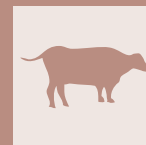


Validation of cow-side chemical tests for early pregnancy diagnosis in dairy cattle in Ethiopia: a comparative field study with transrectal ultrasonography and progesterone rapid test kit



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SUMMARY

Early pregnancy diagnosis in dairy cows is crucial for timely identification of non-pregnant animals, enabling prompt re-breeding or culling to improve reproductive efficiency. Lack of early pregnancy detection tool has been the biggest hurdle in reproductive management of dairy cows. Conventional diagnostic methods such as ultrasonography and hormone assays are often inaccessible at field level and unaffordable to smallholder farmers in low-resource settings. This study aimed to evaluate inexpensive, non-invasive, easy-to-use cow-side chemical tests for early pregnancy detection and compare their accuracy against transrectal ultrasonography (gold standard) and a commercial progesterone rapid test kit. A total of 191 Holstein-Friesian cows were tested using three chemical methods-copper sulfate tests in milk (3%, 5%, 7% CuSO₄ solutions), a barium chloride urine test (1% BaCl₂ plus urine protein dipstick), and a sodium hypochlorite urine test (10% NaClO) alongside a commercial progesterone rapid test kit (P4 Gold), and trans-rectal ultrasonography as gold standard. The overall pregnancy rate was 74.9% (143/191) using ultrasonography. The 3% CuSO₄ and 1% BaCl₂ tests demonstrated excellent diagnostic performance, each achieving 79.2% and 72.3% pregnancy, respectively; followed by NaClO urine test detected 70.2%. The specificity and sensitivity were 80.0% and 100.0% for 3% CuSO₄ test, and 85.4% and 92.3% for 1% BaCl₂ tests, respectively. Test agreement with ultrasonography were 85.9% and 90.6%, for 3% CuSO₄ and 1% BaCl₂, respectively. There was a significant difference in test results with 3% CuSO₄ performing better than all tests followed by 1% BaCl₂ (Chi Sq.=52.8, P<0.001; Chi Sq.=50.5, P<0.001, respectively) though overall test agreement with ultrasonography was better with 1% BaCl₂. The specificity and sensitivity for NaClO were 75.0% and 96.9%, respectively. The rapid P₄ kit identified 91.4% of pregnancies closely matching ultrasound results. This is the first study to directly validate these simple chemical tests in cattle against ultrasonography. The findings demonstrate the practicality, affordability, non-invasive and effectiveness of milk- and urine-based chemical tests for early pregnancy detection in cows, particularly in resource-limited and smallholder environments. These cow-side tests have the potential to substantially reduce open days and improve herd reproductive performance by enabling earlier intervention.

KEY WORDS

Copper sulphate; Barium chloride; Sodium hypochlorite; Pregnancy detection; Non-invasive diagnostics.

INTRODUCTION

Early and accurate pregnancy diagnosis is essential for improving reproductive efficiency in dairy cattle. Identifying open (non-pregnant) cows shortly after breeding allows for timely re-insemination, reduces calving intervals, and minimizes economic losses linked to extended non-productive periods. While the ideal calving interval in well-managed herds is 12-13 months, studies in Ethiopia report extended intervals 15.8 months in intensive farms (1) and 19-24 months in crossbred dairy cows in smallholder systems (2) primarily due to delayed pregnancy detection.

Traditional methods such as transrectal palpation are not reliable before 35-40 days post-insemination and require skilled

technicians. Ultrasonography offers accurate diagnosis from 28 days, but its use is limited in low-resource settings due to equipment cost and technical expertise (3). Behavior-based assessments like non-return to estrus are common among smallholders but often lead to false assumptions or overlooked early embryonic loss (4). Biomarker-based methods such as progesterone and pregnancy-associated glycoprotein (PAG) tests have shown promise but are limited by moderate specificity and dependence on laboratory infrastructure (5). Although omics-based diagnostics using milk and urine metabolites are emerging, their cost and complexity render them unsuitable for routine field use, especially in smallholder systems (6).

Simple chemical-based tests have long been proposed as affordable and non-invasive on-farm alternatives for pregnancy diagnosis. Sodium hypochlorite (NaClO) produces frothing in the urine of pregnant goats (7), and barium chloride (BaCl₂) forms precipitates with estrogen-rich non-pregnant urine in some species (8). Copper sulfate (CuSO₄) has also been explored for its ability to induce milk coagulation in pregnant

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animals (9). Despite these promising anecdotes, few studies have rigorously validated their accuracy in cattle.

