



Prevalence and determinants of false pregnancy diagnosis in buffaloes: Field evidence from Andhra Pradesh, India

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ABSTRACT

Accurate and timely pregnancy diagnosis is vital for efficient reproductive management and economic sustainability in buffalo husbandry. This study examines prevalence and determinants of false pregnancy diagnosis in buffaloes under field conditions across four major buffalo-rearing districts of Andhra Pradesh: Guntur, Prakasam, Krishna and Nellore. A multistage random sampling method yielded data from 240 dairy farmers and 415 buffaloes, capturing information on visual observation of estrus for pregnancy diagnosis (VOB) at 21±3-days intervals and rectal palpation (RP) conducted 60–120 days post-AI. The diagnostic accuracy of both methods was evaluated for sensitivity, specificity, predictive values and overall accuracy. VOB, though widely used, showed high sensitivity but low specificity, resulting in a considerable rate of false positives. In contrast, RP demonstrated significantly higher accuracy and reliability. Binary logistic regression was employed to identify factors influencing false diagnosis. For VOB, increased farmer experience and awareness significantly reduced false diagnosis risk, while larger herd sizes, poor body condition, reproductive disorders and high ambient temperatures elevated the risk. For RP, higher animal parity reduced false diagnosis odds, whereas poor body condition and specific seasonal contexts slightly compromised accuracy.

Keywords: Buffaloes, False diagnosis, Pregnancy diagnosis, Rectal palpation, Visual observation

Water buffalo (*Bubalus bubalis*) is integral to India's agrarian economy, particularly in states like Andhra Pradesh, where it significantly contributes to milk production, draught power and rural livelihoods. Efficient reproductive management, especially accurate and timely pregnancy diagnosis, is crucial for optimizing productivity and ensuring economic sustainability in buffalo husbandry (Abdullah *et al.* 2014). Early and precise detection of pregnancy allows for appropriate nutritional management, timely rebreeding of non-pregnant animals and overall improvement in reproductive efficiency (Abdullah *et al.* 2014, Ingawale *et al.* 2014, Yadav *et al.* 2019, Caton *et al.* 2020). Moreover, animals with extended calving intervals fail to achieve the goal of one calf per year, which is vital for maintaining dairy farm profitability (NDDB 2023). However, buffaloes present unique reproductive challenges, including silent estrus, irregular estrus cycles and seasonal breeding patterns, which complicate the detection of pregnancy (Pannu *et al.* 2022). These challenges underscore the necessity for reliable, field-applicable diagnostic tools and a deeper understanding of the factors influencing pregnancy diagnosis.

Various direct and indirect methods of pregnancy diagnosis are available which vary in their accuracy, cost, ease of use, need for veterinary involvement and applicability at various postpartum stages. The simplest method involves visually observing estrus 18–24 days post-artificial insemination (AI) or natural service. However, the accuracy of visual diagnosis heavily relies on the observer's expertise and experience, introducing subjectivity and potential for error (Orihuela 2000, Banuvalli *et al.* 2015, De Rensis *et al.* 2019). Rectal palpation, typically performed around day 40 after insemination, requires skilled personnel and carries risks of causing stress or injury to the animal if not performed correctly (Jaśkowski *et al.* 2019). Transrectal ultrasonography offers more precise and earlier detection (as early as 28 days post-insemination) but requires expensive equipment and trained operators, limiting its accessibility in rural settings (Balhara *et al.* 2013, Abdullah *et al.* 2014). Similarly, hormonal assays, though useful, are primarily used as research tools (Kumar *et al.* 2021). Thus, limitations such as accuracy, later stages of applicability and the requirement for elaborate instrumentation and laboratory setups are various constraints of present-day methods.

At the field level, visual observation of estrus for pregnancy diagnosis and rectal palpation are the most commonly used pregnancy diagnosis methods in India

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(Patel *et al.* 2016, Kumar *et al.* 2021). However, their accuracy can be affected by false negative and false positive results due to variations in specificity and reliability (Lopez *et al.* 2004, Diskin 2008, Walker *et al.* 2008, Ghuman and Singh 2009, Team Pashudhan Praharee 2023).

