Research Article

Identification of Fertility Rates in Swamp Buffalo Heifers Through Reproductive Tract Score and Cervical Mucus Analysis

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Abstract | Reproductive efficiency is crucial for livestock productivity, particularly in swamp buffaloes, which play a significant role in agriculture in tropical regions. However, their reproductive performance is often hindered by prolonged inter-calving intervals, low conception rates, and high rates of silent estrus. This study aimed to evaluate the relationship between reproductive tract scores (RTS), cervical mucus characteristics, and fertility outcomes in swamp buffalo heifers. A total of 40 heifers were assessed for reproductive tract parameters, including uterine horn diameter, ovary length, and ovary width, along with cervical mucus characteristics such as appearance, viscosity, spinnbarkeit, fern patterns, and pH. Fertility was measured using the non-return rate (NRR) after artificial insemination (AI). Heifers with higher RTS and superior cervical mucus quality demonstrated significantly higher fertility rates, with the GRTS group achieving a 60% NRR compared to 40% in the PRTS group ($p < 0.05$). The GRTS group exhibited larger uterine horns, longer and wider ovaries ($p < 0.05$). The Good Cervical Mucus (GCM) group had better scores for appearance, viscosity, spinnbarkeit, fern patterns, and pH ($p < 0.05$). These findings suggest that RTS and cervical mucus characteristics can serve as valuable fertility predictors in swamp buffalo heifers, enhancing AI success and breeding strategies.

Keywords | Artificial insemination, Cervical mucus, Fertility predictors, Reproductive efficiency, Reproductive tract score, Swamp buffalo heifers

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INTRODUCTION

Reproductive efficiency is a cornerstone of livestock productivity, directly influencing the sustainability and profitability of agricultural systems. In tropical countries like Indonesia, swamp buffaloes (*Bubalus bubalis*) play a critical role not only in meat and milk production but also in traditional agriculture, serving as draught animals and holding cultural significance in many rural communities. Their contribution to food security and rural livelihoods underscores the importance of optimizing their reproductive performance (Reswati *et al*., 2021). However, prolonged inter-calving intervals, low conception rates, and a high incidence of silent estrus hinder optimal productivity (Dhamsaniya and Parmar, 2016). Addressing these reproductive challenges is critical to ensuring the sustainability

of swamp buffalo farming, particularly in resource-limited settings where their economic and cultural importance is profound.

Despite advancements in reproductive technologies such as artificial insemination (AI), success rates in swamp buffaloes remain inconsistent. These inconsistencies are largely attributed to a lack of reliable, non-invasive predictors of fertility, compounded by unique reproductive traits of the species, which distinguish them from other livestock such as cattle. These include longer estrous cycles, subtler estrus signs, and greater sensitivity to environmental stressors such as heat and humidity, making reproductive detection and intervention more complex (Praharani *et al*., 2024; Srirattana *et al*., 2022). Understanding and addressing these factors require robust, species-specific approaches that integrate anatomical and physiological assessments to predict fertility potential more accurately.

Two key parameters have emerged as potential tools for evaluating reproductive health and fertility: reproductive tract score (RTS) and cervical mucus characteristics. RTS is a composite measure of reproductive organ development, assessing the diameter of the uterine horns, as well as the length and width of the ovaries (Yusuf *et al*., 2024). These anatomical traits are directly linked to hormonal regulation, follicular growth, ovulation, and uterine receptivity—processes critical for successful reproduction (Nafziger *et al*., 2021). Meanwhile, cervical mucus characteristics, including appearance, viscosity, spinnbarkeit, pH, and crystallization patterns (fern patterns), serve as physiological markers of estrus (Huang *et al*., 2024). Cervical mucus facilitates sperm transport, motility, and capacitation, and its quality is closely regulated by hormonal fluctuations, particularly estradiol, during the estrous cycle (Abril-Parreño *et al*., 2022).

Although these parameters have been extensively studied in cattle, their application in swamp buffaloes is limited. Buffaloes exhibit distinct reproductive physiology, which necessitates targeted research to adapt and validate these indicators for effective use in this species. Additionally, the potential interactions between RTS and cervical mucus characteristics and their combined influence on fertility outcomes remain underexplored. Understanding these relationships could provide novel insights into the mechanisms underlying reproductive efficiency and inform strategies for improving AI success rates in buffaloes.