This study aimed to assess a comparative field evaluation of the diagnostic performance of three inexpensive, cow-side chemical tests; CuSO_4 in milk, BaCl_2 and protein strips in urine, and NaClO in urine between 25 and 35 days post-insemination. Results were compared with a commercial progesterone rapid test kit and validated against ultrasonography. To our knowledge, this is the first comprehensive validation of these traditional chemical pregnancy tests in cattle and benchmarked their performance against the gold-standard (ultrasound) and a commercial on-farm progesterone kit. The goal is to provide empirical evidence for practical, non-invasive and affordable early pregnancy diagnostics to improve reproductive performance in smallholder dairy systems and resource-limited areas.

MATERIALS AND METHODS

This study was carried out as a field-based validation of chemical pregnancy tests in dairy cows. A purposive experimental design was used to ensure that cows of known breeding dates were tested and then verified for pregnancy status. Twenty-eight dairy farms were randomly selected in the study area, and a total of 191 Holstein-Friesian cows and heifers that had been inseminated 25–35 days prior were enrolled. This selective inclusion ensured that the animals were managed under uniform, controlled conditions, thereby facilitating a precise correlation between the test's performance and the cows' actual reproductive status. Urine and milk samples were collected between 25 and 35 days post-artificial insemination (AI). Urine samples were collected into clean containers following gentle massage of the perineal region ventral to the vulva to induce urination. Milk samples were obtained directly by milking into clean collection cups.

Cow-Side Chemical Pregnancy Tests

Each cow's milk and urine sample was subjected to a set of chemical pregnancy tests as follows:

- **Copper Sulfate (CuSO_4) Milk Test:** Milk from each cow was tested with three concentrations of copper sulfate solution (3%, 5%, and 7% w/v in water) to determine which concentration gave the best reaction. In practice, a small volume of milk (a few milliliters) was mixed with an equal volume of the CuSO_4 solution in a clear test tube and observed immediately. The reaction was scored on a 0–4 scale based on the degree of milk coagulation. A score of 0 indicates solid coagulation (completely clotted milk, considered a strong positive), while 4 indicates no coagulation with only flaky precipitates (considered negative). We defined positive results for pregnancy as scores 0, 1, or 2 (complete or partial coagulation), and negative results as scores 3 or 4 (little to no coagulation with flakes). The test was conducted at ambient farm temperature and results were read within about 1 minute of mixing.
- **Barium Chloride (BaCl_2) Urine Test:** Urine samples were tested by adding a 1% BaCl_2 solution. Approximately 5 mL of fresh urine was mixed with ~5 mL of 1% BaCl_2 in a clean glass tube. The mixture was allowed to stand for a few minutes and observed for the formation of a white precipitate (barium sulfate). In addition, a urine protein dipstick test was performed on each sample concurrently, because uri-

nary protein changes can assist interpretation. A positive indication of pregnancy was defined by the absence or minimal precipitate in the urine (a clear or slightly hazy solution) and a urine protein reading <100 mg/dL on the dipstick. The degree of precipitate ("flakes") was graded 0–4 (0 = no precipitate, 4 = heavy flocculation). Scores 0, 1, 2 (no or minimal flakes) were interpreted as pregnant (since progesterone metabolites in pregnant cows inhibit BaCl_2 precipitation), whereas scores 3, 4 (heavy white floccules) indicated non-pregnant status (higher estrogen levels in open cows cause more precipitate). Thus, a clear solution with low protein was considered pregnant, and a milky, heavily flocculated solution was non-pregnant.

- **Sodium Hypochlorite (NaClO) Urine Test:** This test (sometimes called the "bleach test") is based on a unique reaction between chlorine bleach and pregnant animal urine. For each cow, ~10 mL of urine was carefully poured into ~10 mL of a 10% NaClO solution (household bleach diluted to 10%) in a transparent cup or tube. The mixture was observed for vigorous effervescence (foaming) and the appearance of a distinct white, soap-like "chalky" ring at the liquid-air interface. A positive result for pregnancy was indicated by strong frothing with a chalky white ring within ~30 seconds of mixing, whereas little to no effervescence and no ring was interpreted as negative (non-pregnant). This reaction is thought to occur due to chemical interactions with pregnancy-specific metabolites in urine. All NaClO tests were conducted outdoors or in well-ventilated areas due to chlorine fumes, and results were read immediately as the reactions are rapid.

For each test, the observers were trained to use the predefined scoring criteria, and whenever possible, two technicians cross-checked the interpretation to reduce subjectivity. All cow-side tests were performed blind to the ultrasonography results (i.e., the person performing ultrasound did not know the chemical test outcomes and vice versa) to prevent bias in interpretation.