Most existing studies focus on the efficacy of specific diagnostic tests under controlled conditions, with limited exploration of their applicability and accuracy in field settings. Furthermore, there is a need to understand the factors contributing to false diagnosis, including animal-related factors (e.g., reproductive disorders), environmental conditions and the proficiency of personnel performing the diagnosis. To the best of our knowledge, two studies have been conducted in India to estimate the economic losses due to late pregnancy diagnosis. Both the studies have been carried out in Haryana. One study estimated economic losses in Karan Fries and Sahiwal cattle of an institute cattle yard, while the other focused on losses resulting from delays by farmers in visiting veterinary hospitals for buffalo rectal palpation tests. However, these studies do not account for false diagnosis of pregnancies. Field-level information on the accuracy of pregnancy diagnosis, existing methods and constraints faced by dairy farmers is unavailable. Further, factors affecting the probability of false diagnosis of pregnancies have not been identified and analysed.

This study aims to address the identified research gaps by investigating the prevalence and determinants of pregnancy false diagnosis in buffaloes under field conditions in Andhra Pradesh. By providing field-based evidence on the prevalence and determinants of pregnancy false diagnosis in buffaloes, this study will contribute to the development of more effective and practical diagnostic strategies tailored to the needs of farmers in Andhra Pradesh.

MATERIALS AND METHODS

A multistage random sampling technique was employed to collect data from two random blocks each of the four districts of Andhra Pradesh: Guntur, Prakasam, Krishna and Nellore. Within each selected block, one cluster of villages was randomly chosen. From each village cluster, 30 dairy farmers were randomly selected, resulting in a total sample size of 240 dairy farmers, who owned at least two adult female buffaloes. Only buffaloes that had calved within the six months preceding the data collection were included. Primary data were gathered through personal interviews with the selected dairy farmers using a pretested structured schedule on farmer demographics and experience, buffalo breed types (Graded Murrah, Jaffarabadi, and non-descript), methods and outcomes of pregnancy diagnosis (True Positive; False Positive; False Negative and True Negative). Based on the collected data, diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated following Trevelan (2017) and Baratloo *et al.* (2015). Accuracy was defined as the proportion of correctly identified cases (both

pregnant and non-pregnant); sensitivity as the ability to correctly identify pregnant cases; specificity as the ability to correctly identify non-pregnant cases; PPV as the proportion of positive test results that were true positives; and NPV as the proportion of negative test results that were true negatives. Additionally, expert opinions were solicited to determine attribute levels for constructing an awareness index.

To identify factors associated with incorrect pregnancy diagnoses, a binary logistic regression model was employed. The dependent variable was binary, representing the accuracy of the pregnancy diagnosis:

Y = 1: Incorrect diagnosis (i.e., false positive or false negative)

Y = 0: Correct diagnosis (i.e., true positive or true negative)

Based on existing literature, factors known to influence the accuracy of pregnancy diagnosis were included as independent variables as follows:

Continuous variables: Experience of the practitioner (dairy farmer/veterinarian/paravet) in years, Awareness level of the farmer (measured via a standardized scale), Herd size (number of animals) and Parity of the animal (number of calvings).

Categorical variables: Breed type, Body condition score (BCS), Season during diagnosis, Location, Service count at time of pregnancy diagnosis, Presence of reproductive disorder (Table 1).

The binary logistic regression model for VOB was specified as follows:

$$\log \frac{P(Y=1)}{1-P(Y=1)} = \beta_0 + \beta_1 \cdot \text{Exp} + \beta_2 \cdot \text{Aware} + \beta_3 \cdot \text{Herd} + \beta_4 \cdot \text{Parity} + \sum \beta_i D_i$$

Similarly, the model for Rectal Palpation (RP) diagnosis was specified as:

$$\log \frac{P(Y=1)}{1-P(Y=1)} = \beta_0 + \beta_1 \cdot \text{Exp} + \beta_2 \cdot \text{Parity} + \sum \beta_i D_i$$

Where,

$P(Y=1)$ is the probability of an incorrect pregnancy diagnosis.

