



The Reproductive Tract Score and Characteristic of Cervical Mucus on Fertility Rate of Bali Heifers

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Abstract | The productivity of Bali heifers still needs to be improved, especially in reproductive aspects. Reproductive Tract Score (RTS) is a comprehensive to evaluate the reproductive organs of heifers, while cervical mucus is an environment that influences the sperm for successful fertilization. This study aims to evaluate the reproductive tract score (RTS) and the quality of cervical mucus in relation to the fertility rate of Bali heifers. Assessment of the reproductive tract was carried out using a scoring method in the form of score 1 = size < SE and Score 2 = size > SE, then grouped into Good RTS (GRTS) with a score > 4 and Poor RTS (PRTS) with a score ≤ 4 with a total score of 6. Cervical mucus is evaluated using a scoring on five variables (appearance, viscosity, spinnbarkeit, fern pattern, and pH) with score scale 0 until 3. The parameter will be grouped into Good Cervical Mucus (GMC) with a score of >7 and Poor Cervical Mucus (PMC) with a score of ≤7 for total score of 15. The result showed diameter horn uterine, length ovary, and width ovary in GRTS was significantly higher ($p < 0.05$) from PRTS. The fertility rate of GRTS showed was significantly higher ($p < 0.05$) from PRTS. The characteristic cervical mucus on fertility rate of Bali heifers showed that all variables in GMC was significantly higher ($p < 0.05$) from PMC. The fertility rate of GMC showed was significantly higher ($p < 0.05$) from PMC. Significant differences in the GRTS and PRTS groups, as well as GMC and PMC in Bali cattle, may be due to genetic factors that impact hormones, where an optimal balance of estrogen and progesterone hormones in superior heifers supports larger ovary and uterine sizes and better quality of cervical mucus. Increases fertilization and reproductive success. Assessment of the Reproductive Tract Score (RTS) and cervical mucus characteristics is a very effective and accurate method in Bali cattle breeding and reproductive management programs. In conclusion, both RTS and cervical mucus characteristics are important for predicting reproductive performance in Bali heifers, their interdependence is minimal. Therefore, integrating multiple evaluative criteria is essential for a comprehensive assessment of reproductive potential on Bali heifers. This approach will optimize breeding strategies and enhance productivity and sustainability of Bali heifer populations.

Keywords | Bali cattle, Heifers, Reproductive tract score, Cervical mucus, Fertility, Reproductive

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The productivity of Bali cattle still needs to be improved, especially in reproductive aspects. The increase in livestock population is determined by good reproductive factors related to providing superior calves to ensure that national meat needs are met. The current condition of Bali cattle productivity has been further worsened by the Foot and Mouth Disease (FMD) outbreak, which has resulted in high livestock mortality so that superior livestock cannot be saved. The impact of this resulted in the need for superior Bali heifers as a replacement for broodstock affected by this problem. Therefore, the selection and management of replacement heifers to achieve greater reproductive success is critical to the beef cattle production system (Hindman *et al.*, 2022).

Several efforts to increase the productivity of Bali cattle have been carried out, including the application of reproductive technology, including artificial insemination (AI). AI as a reproductive technology that aims to utilize superior bull, suppress the spread of reproductive diseases, and improve the genetic quality of livestock (Said, 2020). The application of AI technology to cattle with high fertility levels is expected to have a conception/pregnancy rate of up to 60% (Ermen *et al.*, 2021); however, Hasrin *et al.* (2022) study showed that the success rate of AI in Bali cattle is only 23-29%. This can be caused by the problem of less effective parent selection.

The selection of parent seeds in the breeding program per Minister of Agriculture Regulation No.101/Permentan/OT.140/7/2014 is based only on general production and reproductive performance. This will impact the selection of parent seeds that do not have reproductive potential. A selection method for brood stock performance can be developed using the Reproductive Tract Score (RTS) (Jones *et al.*, 2018; Schowe *et al.*, 2023). For example, mature heifers will breed early in their first breeding season, and that is critical for lifetime success and stayability in the maternal herd (Handcock *et al.*, 2021). The results of Dickson *et al.* (2019) and Miller (2020), showed the satisfactory results.