Validation of the Pregnancy test: Pregnancy Confirmation (Ultrasonography)

Following the chemical tests, all cows were examined by transectal ultrasonography to confirm their true pregnancy status. A portable B-mode ultrasound scanner with a 5.0 MHz linear-array transrectal probe (DUS 60, Edan Instruments Inc., Shenzhen, China) was used 25–35 days post-insemination. Pregnancy was confirmed by visualization of a fluid-filled uterine horn containing an embryonic vesicle, often with the detection of an embryo and/or embryonic heartbeat. Cows with no evidence of an embryonic vesicle were classified as not pregnant. Ultrasonography was considered the gold standard diagnostic in this study, and its results (pregnant or not pregnant) were used to calculate performance metrics for each test.

Test Outcome Definitions

Each chemical test result was classified as true positive (TP), true negative (TN), false positive (FP), or false negative (FN) by comparing it to the ultrasonography outcome. We define these as:

- **TP:** Test positive and pregnancy confirmed by ultrasound.
- **TN:** Test negative and no pregnancy confirmed by ultrasound.

- **FP:** Test positive but no pregnancy by ultrasound (a false alarm).
- **FN:** Test negative but pregnancy confirmed by ultrasound (a missed pregnancy).

From these values, we derived the standard diagnostic performance measures for each test:

- **Sensitivity (Se)** - the true positive rate, calculated as $TP / (TP + FN) \times 100\%$. Sensitivity represents the proportion of actual pregnant cows that the test correctly identified as pregnant.
- **Specificity (Sp)** - the true negative rate, calculated as $TN / (TN + FP) \times 100\%$. Specificity represents the proportion of actual non-pregnant cows that the test correctly identified as not pregnant.
- **Positive Predictive Value (PPV)** - the probability that cows testing positive are truly pregnant, computed as $TP / (TP + FP) \times 100\%$.
- **Negative Predictive Value (NPV)** - the probability that cows testing negative are truly not pregnant, computed as $TN / (TN + FN) \times 100\%$.
- **Overall Accuracy (Agreement)** - the overall proportion of correct classifications, $(TP + TN) / \text{total tested} \times 100\%$, which reflects agreement with the ultrasound results.

Statistical Analysis

All data were analyzed using IBM SPSS Statistics (Version 26; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to calculate the pregnancy rates and test outcome frequencies.

The sensitivity, specificity, PPV, NPV, and accuracy of each test were calculated with their 95% confidence intervals (95% CI). Confidence intervals for proportions were obtained using the Wilson score method to account for the sample sizes of each subgroup. A chi-square test was used to compare the performance (proportion of correct results) between different tests and to evaluate differences in detection rates among the three CuSO₄ concentrations. For all analyses, a two-tailed P value < 0.05 was considered statistically significant.

RESULTS

Copper sulphate milk test for early pregnancy detection

The overall pregnancy rate, as determined by ultrasonography, was 74.9% (143/191). The 3% CuSO₄ and 1% BaCl₂ tests exhibited strong diagnostic performance, identifying 79.2% and 72.3% of pregnancies, respectively, with the NaClO urine test detecting 70.2%. Specifically, the 3% CuSO₄ test yielded a specificity of 80.0% and a sensitivity of 100.0%. There was a significant difference in test performance (Chi Sq.=52.8, P<0.001 for 3% CuSO₄; Chi Sq.=50.5, P<0.001 for 1% BaCl₂), with the 3% CuSO₄ test generally outperforming other tests, followed by the 1% BaCl₂ test, although the overall agreement with ultrasonography was slightly higher for 1% BaCl₂. There was a highly significant difference (P<0.001) in the rate of detection of pregnancy by the different concentrations of CuSO₄ as evidenced by the milk coagulation (Figure 1), with 3% CuSO₄