This study aims to evaluate the relationships between RTS, cervical mucus characteristics, and fertility outcomes in swamp buffalo heifers. Specifically, the study seeks to quantify the influence of reproductive organ dimensions and cervical mucus quality on fertility performance, as measured by the non-return rate (NRR). By investigating the correlations between these parameters, the study provides a comprehensive analysis of the anatomical and physiological factors that contribute to reproductive efficiency in swamp buffaloes.

The findings of this research have the potential to enhance reproductive management practices in swamp buffaloes, offering practical tools for fertility prediction and selection in AI programs. Furthermore, these insights contribute to a deeper understanding of buffalo reproductive biology, with implications for improving productivity, sustainability, and genetic advancement in tropical livestock systems.

MATERIALS AND METHODS

The study was conducted from May to November 2024 in Kajang district, Bulukumba Regency, South Sulawesi Province, Indonesia. Kajang District, located in Bulukumba Regency, South Sulawesi Province, lies approximately between 5°20′–5°40′ South Latitude and 119°58′–120°28′ East Longitude. The area features a mixed topography of lowlands and hills, surrounded by forests and relatively natural agricultural land. Environmental and climatic conditions in Kajang play a crucial role in the buffalo farming system, which is predominantly practiced extensively in open grazing areas. Natural vegetation supports forage availability; however, high temperatures, humidity, and rainfall pose challenges to reproductive management, particularly in estrus detection and the success of artificial insemination. Therefore, the application of adaptive technologies combined with local wisdom is essential to maintain the productivity and sustainability of buffalo farming in this region as well as at the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia.

The study utilized 40 healthy swamp buffalo heifers, aged 2 to 5 years, all confirmed to be reproductively healthy. All swamp buffalo heifers were provided with forage-based feed, drinking water, and routine vaccinations to ensure optimal health conditions. All experimental procedures adhered to ethical standards and were approved by the Animal Ethics Commission of the National Research and Innovation Agency (Certificate No. 050/KE.02/SK/03/2023). Considering the known impact of environmental factors on buffalo reproductive performance, ambient temperature and humidity were monitored daily throughout the study using digital thermohygrometers installed within the livestock housing areas. During the study period, average temperatures ranged from 27°C to 33°C, while relative humidity varied between 70% and 85%. Although the environment was not artificially controlled, measures were taken to maintain animal comfort, including the provision of shade, adequate ventilation, and regular watering schedules.

Assessment of Reproductive Tract Score

The reproductive tract score (RTS) was assessed using a modified Miller (2020) procedure through rectal palpation during estrus. Estrus detection was conducted visually twice daily, in the morning and late afternoon, by observing external signs such as vulvar swelling, mucus discharge, restlessness, and standing heat behavior. Once estrus was confirmed, rectal palpation and ultrasonography were performed within the first 12 hours to ensure accurate timing for RTS evaluation. The evaluation included measuring the diameter of the uterine horn and the length and width of the ovaries using ultrasonography (USG) equipment (Honda, Japan). Based on the RTS scoring procedure following the modified Binelli *et al*. (2021), swamp buffalo heifers were classified into two groups: Good Reproductive Tract Score (GRTS), defined as a score >4, and Poor Reproductive Tract Score (PRTS), defined as a score ≤4, with the total score ranging up to 6. The scoring criteria were as follows: a score of 1 was assigned for sizes below the standard error (SE), and a score of 2 was assigned for sizes exceeding the SE. This method provided a systematic approach to categorize the reproductive potential of the heifers.

Assessment of Cervical Mucus

Cervical mucus samples were collected from swamp buffalo heifers 5 to 30 minutes before the first artificial insemination (AI) and during their first estrus. As with RTS, estrus detection for cervical mucus sampling was based on visible signs and confirmed by experienced personnel through behavioral observation and manual rectal examination. The evaluation of cervical mucus followed a modified procedure by Yusuf *et al*. (2024), scoring parameters such as characteristic appearance of cervical mucus can be observed by examining its color and condition. The viscosity was observed by dropping 2-3 drops of mucus onto a reaction plate, then tilting it at a 45°. Spinnbarkeit was observed by stretching cervical mucus between two glass slides, moving the second slide away from the first until the mucus is cut off. The fern pattern observed with 2-3 drops of cervical mucus evenly spread over the object glass and dried. The slides were then observed using a microscope, and pH was observed using pH paper with a drop of cervical mucus which gives an accurate pH reading. Based on these criteria, swamp buffalo heifers were classified into two groups: Good Cervical Mucus (GCM) with a score >7 and Poor Cervical Mucus (PCM) with a score ≤7, with a total possible score of 15.