β_0 is the intercept term.

$\beta_1, \beta_2, \beta_3, \beta_4$ are coefficients for the continuous independent variables

D_i represents the dummy variables for the categorical predictors.

Categorical variables were transformed into dummy variables to facilitate their inclusion in the binary logistic regression model. For each categorical variable with k categories, $k - 1$ dummy variable were created, with one category serving as the reference group (Table 1). Each dummy variable takes the value of 1 if the observation belongs to the specified category and 0 otherwise. The reference group is represented when all dummy variables for that categorical variable are 0. The logistic regression analysis was conducted using SPSS software. The coefficients (β) for each predictor were estimated and their statistical significance was assessed using Wald tests. Odds ratios (ORs) with 95% confidence intervals (CIs)

Table 1. Dummy variable coding for categorical variables

Variable	Category	Dummy variable name	Reference group
Breed type (VOB)	Graded Murrah	Graded_Murrah	Jaffarabadi
	Non-descript	Non_descript	
Breed type (RP)	Graded Murrah	Non_descript	Graded Murrah
	Non-descript		
Body condition Score (BCS)	BCS > 3	BCS_gt_3	BCS = 3
	BCS < 3	BCS_lt_3	
Season of diagnosis	Summer (March–May)	Summer	Winter (January–February)
	Autumn (October–December)	Autumn	
	Rainy (June–September)	Rainy	
Service count at time of pregnancy diagnosis	1	PD_1	3
	2	PD_2	
	Guntur	Guntur	
Location of study	Krishna	Krishna	Prakasam
	Nellore	Nellore	
	Presence	RD_P	Absence
Reproductive disorder			

were calculated to interpret the strength and direction of associations between predictors and the likelihood of an incorrect diagnosis.

To measure the differential levels of awareness regarding pregnancy diagnosis, a structured schedule was prepared, consisting of statements deemed irrelevant by experts. Weights were assigned to the selected statements based on expert input. Farmers, who typically perform visual observation methods for pregnancy diagnosis, were asked to score each statement (1 if they were aware, 0 if not aware). Awareness scores for rectal palpation were collected from a total of 20 veterinary professionals involved in the diagnosis process. Thereafter, the awareness index was calculated using the following formula:

$$\text{Awareness index} = \sum_{i=1}^n (p_i/p_m) \times W_i$$

Where,

p_i : Score obtained for i^{th} statement

p_m : Maximum score attainable for i^{th} statement

W_i : Weight assigned to each statement

N : Number of statements

Farmer awareness index values were utilized to model factors influencing the accuracy of pregnancy diagnosis through visual observation. However, due to

multicollinearity issues, the awareness index variable for rectal palpation was excluded from the regression analysis.

RESULTS AND DISCUSSION

Description of study animals and diagnostic procedures for pregnancy: Table 2 reveals that Graded Murrah buffaloes constitute the majority of the samples across all districts, accounting for approximately 68% of the total animals examined through both VOB and RP. This predominance underscores the breed's significance in the region's dairy farming practices. Jaffarabadi buffaloes represent around 13% of the sample, while non-descript buffaloes make up about 19%, with a notably higher presence in Nellore district. In the study region, farmers diagnosed pregnancy in buffaloes using two methods: VOB and RP method. VB method was employed to detect estrus signs—such as mucus discharge, reduced feed intake, restlessness and bellowing—at regular intervals of 21 ± 3 days until confirmed conception, whereas RP was performed 60–120 days after artificial insemination (AI). All buffaloes (100.00%) underwent pregnancy diagnosis using VOB method, while 403 buffaloes (97.10%) were also diagnosed using RP method. This indicates that the majority of buffaloes in the sample were subjected to