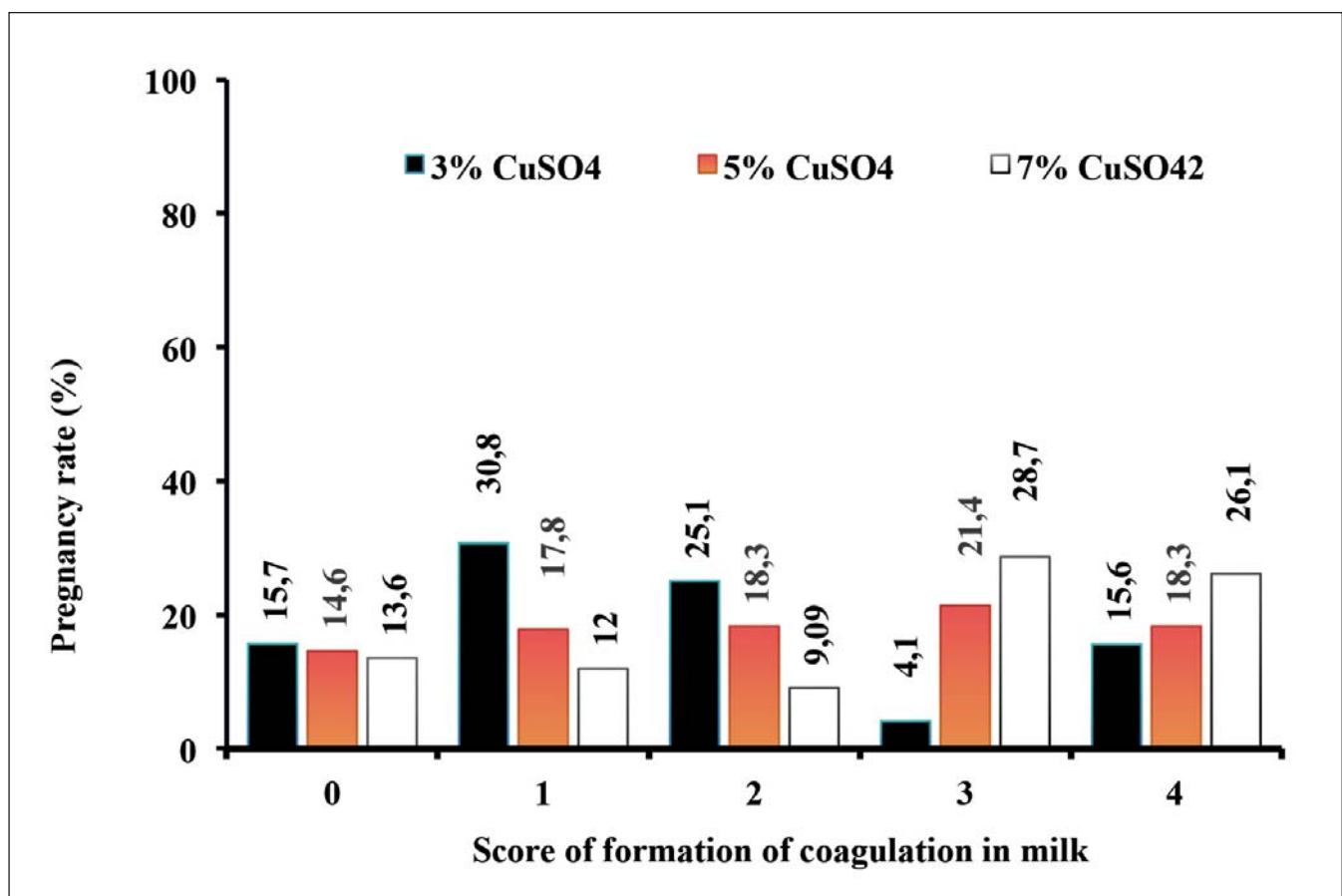


Figure 1 - Results of different concentration of CuSO₄ pregnancy test in milk in dairy cattle.

demonstrating superior performance (Fig. 1) Agreement rates with ultrasonography were high, at 85.9% for 3% CuSO₄ compared with 90.6% for 1% BaCl₂. Notably, most of these pregnant cows, accounting for 62.5% positive test, had a score of 0 and 1 exhibiting solid coagulation; a strong indicator of pregnancy in this testing method. In the 7% CuSO₄, nearly half of the test results (48.95%) had a score of 3 and 4 exhibiting minor visible flakes wrongly assigning pregnant cows as non-pregnant. The difference in score are distinct making the choice for concentration relatively easier during field pregnancy test.

Barium Chloride urine test for early pregnancy detection

The overall positive pregnancy test was 72.3%. The specificity and sensitivity 1% BaCl₂ test were 85.4% and 92.3%. Apparently, most of the pregnant cows, accounting for 83.9% positive test, had a score of 0 and 1 exhibiting no or only minor flakes; a strong indicator of pregnancy in this testing method. Fortunately, only a small proportion of cows and heifers (7.7%) had a score of 3 and 4 exhibiting hefty visible flakes; a strong indicator of non-pregnancy in this testing method (Fig. 2). The test agreement with the gold standard (Ultrasonography) was 90.6%. The relatively clear dichotomy between scores indicative of pregnancy and non-pregnancy was advantageous compared to other tests in terms of interpretation and reduces ambiguity in result classification.