Furthermore, another crucial aspect contributing to reproductive success is the characteristics of cervical mucus, which is vital in the reproductive process because cervical mucus plays a role in fertilization and is also being the place for sperm capacitation (Maher *et al.*, 2018). In line with Chaudhary *et al.* (2024), stated that cervical mucus is a biological environment that affects sperm survival and determines the ability of cattle to become pregnant. The condition of the cervical mucus, which is influenced by hormonal factors, nutrition, and the cattle general health condition, can significantly influence the success rate of fertilization and pregnancy. Combining RTS assessment and cervical

mucus characterization in the reproductive management of Bali cattle provides a more effective strategy. By monitoring these two aspects, farmers can identify whether the cattle is physically ready for reproduction and whether her internal conditions are favorable for successful fertilization.

The integration of information from RTS and cervical mucus characterization allows the development of breeding protocols that are more dynamic and responsive to each animal's individual conditions. Gulati and Bhagat (2022), stated that artificial insemination based on cervical mucus characteristics may increase pregnancy rate. This increases reproductive efficiency and minimizes the risk of pregnancy failure, which is often a challenge in cattle breeding. However, information regarding RTS and characteristics of cervical mucus in Bali heifers is still limited and has yet to be published. This study aims to evaluate the reproductive tract score (RTS) and the quality of cervical mucus in relation to the fertility rate of Bali heifers. The information approach of RTS and characteristics of mucus cervical on Bali heifers can enable a more efficient and accurate selection of superior seeds.

MATERIALS AND METHODS

STUDY AREA AND PERIOD

This study was conducted from October 2023 until April 2024 at the Lappariaja District, Bone Regency, and the Laboratory of Animal Reproduction, Faculty of Animal Science, Hasanuddin University, Makassar, Indonesia.

EXPERIMENTAL DESIGN AND CONTROL

The Animal Ethics Committee at Hasanuddin University approved all procedures performed in this study. Forty Bali heifers aged 2 to 3 years old were studied from farms in Lappariaja District, Bone Regency. Bali heifers were kept using a semi-extensive system. Bali heifers were grouped into groups based on RTS and cervical mucus characteristics. The group based on RTS was divided into GRTS (Good RTS) and PRTS (Poor RTS) according to the results of reproductive target measurements. At the same time, the group based on cervical mucus characteristics was divided into GMC (Good Mucus Cervical) and PMC (Poor Mucus Cervical) according to the results of the cervical mucus characteristics assessment. The grouped Bali heifers were subjected to in vivo fertility test.

SCORING ASSESSMENT

The reproductive tract score assessment followed the modified Miller (2020) procedure by rectal palpation during estrus. The assessment of the reproductive tract score was used Ultrasonography (USG) (Honda, Japan). Bali heifers will be grouped into the Good RTS (GRTS) group with a score of >4 and Poor RTS (PRTS) with a score of ≤4 with

Table 1: Score assessment of cervical mucus.

Score	Appearance	Viscosity	Spinnbarkeit	Fern Pattern	pH
0	Dirty	Thick, highly visous	<6 cm	No crystallization	<7 or >8.6
1	Translucent/ cloudy	Mucus of intermediate	7-12 cm	Atypical fern formation	8.1-8,5
2	Slight cloudiness	Mildly viscous mucus	19 cm or more	Primary and secondary stem ferning	7.0-7.5
3	Clear/ transparent	Watery minimally viscous	13-18 cm	Tertiary and quintenary stem ferning	7.6-8.0

total score at 6. RTS was scored by calculating the standard error (SE) to get the threshold in both groups. Score calculations were carried out under the condition that the score is 1 for the size < SE, and conversely, the score is 2 for the size > SE. While on cervical mucus samples were collected from Bali heifers 5 to 30 min before the first AI. Additionally, samples of cervical mucus were collected at their first estrus. The assessment was scored on the appearance, viscosity, spinnbarkeit, fern pattern, and pH (Table 1). Bali heifers will be grouped into the Good Mucus Cervical (GMC) group with a score of >7 and Poor Mucus Cervical (PMC) with a score of ≤ 7 for a total score of 15.