Table 2. Description of sample animals and their pregnancy diagnosis methods

Breed	Visual observation (VOB)					Rectal palpation (RP)				
	Guntur	Prakasam	Krishna	Nellore	Overall	Guntur	Prakasam	Krishna	Nellore	Overall
Graded Murrah (No.)	78 (69.64)	84 (68.85)	75 (75.00)	44 (54.32)	281 (67.71)	77 (70.00)	80 (69.57)	75 (75.00)	43 (55.13)	275 (68.24)
Jaffarabadi (No.)	25 (22.32)	12 (9.84)	10 (10.00)	8 (9.87)	55 (13.25)	24 (21.81)	12 (10.43)	10 (10.00)	8 (10.26)	54 (13.40)
Non-descript (No.)	9 (8.04)	26 (21.31)	15 (15.00)	29 (35.80)	79 (19.04)	9 (8.18)	23 (20.00)	15 (15.00)	27 (34.62)	74 (18.36)
Total	112 (26.99)	122 (29.40)	100 (24.10)	81 (19.52)	415 (100.00)	110 (27.30)	115 (28.54)	100 (24.81)	78 (19.35)	403 (100.00)

Note: Figures in the parentheses indicate the percentage of the column total

Table 3. Awareness on pregnancy diagnosis among farmers and veterinarians/paravets

Indicator (Weight)	Index value									
	Farmer					Veterinarians/Paravets				
	Guntur	Prakasam	Krishna	Nellore	Overall	Guntur	Prakasam	Krishna	Nellore	Overall
Physiological changes (0.12)	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Behavioural changes (0.16)	0.166	0.166	0.159	0.159	0.162	0.160	0.160	0.160	0.160	0.160
Pregnancy diagnosis method and its features (0.22)	0.099	0.101	0.099	0.096	0.098	0.220	0.220	0.220	0.220	0.220
Merits and demerits of pregnancy diagnosis methods (0.12)	0.027	0.026	0.025	0.023	0.025	0.105	0.120	0.090	0.105	0.105
Source and price of pregnancy diagnosis method/kit (0.12)	0.053	0.052	0.052	0.051	0.052	0.105	0.075	0.075	0.075	0.082
Diseases and environmental effect (0.10)	0.095	0.095	0.093	0.090	0.093	0.100	0.100	0.100	0.100	0.100
Monitoring and maintenance of breeding record (0.16)	0.106	0.110	0.104	0.104	0.106	0.160	0.160	0.160	0.160	0.160
Pregnancy diagnosis awareness index	0.671	0.675	0.657	0.648	0.661	0.975	0.960	0.930	0.945	0.952

both diagnostic methods, with only 12 buffaloes (2.89%) diagnosed exclusively through the VOB method.

Awareness level of farmers and veterinarians/paravets on pregnancy diagnosis methods: The analysis of pregnancy diagnosis awareness among farmers and veterinarians/paravets revealed notable disparities in knowledge levels. Veterinarians and paravets consistently demonstrated higher awareness index scores, with an overall average of 0.952, compared to farmers' average of 0.661 (Table 3). This gap was particularly evident in technical domains. For instance, in the category of "Pregnancy diagnosis method and its features," veterinarians/paravets achieved a perfect score of 0.220, whereas farmers averaged only 0.098. Similarly, regarding the "Merits and demerits of pregnancy diagnosis methods," veterinarians/paravets scored an average of 0.105, significantly surpassing the farmers' average of 0.025. These findings indicated that while farmers possessed a basic understanding of physiological and behavioral indicators of pregnancy, their knowledge of diagnostic methods and their implications was limited. This disparity underscored the necessity for targeted educational interventions aimed at enhancing farmers' comprehension of pregnancy diagnosis techniques, thereby potentially improving reproductive management and productivity within the dairy sector.

Diagnostic Accuracy of VOB and RP methods: The pregnancy diagnosis using VOB method was performed one to three times per animal, resulting in a total of 927 diagnostic cases with 292 false diagnosis cases for 415 buffaloes. RP was conducted one to two times per animal on 403 buffaloes, yielding 482 diagnostic cases with 28 false diagnosis cases. Farmers monitored buffaloes for estrus signs at 21 ± 3 -day intervals until conception was confirmed. The diagnostic effectiveness of both methods was evaluated using several key statistical measures, as summarized in Table 4 and elaborated in the following

paragraphs.