Sodium hypochlorite urine test for early pregnancy detection

The test was carried out in urine samples of 84 animals and pregnancy rate was 70.2 % (59/84). The specificity and sensitivity for NaClO were 75.0% and 96.9%, respectively. The test agreement with the gold standard (Ultrasonography) was 88.1%. A high sensitivity suggesting the ability of the test to

accurately identify positive animals while still preserving a useful proportion of non-pregnant classifications, thereby avoiding the excessive false-positive rate seen with overly reactive test mixtures. In practical terms, such a balance makes the NaClO test attractive for on-farm screening: it is inexpensive, rapid, and can be conducted without specialized equipment, enabling herd managers to identify most pregnant cows early and allocate feed, housing, and breeding decisions accordingly.

Performance of the Rapid progesterone pregnancy test kit

The commercial kit for rapid pregnancy assay (p4Gold, , UK) was also used to test pregnancy in milk drops (Table 1) in 58 animals for comparative study. Pregnancy rate was 89.7%, with 75.0% specificity and 98.1% sensitivity. Few test results (3.4 %) were doubtful. The test agreement with the gold standard (Ultrasonography) was 93.1%. The kit's near-perfect sensitivity highlights progesterone as a robust marker of pregnancy, but its moderate specificity reflects false positives in some non-pregnant cows (e.g., persistent CL or early embryonic loss). While highly accurate, the test is relatively expensive and requires proper storage, limiting its use in smallholder settings. Thus, although the progesterone kit confirms the feasibility of on-farm diagnostics approaching laboratory accuracy, the simpler chemical tests (CuSO₄, BaCl₂, NaClO) remain cheaper and more scalable alternatives.

Comparison of predictive values and relative accuracy of the tests

Positive Predictive Value (PPV) and Negative Predictive value (NPV) were computed for all the chemical tests and compared with the gold standard (Figure 3). There was a significant difference in accuracy of pregnancy detection among the various tests conducted with 3% CuSO₄ performing better than all tests followed by 1% BaCl₂ (Chi Sq.=52.8, P<0.001; Chi Sq.=50.5,