FERTILITY RATE

A fertility test was performed by in vivo using success rate artificial insemination (AI). All Bali heifers were given forage feed, drinking water, and vaccinations. According to Diansyah *et al.* (2023), the fertility test observed a Non-Return Rate (NRR). Different factors that may affect the success rate of AI were not assessed.

STATISTICAL ANALYSIS

The data obtained in this study were tabulated in Microsoft Excel. Furthermore, the reproductive tract score and cervical mucus characteristics were analyzed by One-Way Analysis of Variance (ANOVA) and their correlation were analyzed by regression analysis using SPSS version 25. The fertility rate was analyzed by Chi-Square using GraphPad.

RESULTS AND DISCUSSION

THE REPRODUCTIVE TRACT SCORE OF BALI HEIFERS

The reproductive tract score on fertility rate of Bali heifers in this study can be seen in Table 2.

Table 2 shows the reproductive tract score on fertility rate of Bali heifers. Statistical analysis showed that diameter

horn uterine in GRTS was significantly higher (p < 0.05) from PRTS (20.70 mm vs. 17.00 mm). Likewise, length ovary (13.20 mm vs. 10.10 mm), and width ovary (12.30 mm vs. 9.20 mm) was significantly between the two groups (p < 0.05). The fertility rate of GRTS showed was significantly higher (p < 0.05) from PRTS (70% vs 45%).

Table 2: The reproductive tract score on fertility rate of bali heifers.

Group	Mean±SD			NRR (%)
	Diameter Horn Uterine (mm)	Length Ovary (mm)	Width Ovary (mm)	
GRTS	20.70 ± 1.63 ^a	13.20 ± 2.29 ^a	12.30 ± 0.73 ^a	70 ^a
PRTS	17.00 ± 1.03 ^b	10.10 1.02 ^b	9.20 ± 1.88 ^b	45 ^b

Note: Means in a column with different superscripts differ significantly at p < 0.05; GRTS (Good Reproductive Tract Score); PRTS (Poor Reproductive Score).

THE CHARACTERISTICS CERVICAL MUCUS OF BALI HEIFERS

The characteristic cervical mucus on fertility rate of Bali heifers in this study can be seen in Table 3.

Table 3 shows the characteristic cervical mucus on fertility rate of Bali heifers. Statistical analysis showed that appearance in GMC was significantly higher (p < 0.05) from PMC (2.60 vs. 1.20). Likewise, viscosity (2.30 vs. 1.45), spinnbarkeit (2.20 vs. 1.10), fern pattern (2.25 vs. 1.40), and pH (2.10 vs. 1.65) was significantly between the two groups (p < 0.05). The fertility rate of GMC showed was significantly higher (p < 0.05) from PMC (70% vs. 45%).

THE CORRELATION BETWEEN RTS AND CERVICAL MUCUS OF BALI HEIFERS

The correlation between RTS and Cervical Mucus of Bali Heifers in this study can be seen in Table 4.

Table 3: The characteristic cervical mucus on fertility rate of bali heifers.

Group	Mean±SD					NRR (%)
	Appearance	Viscosity	Spinnbarkeit	Fern Pattern	pH	
GMC	2.60 ± 0.60 ^a	2.30 ± 0.80 ^a	2.20 ± 0.77 ^a	2.25 ± 0.91 ^a	2.10 ± 0.91 ^a	70 ^a
PMC	1.20 ± 0.70 ^b	1.45 ± 0.76 ^b	1.10 ± 0.70 ^b	1.40 ± 0.68 ^b	1.65 ± 0.75 ^b	45 ^b

Note: Means in a column with different superscripts differ significantly at p < 0.05; GMC (Good Mucus Cervical); PMC (Poor Mucus Cervical).

Table 4: The correlation between RTS and cervical mucus of bali heifers.