The performance metrics for pregnancy diagnosis using VOB method across four districts highlight both the strengths and limitations of this approach. The method demonstrated consistently high sensitivity, exceeding 90% in all districts, indicating its effectiveness in correctly identifying pregnant animals. NPV values were also high, suggesting that when the VOB method indicated a negative result, it was generally reliable. This reduced the chances of missing actual pregnancies. However, specificity values were relatively low, particularly in Nellore district, which recorded the lowest specificity at 43.14%. This indicated a higher rate of false positives, where non-pregnant animals were incorrectly classified as pregnant. Positive predictive values (PPVs) ranged from 57.04% to 62.22%, underscoring that a considerable proportion of positive diagnoses may not have reflected true pregnancies. Prakasam district exhibited the highest sensitivity (94.12%) and NPV (91.86%), reflecting superior diagnostic performance in both identifying pregnant animals and excluding non-pregnant ones. Conversely, Nellore district recorded the lowest specificity (43.14%) and overall accuracy (64.71%), highlighting the need for improved diagnostic strategies in that region. Thus, while the VOB method proved effective for initial pregnancy screening due to its high sensitivity and NPV, its low specificity and moderate PPV pointed to a significant potential for false positives. These findings underscored the importance of complementing VOB with more specific diagnostic tools, especially in districts like Nellore.

In contrast, the RP method demonstrated markedly higher diagnostic accuracy and reliability. It exhibited an overall sensitivity of 97.14% and specificity of 90.10%, indicating its strong ability to correctly identify both pregnant and non-pregnant animals. Both PPV (93.15%) and NPV (95.79%) were high, affirming the trustworthiness

Table 4. Performance metrics of VOB and RP method of pregnancy diagnosis

	Visual observation (VOB)					Rectal palpation (RP)				
	Guntur	Prakasam	Krishna	Nellore	Overall	Guntur	Prakasam	Krishna	Nellore	Overall
Total cases	254	266	220	187	927	126	127	132	97	482
True positive (TP)	107 (42.12)	112 (42.10)	85 (38.63)	77 (41.17)	381 (41.10)	72 (57.14)	80 (62.99)	72 (54.54)	48 (49.48)	272 (56.43)
True negative (TN)	69 (27.16)	79 (29.69)	62 (28.18)	44 (23.52)	254 (27.40)	48 (38.09)	43 (33.85)	50 (37.87)	41 (42.26)	182 (37.75)
False positive (FP)	68 (26.77)	68 (25.56)	64 (29.09)	58 (31.01)	258 (27.83)	3 (2.38)	4 (3.14)	7 (2.27)	6 (6.18)	20 (4.16)
False negative (FN)	10 (3.93)	7 (2.63)	9 (4.09)	8 (4.27)	34 (3.66)	3 (2.38)	0	3 (2.27)	2 (2.06)	8 (1.66)
Positive predictive value (%)	61.14	62.22	57.05	57.04	59.62	96.00	92.23	91.14	88.89	93.15
Negative predictive value (%)	87.34	91.86	87.32	84.62	88.19	94.11	100.00	94.34	95.35	95.79
Sensitivity (%)	91.45	94.12	90.43	90.59	91.81	96.00	100.00	96.00	96	97.14
Specificity (%)	50.36	53.74	49.21	43.14	49.61	94.11	91.49	87.72	87.23	90.1
Accuracy (%)	69.29	71.80	66.82	64.71	68.50	95.23	96.85	92.42	91.75	94.19

Note: Figures in the parentheses indicate the percentage of the total cases under respective column

of the diagnostic outcomes. The method achieved an overall accuracy of 94.19%, making it a dependable diagnostic approach across different districts. Notably, Prakasam district achieved perfect sensitivity (100%) and NPV (100%), reflecting exceptional diagnostic performance. Although accuracy remained high in Krishna and Nellore districts, these regions exhibited slightly lower specificity and PPV, suggesting a marginally higher incidence of false positives compared to Guntur and Prakasam districts.