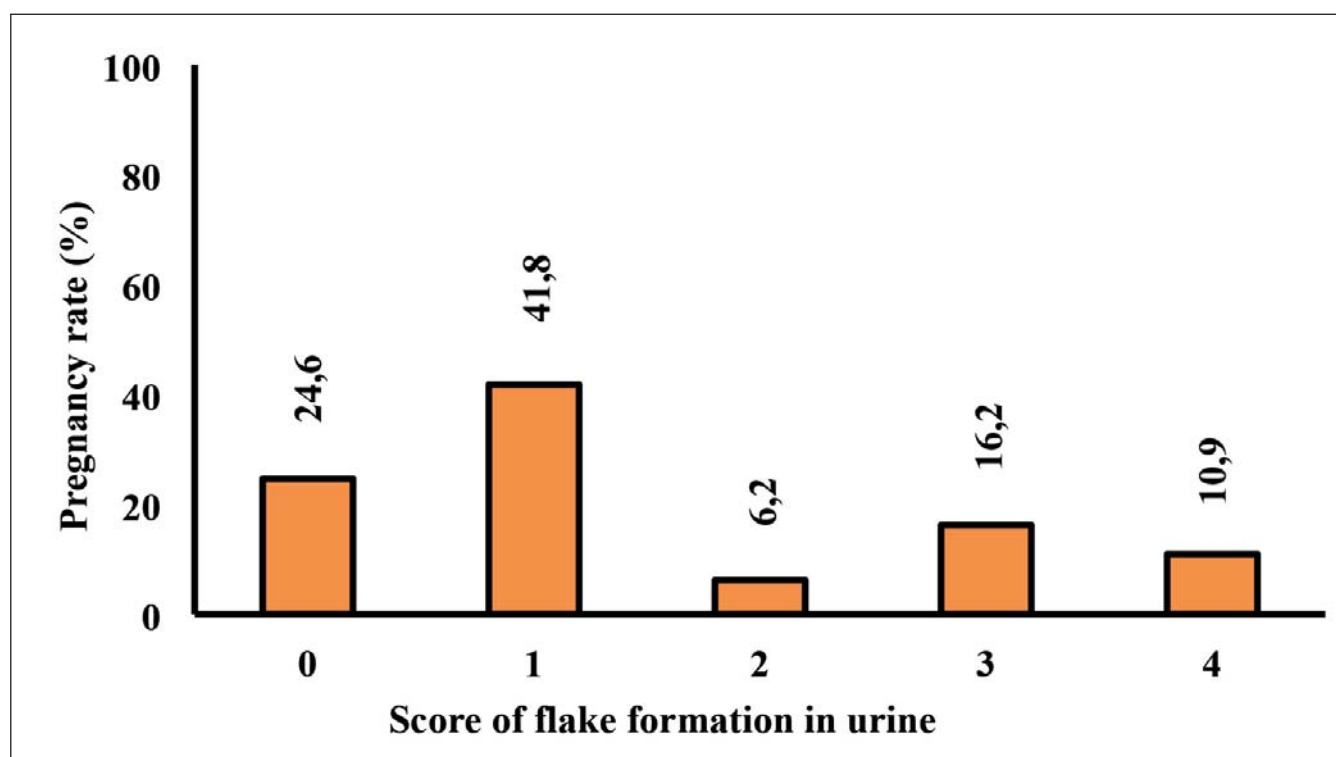


Figure 2 - Pregnancy test results in urine of dairy cows and heifers using 1% BaCl₂.

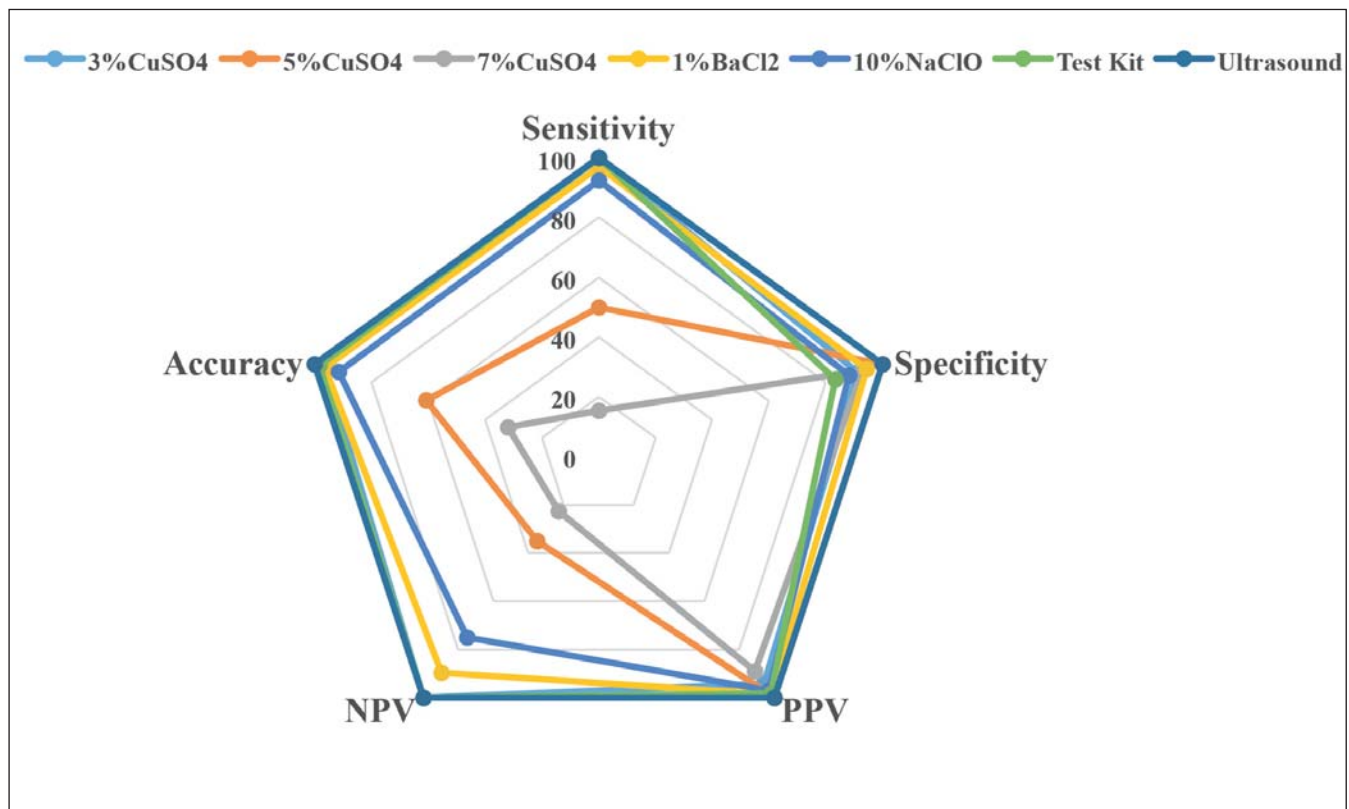


Figure 3 - Radar plot comparing the diagnostic performance of different methods used to detect pregnancy in cows and heifers. Metrics include Sensitivity, Specificity, PPV, NPV, and overall accuracy. Ultrasound is included as a reference standard.