The scoring system for cervical mucus was as follows: Appearance was graded from dirty (score 0) to clear/transparent (score 3). Viscosity ranged from thick and highly viscous (score 0) to watery and minimally viscous (score 3). Spinnbarkeit was measured in centimeters, with less than 6 cm scoring 0, 7-12 cm scoring 1, 13-18 cm scoring 3, and 19 cm or more scoring 2. The fern pattern was assessed from no crystallization (score 0) to tertiary and quinary stem ferning (score 3). Finally, pH was scored based on ranges, with <7 or >8.6 scoring 0, 8.1–8.5 scoring 1, 7.0–7.5 scoring 2, and 7.6–8.0 scoring 3.

The study involved three trained evaluators performing rectal palpation and ultrasonography to assess the reproductive tract scores (RTS). All evaluators underwent standardized training to ensure consistency in evaluation and minimize inter-observer variability, with all evaluators undergoing standardized training to minimize variability. RTS assessments were conducted during the estrus phase, immediately prior to artificial insemination (AI), as this timing is crucial for evaluating fertility status and determining optimal insemination timing. The sample size of 40 heifers was chosen based on a power analysis, which indicated it was sufficient to detect significant differences in fertility outcomes with reasonable statistical power. While 40 heifers may be considered on the lower end for detecting subtle differences, this sample size is commonly used in fertility studies with similar parameters and is expected to yield meaningful insights into the reproductive health of the buffalo.

Assessment of Fertility Rate

The fertility test was conducted by evaluating the success rate of artificial insemination (AI). According to Diansyah *et al*. (2023), fertility performance was measured using the non-return rate (NRR), NRR observation is the percentage of heifers that have been artificially inseminated and do not come back in heat for 17-25 days after being inseminated are considered pregnant. The Non Return Rate is calculated using the formula: NRR = (Number of heifers that did not return to estrus / Number of heifers inseminated) × 100. in accordance with established protocols. However, other potential factors influencing the success rate of AI were not analyzed in this study.

Statistical Analysis

The data collected in this study were organized and tabulated using Microsoft Excel. The chi-square test was employed to compare reproductive tract scores and cervical mucus characteristics between swamp buffalo groups. Pearson correlation analysis was conducted to determine the relationship between the various parameters. All statistical analyses were performed using SPSS software (version 25) for Windows (IBM Corp., Chicago, IL, USA).

RESULTS

The Reproductive Tract Score of Swamp Buffalo Heifers

The reproductive tract score on the fertility rate of swamp

buffalo heifers in this study can be seen in Table 1. The results showed that the diameter of the uterine horn in GRTS differed significantly higher ($p < 0.05$) from PRTS (24.50 mm vs. 18.00 mm). Likewise, the length of the ovary, (14.80 mm vs. 11.00 mm) and the width of the ovary (14.00 mm vs. 11.50 mm), in GRTS was statistically higher ($p < 0.05$) from GRTS. Furthermore, the fertility rate was significantly higher ($p < 0.05$) in the GRTS group compared to the PRTS group (60% vs. 40%).

Table 1: Reproductive tract score on fertility rate of swamp buffalo heifers.

Parameter	Group (Mean ± SD)	
	GRTS	PRTS
Diameter of Uterine Horn (mm)	24.50 ± 5.50[a]	18.00 ± 6.32[b]
Length of Ovary (mm)	14.80 ± 3.05[a]	11.00 ± 2.11[b]
Width of Ovary (mm)	14.00 ± 3.94[a]	11.50 ± 2.42[b]
NRR (%)	60[a]	40[b]

Note: Values within a row followed by different superscripts were significantly different ($p < 0.05$); GRTS = Good Reproductive Tract Score; PRTS = Poor Reproductive Tract Score; NRR = Non-return Rate.

Table 2: Characteristics of cervical mucus for fertility rate of swamp buffalo heifers.

Parameter	Group (Mean ± SD)	
	GCM	PCM
Appearance	2.60 ± 0.70[a]	1.40 ± 0.52[b]
Viscosity	2.20 ± 0.92[a]	1.30 ± 0.67[b]
Spinnbarkeit	2.10 ± 0.88[a]	1.30 ± 0.48[b]
Fern Pattern	2.20 ± 0.79[a]	1.30 ± 0.48[b]
pH	2.20 ± 0.63[a]	1.60 ± 0.70[b]
NRR (%)	60[a]	40[b]

Note: Values within a row followed by different superscripts were significantly different ($p < 0.05$); GCM = Good Cervical Mucus; PCM = Poor Cervical Mucus; NRR = Non-return Rate.