When comparing the two methods, RP clearly outperformed VOB in all key diagnostic metrics. The overall accuracy of the VOB method (68.50%) was considerably lower than that of RP (94.19%). The lower PPV and specificity associated with VOB indicated a higher rate of false positive diagnoses, which could lead to inappropriate management decisions, such as unnecessary treatments or the premature culling of animals mistakenly identified as pregnant. Given these limitations, the use of VOB as a standalone diagnostic tool is not advisable. Instead, it should be employed as an initial, cost-effective screening mechanism, followed by confirmatory diagnosis through RP. This tiered diagnostic approach harnesses the accessibility of VOB and the precision of RP, thereby optimizing resource utilization and improving reproductive decision-making in herd management. However, the application of RP should be performed judiciously and only by trained professionals to ensure diagnostic accuracy and safeguard animal welfare.

Factors influencing accuracy of pregnancy diagnosis in the visual observation method: The logistic regression analysis (Table 5) identified several significant risk factors associated with incorrect pregnancy diagnoses when employing the visual observation (VOB) method. Specifically, increased experience and higher awareness levels of dairy farmers were found to significantly reduce the likelihood of false diagnosis. Each additional year of

experience corresponded to a 9.9% decrease in the odds of an incorrect diagnosis ($\text{Exp}(B) = 0.901$, $p < 0.001$). Higher awareness levels among the farmers led to an 8% reduction in the likelihood of false diagnosis, highlighting the role of continuous education and training. Conversely, larger herd sizes, presence of reproductive disorders and poor body condition increased the likelihood of incorrect pregnancy diagnosis. Larger herd sizes elevated the odds of incorrect diagnosis by 21.3%, potentially due to challenges in individually monitoring animals in bigger herds. The presence of reproductive disorders nearly doubled the risk of false diagnosis, underscoring the need for meticulous assessment in such cases. Body condition score (BCS) also played a critical role; animals with a BCS below 3 had over three times the odds of being incorrectly diagnosed, emphasizing the impact of poor nutritional status on diagnostic accuracy.

Seasonal and regional factors also influenced false diagnosis rates. Although previous literature reports stronger behavioral signs of estrus during winter (January – February), our results showed a 56.7% reduction in the odds of false pregnancy diagnosis during the rainy (June – September) season. This difference may be explained by the fact that false diagnosis is affected not only by the intensity of estrus expression but also by management practices, observation frequency and confounding health or environmental factors. In contrast, the summer (March – May) season increased the odds of incorrect diagnosis by 140.2%, which may be attributed to heat stress affecting animal behavior and observation accuracy. Regionally, animals in Nellore district had an 84.4% higher chance of false diagnosis compared to the reference district, Prakasam, suggesting regional differences in diagnostic practices or animal management. The model demonstrated a good fit, with a Nagelkerke R Square of 0.517, indicating that approximately 51.7% of the variance in diagnostic

Table 5: Factors influencing accuracy of pregnancy diagnosis in the visual observation method

