cows correctly diagnosed) suggests that the sodium hypochlorite solution effectively reacts with pregnancy-specific compounds present in the urine. Although the exact chemical mechanism is not fully understood, it is believed that hormonal or protein changes in the urine during pregnancy might interact with sodium hypochlorite to produce a distinguishable color or precipitation reaction (Siad *et al.*, 2023). These results align with previous studies in other cattle breeds where urine-based pregnancy tests using oxidizing agents showed promising diagnostic performance (Sangwan *et al.*, 2024).

The relatively high specificity (71 out of 80 non-pregnant cows correctly identified) indicates a low rate of false positives, which is important in minimizing the mismanagement of non-pregnant animals. However, the presence of 9 false positives and 8 false negatives suggests that the method is not yet perfectly reliable. False positives may lead to the retention of non-pregnant cows in breeding herds, reducing herd fertility efficiency. Conversely, false negatives could result in the premature culling or rebreeding of already pregnant cows, which could be costly (Borş an Bors, 2022).

Several factors could have contributed to these diagnostic errors. Variations in urine composition due to diet, hydration status, time of collection, or underlying metabolic conditions may affect the chemical reaction (Balhara *et al.*, 2023). Additionally, environmental factors such as temperature and

lighting during the visual assessment of the reaction could influence interpretation. Operator subjectivity and lack of standard color reference may also contribute to inconsistent results, indicating a need for test standardization. Maybe the reason for variation the sensitivity of sodium chloride test for pregnancy diagnosis due to the variation in plasma protein production among the individual cattle.

Despite these limitations, the test has strong potential as a preliminary screening method, particularly for smallholder farmers and rural veterinary practices that may lack access to ultrasonography or hormonal assays. The simplicity, low cost, and rapid results of urine analysis tests could make it a valuable tool in reproductive management (Haque *et al.*, 2023). Importantly, it can help in the early identification of pregnant cows, allowing for timely adjustments in feeding, and health care, and to be rebred as soon as possible (Delhez *et al.*, 2020).

Further research is necessary to refine the method. This should include larger-scale validation, comparisons with gold-standard techniques such as ultrasound or progesterone testing, and investigation of the biochemical basis of the test reaction. It may also be useful to explore different concentrations of sodium hypochlorite, the timing of the reaction, and the development of a standardized color chart or semi-automated detection system to improve accuracy and reduce observer bias. It appears that non-invasive methods of pregnancy diagnosis need to be further improved to minimize or eliminate the disadvantages cited (Jaśkowski *et al.*, 2024).

Conclusion

The results of this preliminary trial suggest that the use of 16% sodium hypochlorite solution offers a promising, simple, and low-cost method for pregnancy diagnosis in Bali cattle. With a sensitivity of 90.0% and specificity of 88.75%, the test demonstrated relatively high accuracy in differentiating between pregnant and non-pregnant cows. These findings highlight its potential as a field-level diagnostic tool, particularly useful in rural or resource-limited settings where access to conventional diagnostic technologies such as ultrasonography is restricted. However, the occurrence of false positives and false negatives indicates the need for caution when interpreting results and suggests that the sodium hypochlorite test

should be used as a preliminary screening tool rather than a definitive diagnostic method. Factors such as urine composition, environmental conditions, and subjective interpretation may influence test outcomes and require further investigation and standardization.

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Novelty Statement

This study explores the preliminary use of sodium hypochlorite as a cost-effective and accessible method for pregnancy diagnosis in Bali cattle, offering an alternative to conventional veterinary techniques.

Author's Contribution

IWS conceived the ideas, designed the experiments and write original manuscript IGNBT and IBKS supervised the field and data collection; PDJ collected and visualized the data; IWNFG and IKP analyze and write original draft of manuscript. All authors have read and approver the version of manuscript.

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Conflict of interest

The authors have declared no conflict of interest.

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