The Characteristics of Cervical Mucus of Swamp Buffalo Heifers

The characteristics of cervical mucus that effect on the fertility rate of swamp buffalo heifers were analyzed in this study and results were presented in Table 2. The statistical analysis of the results showed that the appearance score in the GCM group was notably higher ($p < 0.05$) compared to the PCM group (2.60 vs. 1.40). Likewise, the viscosity score was significantly higher in the GCM group (2.20 vs. 1.30; $p < 0.05$), as was the spinnbarkeit score (2.10 vs. 1.30; $p < 0.05$). The fern pattern scores also differed significantly, with the GCM group scoring higher (2.20 vs. 1.30; $p < 0.05$). The pH value was showed a significant rise in the GCM group (2.20 vs. 1.60; $p < 0.05$). Furthermore, the fertility rate, measured as the non-return rate (NRR), was significantly greater in the GCM group compared to the PCM group (60% vs. 40%; $p < 0.05$).

The Correlation Between Reproductive Tract Score and Cervical Mucus of Swamp Buffalo Heifers

The study investigated the relationship between reproductive tract score and cervical mucus in swamp buffaloes. The correlations between various parameters are detailed and visually represented in Figure 1, providing insights into their interactions and significance.

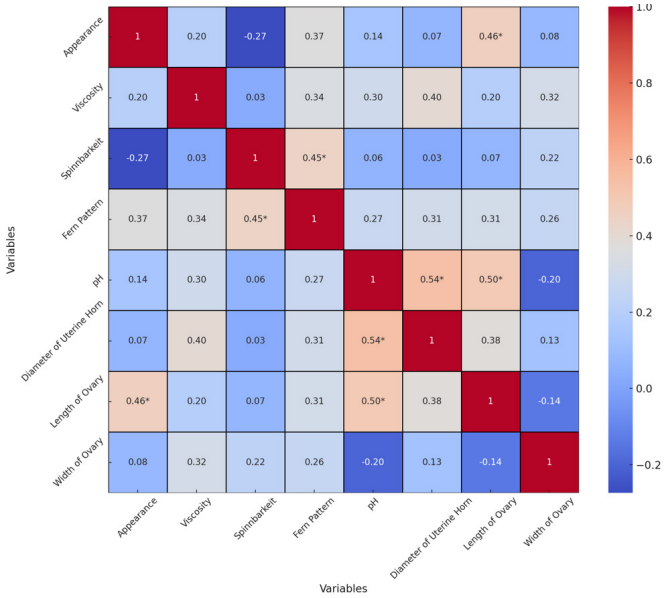

Figure 1: Heatmap showing the Pearson correlation between reproductive tract scores and cervical mucus characteristics in swamp buffalo heifers.

The correlation analysis presented in the heatmap reveals key relationships between the studied variables. Notably, a moderate positive correlation exists between pH and Diameter of Uterine Horn ($r = 0.54$), as well as pH and Length of Ovary ($r = 0.50$), suggesting that pH levels might influence or relate to reproductive organ dimensions. Appearance and Length of Ovary also show a moderate positive correlation ($r = 0.46$), indicating that external traits may be indicative of internal reproductive features. On the other hand, Spinnbarkeit exhibits a negative correlation with Appearance ($r = -0.27$), highlighting potential inverse relationships between these variables. Most other correlations are weak or negligible, with coefficients near zero, indicating minimal linear relationships. These findings provide a foundational understanding of inter-variable interactions, which could guide further studies into physiological or reproductive processes. No extreme outliers were observed in the dataset. All measurement values fell within the expected biological range for swamp buffalo heifers, and therefore, no data were excluded from statistical analysis.

DISCUSSION

The findings of this study provide compelling evidence for the biological significance of reproductive tract scores (RTS) and cervical mucus characteristics as predictors of fertility outcomes in swamp buffalo heifers. RTS represents a composite measure of reproductive organ development, which reflects both structural integrity and functional readiness for reproduction, including hormonal responsiveness and follicular activity (Thomson *et al*., 2023). Anatomical features such as the diameter of the uterine horn and the dimensions of the ovaries serve as critical markers of reproductive capacity. Larger uterine horns are indicative of enhanced endometrial surface area, which supports increased nutrient exchange and embryo implantation. Similarly, larger ovaries are directly associated with greater follicular activity and ovulatory potential, as they provide a more conducive environment for oocyte maturation and release (Tamura *et al*., 2021).