$P < 0.001$, respectively). Comparison of the 7% CuSO₄ pregnancy test against the ultrasound result (Chi sq. = 8.45, $P = 0.076$) giving approximately a 32% accuracy. The low NPV value indicates that a negative test result did not reliably rule out pregnancy. The high positive predictive value on 3% CuSO₄ and 1% BaCl₂ tests suggest that when the tests were positive, they were usually correct. While the 7% CuSO₄ test does well at confirming pregnancy when it gives a positive result, its performance in ruling out pregnancy is weak making it less reliable.

The comparison of the 1% BaCl₂ pregnancy test against the ultrasound result (Chi sq. = 67.77, $P = 0.000$) giving approximately a 96.4% accuracy. In practical terms, the BaCl₂ test is highly likely in identifying pregnant animals and seldom mislabels an open cow as pregnant, making it a more reliable, low-cost alternative to ultrasound when rapid or large-scale field screening is required. The comparison of NaClO test with the ultrasonography (Chi Sq. = 48.7665, $P = 0.000$) also revealed a promising result, positioning the test as a viable and efficient tool for early pregnancy detection in cattle with a higher ac-

curacy. The NPV of NaClO test is moderately strong, suggesting that while the test is better at confirming pregnancy, it is somewhat less reliable at ruling it out. Nonetheless, the high overall accuracy indicates that the NaClO test is generally dependable (Fig. 3).

Interpretation of Table 1: Sensitivity (Se) is the proportion of pregnant cows correctly identified by the test. Specificity (Sp) is the proportion of non-pregnant cows correctly identified. PPV is the likelihood that a positive test corresponds to a truly pregnant cow, while NPV is the likelihood that a negative test corresponds to a truly open cow. Accuracy is the overall percentage of cows correctly classified by the test. The 3% CuSO₄ milk test and 1% BaCl₂ urine test showed high Se and Sp, indicating strong reliability. The NaClO urine test had exceptional Se (few pregnancies missed) but more moderate Sp (some false positives), whereas the P4 rapid kit had very high Se and moderate Sp in this trial. The 95% CIs reflect the precision of these estimates, which is narrower for the larger sample sizes (CuSO₄, BaCl₂) and wider for the smaller subsets (NaClO, kit). For example, the CuSO₄ test's sensitivity is 100% with a low-

Table 1 - Diagnostic performance of early pregnancy tests (cow-side chemical tests and a rapid progesterone kit) compared to transrectal ultrasonography (gold standard). Values are shown as percentages with 95% confidence intervals (CI) in parentheses. The table includes Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV), and Overall Accuracy for each test method.

Test (sample)	N	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
3% CuSO ₄ test (milk)	191	100.0% (97.4-100)	80.0% (65.7-89.8)	93.5% (88.3-96.5)	100.0% (90.8-100)
1% BaCl ₂ test (urine)	191	92.3% (86.6-95.7)	85.4% (71.2-93.5)	94.9% (89.4-97.7)	78.8% (64.4-88.6)
10% NaClO test (urine)	84	96.9% (88.4-99.1)	75.0% (53.3-88.7)	90.5% (80.4-95.5)	90.0% (68.3-98.0)
Progesterone kit (milk)	58	98.1% (89.9-99.7)	75.0% (30.1-95.4)	98.1% (89.9-99.7)	75.0% (30.1-95.4)

er 95% CI of ~97.4%, acknowledging a small margin of error given the sample size.

DISCUSSION

The evaluation of cow-side early pregnancy tests, particularly those utilizing chemical analysis of urine and milk samples, reveal a promising avenue for enhancing reproductive efficiency in dairy cattle. Across the various methods assessed, the 3% CuSO₄ and 1% BaCl₂ tests consistently demonstrated the most favorable balance of sensitivity, specificity, and practical applicability under field conditions.

The 3% CuSO₄ test exhibited a sensitivity of 100% and specificity of 80%, corroborating previous anecdotal and experimental evidence linking milk protein coagulation to pregnancy-related biochemical changes (9). This finding aligns with a previous research that supports the use of milk and urine as affordable diagnostic tool for early pregnancy detection particularly for smallholder settings (10). CuSO₄ for milk coagulation was successfully correlated with early gestation stages (11). However, not all studies have found consistent reliability in CuSO₄-based pregnancy tests. El-Wishy et al (12) reported that visual coagulation methods are prone to subjective interpretation, leading to observer variability and potential false positive or negative results. Singh et al (13) similarly found that variation in milk composition due to factors like diet or stage of lactation, rather than pregnancy, may interfere with the accuracy of CuSO₄ tests. These concerns highlight the need for clear testing protocols and potential integration with digital image analysis for more standardized interpretation.