	Reference	B	S.E.	Wald	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Experience	—	−0.104***	0.012	70.005	<0.001	0.901	0.879	0.923
Awareness	—	−0.083***	0.015	32.838	<0.001	0.920	0.894	0.947
Herd size	—	0.193***	0.028	46.030	<0.001	1.213	1.147	1.283
Parity	—	0.072	0.042	3.018	0.082	1.075	0.991	1.167
RD_P	—	0.686***	0.214	10.276	0.001	1.985	1.305	3.019
PD_1	Pregnancy diagnosis after 3 rd service	0.289	0.303	0.911	0.340	1.335	0.737	2.418
PD_2		0.396	0.326	1.476	0.224	1.486	0.784	2.813
Nondescript	Jaffarabadi	0.581	0.482	1.457	0.227	1.788	0.696	4.596
Graded Murrah		−0.393	0.468	0.708	0.400	0.675	0.270	1.688
Guntur	Prakasam	−0.045	0.267	0.028	0.867	0.956	0.567	1.613
Nellore		0.612**	0.285	4.603	0.032	1.844	1.054	3.224
Krishna	BCS=3	0.495	0.276	3.220	0.073	1.641	0.955	2.818
BCS_gt_3		−0.394	0.270	2.135	0.144	0.674	0.397	1.144
BCS_lt_3	Rainy	1.271***	0.268	22.489	<0.001	3.563	2.108	6.025
Rainy		−0.836***	0.340	6.060	0.014	0.433	0.223	0.843
Autumn	Winter	−0.138	0.313	0.194	0.660	0.871	0.472	1.608
Summer		0.876**	0.443	3.912	0.048	2.402	1.008	5.724
Constant		3.873	1.179	10.783	0.001	48.066		
<i>Model summary</i>								
−2 Log Likelihood	Cox & Snell R Square	Nagelkerke R Square	Chisquare	df	Sig.			
727.74	0.368	0.517	425.057	17	<.001			

***Significant at 1%, **Significant at 5%, S.E.: Standard error, C.I.: Confidence interval, df: Degree of freedom

accuracy could be explained by the variables included. These findings suggest that enhancing practitioner training, effectively managing herd sizes, ensuring good animal nutrition, and considering seasonal and regional factors can improve the accuracy of pregnancy diagnosis using visual observation.

Factors influencing accuracy of pregnancy diagnosis in the rectal palpation method: The analysis of risk factors associated with incorrect pregnancy diagnoses using the rectal palpation (RP) method also revealed several significant findings (Table 6). Higher parity was associated with a reduced likelihood of false diagnosis, with each additional calving decreasing the odds by approximately 29.6%. Experience of the dairy farmer similarly contributed to accuracy, with each additional year reducing the odds of incorrect diagnosis by about 10.4%. This finding aligns with Lech *et al.* (2022), who highlighted the vital role of farmer experience in improving diagnostic accuracy. In contrast, certain factors increased the risk of false diagnosis. Animals with reproductive disorders were nearly four times more likely to be incorrectly diagnosed. Conducting RP after 1st service significantly reduced the risk of false diagnosis by 76.9% compared to pregnancy diagnosis conducted after the 3rd service. However, the summer season was associated with a substantial increase in false diagnosis risk, with the

likelihood of an incorrect diagnosis being over 24 times higher compared to the winter reference season. This could be attributed to environmental stress and physiological effects on buffaloes that reduce the clarity of pregnancy signs, thereby decreasing the accuracy of rectal palpation-based diagnosis (Kumar *et al.* 2019).

Breed and body condition also influenced diagnostic accuracy. Non-descript breeds were nearly four times more likely to be incorrectly diagnosed compared to the reference breed, graded Murrah. Animals with a BCS greater than 3 had an 83% reduction in the likelihood of an incorrect diagnosis, while those with a BCS less than 3 were nearly 14 times more likely to be false diagnosed. Diagnosis of follicles through rectal palpation is influenced by body condition score. Animals with better reproductive health and follicle development typically those with a BCS greater than 3 are easier to accurately assess. In contrast, animals with a BCS less than 3 often exhibit poorer reproductive status and underdeveloped follicles, leading to a significantly higher likelihood of incorrect diagnosis (Hanzen *et al.* 2000). District-level analysis indicated that the coefficients for Guntur, Krishna, and Nellore were not statistically significant, suggesting no substantial district-level influence on false diagnosis rates in this analysis. The model demonstrated a good fit, with a −2 Log Likelihood

Table 6. Factors influencing accuracy of pregnancy diagnosis in the rectal palpation method