The significantly higher measurements of these parameters in the Good Reproductive Tract Score group suggest that well-developed reproductive organs, governed by optimal hormonal regulation, are essential for efficient reproduction. Hormones such as estradiol and progesterone play pivotal roles in stimulating ovarian follicle growth, regulating uterine tissue development, and synchronizing estrus cycles (Kelly *et al*., 2020). The higher fertility rate (60% NRR) observed in the GRTS group underscores the functional importance of these anatomical traits, as they enable better coordination between gamete transport, fertilization, and implantation. This highlights RTS as a practical and scientifically robust tool for identifying heifers with superior reproductive potential (Young *et al*., 2017).

Cervical mucus, a key biomarker of estrus and reproductive health, also plays a central role in facilitating successful reproduction. Cervical mucus acts as a medium for sperm transport, providing a supportive environment that ensures sperm motility and longevity (Gulati and Bhagat, 2022). Its properties are closely linked to the hormonal milieu during estrus, particularly estrogen levels, which modulate the secretion, composition, and physical properties of mucus. The superior scores for appearance, viscosity, spinnbarkeit, fern pattern, and pH observed in the Good Cervical Mucus group indicate a hormonally optimal reproductive state, which enhances the likelihood of successful fertilization. The high spinnbarkeit observed in the GCM group, for example, reflects the elasticity of cervical mucus, which facilitates the upward movement of sperm through the cervix and into the uterine cavity (Ningwal *et al*., 2018; Gonçalves *et al*., 2024). The well-defined fern patterns represent the crystallization of mucus glycoproteins under the influence of elevated estrogen levels, further signifying the optimal timing of insemination. The slightly alkaline pH values recorded in the GCM group are particularly important, as they neutralize the acidic vaginal environment, protecting sperm from degradation and enhancing their motility (Ferdiansyah *et al*., 2022). Together, these characteristics make cervical mucus an indispensable factor in reproductive success, both as a physiological medium and as a diagnostic tool for fertility evaluation (Chaudhary *et al*., 2024).

The correlation analysis provides a deeper understanding of the interrelationships between reproductive tract parameters, cervical mucus characteristics, and fertility outcomes. The moderate positive correlation between pH and the diameter of the uterine horn (r = 0.54) suggests that a favorable pH environment supports uterine development and functionality. A slightly alkaline pH may optimize the uterine environment for sperm survival and embryo implantation by maintaining the structural integrity of uterine tissues and reducing oxidative stress (García-Galán *et al*., 2020). Similarly, the positive correlation between pH and ovary length (r = 0.50) highlights the role of systemic hormonal regulation in synchronizing reproductive organ development with optimal biochemical conditions for conception. The positive correlation between cervical mucus appearance and ovary length (r = 0.46) further underscores the hormonal interdependence of internal and external reproductive traits. Clear, high-quality cervical mucus is often a visual manifestation of elevated estrogen levels, which also drive ovarian follicular development and ovulation (Crowe, 2016). This synchronization ensures that external signs of estrus, such as mucus clarity, align with internal reproductive readiness, thereby maximizing fertility potential (Abd-ElHafeez *et al*., 2020).

The negative correlation between spinnbarkeit and appearance (r = -0.27) is particularly noteworthy. While spinnbarkeit reflects the elasticity and extensibility of cervical mucus under estrogenic influence, increased mucin polymerization and hydration during peak estrus may reduce its visual clarity. This trade-off may explain why mucus that is biologically favorable for sperm transport may appear less visually clear. Such an inverse relationship has been suggested in studies of crossbred cattle (Ningwal *et al*., 2018) and glycoprotein dynamics during estrus (Abril-Parreño *et al*., 2022).

Interestingly, the negative correlation between spinnbarkeit and appearance (r = -0.27) reveals a potential trade-off between mucus elasticity and transparency. This relationship may be influenced by variations in mucin concentration and hydration levels during different phases of estrus. Higher spinnbarkeit, while beneficial for sperm motility, may reduce mucus clarity due to increased mu-

cin polymerization. These findings suggest a complex and dynamic regulation of cervical mucus properties, which warrants further investigation to understand how these factors interact to optimize fertility. The weak or negligible correlations observed between some variables (e.g., viscosity and reproductive tract dimensions) indicate that certain traits may function independently or be influenced by factors not captured in this study. For instance, mucus viscosity may be more sensitive to transient hormonal changes, while reproductive organ dimensions are likely influenced by long-term factors such as genetics, nutrition, and overall health (Kalita *et al*., 2022). These observations highlight the need for multifactorial approaches to fertility assessment, combining both static anatomical markers and dynamic physiological indicators.