The 1% BaCl₂ test, which achieved 92.3% sensitivity and 85.4% specificity, displayed one of the strongest agreements (90.6%) with ultrasonographic outcomes. This result is consistent with prior findings in goats, sows, and alpacas, suggesting a conserved estrogen-barium chloride interaction across species (11). In non-pregnant cows, elevated urinary estrogen levels promote barium sulfate flocculation, whereas pregnant cows with higher progesterone metabolite concentrations inhibit this reaction (14). However, dietary sulfates, urinary pH, and protein contamination may still influence the precipitation reaction (15). The inclusion of protein test strips in this study added a diagnostic safeguard, enhancing the interpretive clarity of the BaCl₂ test a practical improvement over earlier versions.

The NaClO test also proved valuable, with a high sensitivity (96.9%) and moderate specificity (75.0%), identifying most pregnant animals through visible foaming and chalky ring formation in urine. This aligns with reports by Adeoye et al (7), who validated similar reactions in goats and sows. The test's rapid turnaround and ease of use make it attractive for widespread screening. However, as noted by Tiwari et al (16), false positives can arise from urinary contaminants, elevated ammonia, or inconsistent reagent preparation. These issues necessitate cautious interpretation, especially when used before Day 25 post-insemination when hormone-induced metabolic changes may be insufficiently pronounced (7).

The commercial progesterone rapid kit (P4Gold) showed the highest test agreement (93.1%) and nearly perfect sensitivity (98.1%), confirming its value as a reliable diagnostic tool. Yet, its limitations cost, storage requirements, and supply chain constraints restrict scalability in rural Ethiopia (3).

Collectively, these findings carry important implications.

First, they support a shift from reliance on behavioral or delayed clinical signs to proactive, biochemical pregnancy confirmation. This can substantially shorten calving intervals, reduce open days, and improve reproductive efficiency, particularly in smallholder systems (4). Second, they align with national livestock productivity goals, especially within digital and mobile-based Smart Dairy Systems currently piloted in Ethiopia (17). Simple, rapid tests could be easily integrated into mobile veterinary services or farmer-cooperative health packages.

Third, these methods democratize access to reproductive diagnostics. While Europe and North America are advancing AI-driven estrus and pregnancy sensors (4, 18), chemical-based field tools offer a realistic innovation pathway for smallholder-dominated systems. Their non-invasive nature enhances animal welfare, while their ease of use encourages farmer participation, especially among women and youth a key consideration in community-led livestock development.

CONCLUSION

This study provides robust evidence that 3% copper sulfate (CuSO₄) and 1% barium chloride (BaCl₂) tests are highly reliable, affordable, and field-adaptable alternatives for early pregnancy detection in dairy cattle. Their high diagnostic accuracy, ease of use, and strong agreement with ultrasonography position them as transformative tools for reproductive management, particularly in smallholder and low-resource settings. By enabling timely identification of non-pregnant cows, these tests can substantially reduce open days, shorten calving intervals, and boost farm-level productivity. In an environment where access to advanced diagnostics is limited, these chemical-based solutions offer a breakthrough in democratizing reproductive health technologies for resource-limited rural livestock systems. To accelerate adoption, large-scale validation across diverse breeds and environments, integration into AI technician training, and linkage with digital herd monitoring systems are essential. Further research should focus on developing standardized, ready-to-use kits and scaling low-cost diagnostic innovations for practical use in smallholder dairy systems.

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Author Contributions

Berihu Gebrekidan: Conceptualization, methodology, field data collection, data analysis, writing - original draft.

Jemal Dereje: Investigation, data collection, resources, writing - review & editing.

Kibir Mengiste: Laboratory analysis, validation of test methods, writing - review & editing. Seid Ali: Field coordination, sample collection, data curation.

Gebrehiwot Tadesse: supervising the statistical analysis of the research and reviewing of the manuscript.

Alemayehu Lemma: Ultrasonography and clinical validation Project administration, supervision, writing - review & editing. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare no conflicts of interest related to this work. The chemical reagents and commercial kit used in this study were obtained from standard suppliers, and none of the authors have any financial or personal relationship that could inappropriately influence or bias the content of this paper.

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