	Reference	B	S.E.	Wald	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Experience	—	−0.109**	0.052	4.484	0.034	0.896	0.810	0.992
Parity	—	−0.351***	0.123	8.128	0.004	0.704	0.553	0.896
Nondescript	—	1.357***	0.444	9.361	0.002	3.886	1.629	9.271
RD_P	—	1.362***	0.466	8.560	0.003	3.906	1.568	9.729
PD_1	Pregnancy diagnosis after 3 rd service	−1.465**	0.670	4.784	0.029	0.231	0.062	0.859
PD_2		0.698	0.520	1.802	0.179	2.010	0.725	5.573
Rainy	Winter	1.498	0.925	2.622	0.105	4.474	0.730	27.433
Autumn		0.976	0.973	1.005	0.316	2.653	0.394	17.866
Summer	Prakasam	3.186***	0.925	11.874	0.001	24.197	3.951	148.195
Guntur		−0.321	0.641	0.250	0.617	0.725	0.206	2.551
Krishna	Nellore	0.122	0.599	0.041	0.839	1.130	0.349	3.651
Nellore		0.232	0.609	0.145	0.704	1.261	0.382	4.158
BCS_gt_3	BCS=3	−1.771**	0.830	4.553	0.033	0.170	0.033	0.866
BCS_lt_3		2.631***	0.485	29.385	<0.001	13.887	5.364	35.954
Constant		−2.642	1.301	4.121	0.042	0.071		
<i>Model summary</i>								
−2 Log Likelihood	Cox & Snell R Square	Nagelkerke R Square		Chi-square	df		Sig.	
163.21	0.298	0.597		170.74	14		<0.01	

***Significant at 1%, **Significant at 5%, S.E.: Standard error, C.I.: Confidence interval, df: Degree of freedom

of 163.21 and a Nagelkerke R Square of 0.597, indicating that approximately 59.7% of the variance in diagnostic accuracy was explained by the included variables.

In conclusion, this study provides critical field-based insights into the prevalence and determinants of pregnancy false diagnosis in buffaloes across key districts of Andhra Pradesh, highlighting significant implications for reproductive management and economic sustainability in the region's dairy sector. The analysis identified visual observation of estrus (VOB) and rectal palpation (RP) as the two predominant methods currently employed for pregnancy diagnosis. While RP demonstrated superior diagnostic accuracy, sensitivity, and specificity, its utility is constrained by the requirement to wait at least 60 days post-artificial insemination (AI). In contrast, VOB allows for earlier detection at 21±3 days post-AI but suffers from lower specificity and moderate overall accuracy, often resulting in a high incidence of false positives. These diagnostic limitations can lead to inappropriate management decisions, including premature rebreeding or unwarranted culling, ultimately contributing to prolonged calving intervals and economic inefficiencies. Despite the development of more advanced diagnostic technologies, their widespread adoption remains limited in resource-constrained settings due to cost, technical complexity, and infrastructural limitations. Thus, there is a pressing need to enhance access to early, accurate, and field-appropriate diagnostic tools that can be feasibly implemented by smallholder dairy farmers and frontline veterinary personnel.

The multivariate logistic regression analysis elucidated

a complex array of factors influencing diagnostic accuracy. Higher farmer experience and awareness were strongly associated with a lower likelihood of false diagnosis, underscoring the critical role of targeted extension and training programs. In contrast, larger herd sizes, suboptimal body condition scores, reproductive disorders, seasonal stressors—particularly heat during summer—and breed-specific factors were positively correlated with diagnostic errors. These findings point to the importance of adopting an integrated approach that not only improves diagnostic protocols but also addresses animal health, nutrition, and environmental management.

In light of the respective strengths and limitations of each diagnostic method, the study recommends a tiered strategy in which VOB serves as an initial screening mechanism, followed by confirmatory diagnosis using RP. This approach offers a pragmatic balance between early detection and diagnostic reliability. The findings also highlight a critical knowledge gap among farmers in technical aspects of pregnancy diagnosis, underscoring the need for targeted educational interventions to enhance their understanding and application of effective reproductive management practices. Thus, strengthening farmer education, enhancing veterinary training and improving herd management practices are essential components of a broader strategy to reduce pregnancy false diagnosis and enhance reproductive performance in buffalo-based dairy systems.

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