This study was conducted to address critical gaps in understanding the factors influencing reproductive efficiency in swamp buffaloes. Swamp buffaloes exhibit unique reproductive challenges, including prolonged inter-calving intervals and high rates of silent estrus, which limit their productivity. Artificial insemination (AI) programs, while promising, have shown inconsistent success rates in this species, partly due to the lack of reliable predictors of fertility. By focusing on RTS and cervical mucus characteristics, this research provides a practical and scientifically grounded approach to improving reproductive management in swamp buffaloes.

The RTS reflects the developmental status of the reproductive organs, which are influenced by genetics, nutrition, and hormonal cycles (Ault-Seay *et al*., 2023). Cervical mucus characteristics, on the other hand, directly indicate the physiological readiness for conception, with quality metrics such as viscosity, elasticity, and pH serving as proxies for estrogen dominance and estrus intensity. Together, these parameters offer a comprehensive evaluation of reproductive health and fertility potential (Maher *et al*., 2018).

From a scientific perspective, the study underscores the importance of integrating anatomical and physiological markers to predict fertility. Future research should focus on elucidating the molecular and hormonal mechanisms underlying the observed relationships. For instance, studies investigating the role of specific hormones, such as estradiol and progesterone, in modulating reproductive tract dimensions and cervical mucus properties could provide deeper insights (Huang *et al*., 2024). Additionally, genomic studies could identify genetic markers associated with high RTS and cervical mucus quality, enabling selective breeding for improved fertility traits.

In practical terms, the findings have significant implications for livestock management. The integration of RTS and cervical mucus assessments into routine reproductive evaluations offers a cost-effective and non-invasive method for identifying heifers with high reproductive potential. This approach can enhance the success rates of AI programs, reduce economic losses associated with failed inseminations, and improve overall productivity. Furthermore, the use of these parameters in breeding programs can help prioritize animals with superior fertility traits, contributing to genetic improvement and sustainable livestock production.

Despite the valuable insights obtained, this study has several limitations that should be acknowledged. First, the sample size of 40 heifers, while adequate for preliminary analysis, may not fully capture the broader variation present in the swamp buffalo population. Second, although environmental factors such as ambient temperature and humidity were monitored, they were not experimentally controlled, which may have influenced physiological responses. Third, other critical factors that could affect fertility outcomes—such as semen quality, insemination technique, or the skill level of AI technicians—were not evaluated in this study. These limitations suggest that future research should incorporate larger sample sizes, controlled environmental conditions, and a more comprehensive set of fertility determinants to strengthen and validate the findings.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, this study showed that reproductive tract scores (RTS) and cervical mucus characteristics are reliable fertility predictors in swamp buffalo heifers. Higher RTS and optimal mucus quality were linked to improved reproductive organ dimensions and fertility rates. Correlations revealed positive links between pH and organ size, highlighting their biological significance. These findings support integrating RTS and mucus evaluations into reproductive management to enhance artificial insemination success and promote sustainable buffalo farming. Further research involving larger populations, hormonal profiling, and molecular analyses of cervical mucus composition is recommended to validate and expand upon these findings.

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NOVELTY STATEMENTS

This study presents a novel integration of Reproduc-

tive Tract Scoring (RTS) and cervical mucus analysis as a predictive tool for assessing fertility potential in swamp buffalo (*Bubalus bubalis*) heifers. While previous studies have largely focused on these parameters in isolation, our research uniquely combines both morphological and biochemical indicators to enhance the precision of fertility evaluation. This integrative approach offers a more comprehensive assessment of reproductive readiness, providing a valuable reference for improving selection strategies and reproductive management in swamp buffalo, a species that remains underrepresented in fertility research.

AUTHOR'S CONTRIBUTIONS

Athhar Manabi Diansyah, Muhammad Yusuf, and Abdul Latief Toleng: Designed the experiment concept, reviewed and finalized the draft.
Hasrin Hasrin and Muhammad Fajar Amrullah: Statistical analysis, reviewed and finalized the draft.
Rahmat Rahmat, Andi Muhammad Alfian, and Ahmad Alfaruqi Syahrandi Adam: Collecting data and prepared the draft and reference.

Conflict of Interest

The authors have declared no conflict of interest.

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