Correlation of RTS with Mucus Cervical	
Coefficient of correlation (r)	0.0610
Coefficient of Determination (r ²)	0.003
Regression equations	Y=4.20+0.03X

Table 4 shows the correlation between RTS and cervical mucus of Bali heifers. Statistical analysis showed that correlation between RTS and cervical mucus did not differ significant ($p < 0.05$) in Coefficient of correlation (0.0610), Coefficient of Determination (0.003), Regression equations ($Y = 4.20 + 0.03X$).

Reproductive efficiency is a crucial determinant of productivity in cattle breeding, particularly for Bali heifers. Two crucial parameters often evaluated to assess reproductive potential are the Reproductive Tract Score (RTS) and cervical mucus characteristics. This discussion aims to elucidate the relationship between these parameters and their implications for improving breeding outcomes in Bali heifers. In this study, it is implied that significant differences in the GRTS and PRTS groups, as well as GMC and PMC groups, may be due to genetic factors that will have an impact on hormonal factors. In Bali heifers superior genetics have good hormone regulation. Hormones have a very important role in regulating the development and function of reproductive organs (Kelly *et al.*, 2020), so significant differences occur. This has an impact on superior Bali heifers to larger ovary size associated with a higher number of follicles and better egg quality (Tamura *et al.*, 2021), and larger uterine size correlates with increased pregnancy rates because it provides a more favorable environment for embryo development (Geary *et al.*, 2016). Also, in cervical mucus, the difference between GMC and PMC is due to hormonal balance. In superior Bali heifers, the balance of the hormones estrogen and progesterone is at optimal levels. Differences in the quality of cervical mucus between superior and poor heifers include genetic factors that play an important role in regulating the characteristics of this cervical mucus (Mohammad Al-Delemi, 2022). In superior heifers, good genetic factors allow for an optimal balance of the hormones estrogen and progesterone. This condition greatly supports the movement of sperm through the cervix to the uterus, increasing the chances of fertilization and reproductive success.

The Reproductive Tract Score (RTS) is a comprehensive measure used to evaluate the reproductive organs of heifers. It is based on a physical examination of the uterus and ovaries, with scores reflecting the maturity and functionality of these organs. RTS is crucial for predicting the reproductive efficiency and readiness of heifers for breeding (Thomson *et al.*, 2023). Table 2 shows findings suggest that heifers with

higher RTS possess better reproductive organ development and functionality, which are crucial for optimal reproductive performance. Studies have shown that a well-developed reproductive tract, indicated by higher RTS, is associated with increased fertility and reproductive success (Table 2). Higher RTS values have been linked to earlier conception and higher pregnancy rates in heifers. Young *et al.* (2017) stated that heifers with large reproductive tracts have higher fertility rates than with smaller reproductive tracts. This correlation underscores the importance of using RTS as a reliable indicator for selecting heifers with superior reproductive potential.

Cervical mucus plays a critical role in the reproductive process by providing a suitable environment for sperm. Cervical mucus also has roles in sperm endurance and transportation to the uterine cavity (Gonçalves *et al.*, 2024). The quality of cervical mucus can significantly impact fertility, with crucial characteristics including appearance, viscosity, spinnbarkeit (stretchability), fern pattern, and pH. In addition, the physical properties of cervical mucus are directly related to the fertility status of animals (Kalita *et al.*, 2022). Table 3 shows that included higher scores in appearance, viscosity, spinnbarkeit, fern pattern, and pH levels, aligning with their higher NRR values. These results imply that superior cervical mucus characteristics are associated with enhanced reproductive performance. Research supports the significance of cervical mucus characteristics in fertility (Table 3). The results of research by Siregar *et al.* (2019) found that cervical mucus with a medium to thick viscosity was classified as good, while the viscosity of thin mucus was poor. Cervical mucus with higher spinnbarkeit and favorable pH levels has been shown to enhance sperm motility and viability, which are critical for successful fertilization. Poor mucus scores in heifer result with no pregnancy, which may be due to impaired penetration and progressive motility of spermatozoa (Mahdavinezhad *et al.*, 2021). This can also be caused by the viscosity of the moving environment so that spermatozoa can penetrate and move (Rahmat *et al.*, 2024). These results are in accordance with Ningwal *et al.* (2018) who observed higher spinnbarkeit in the cervical mucus of cattle with good fertility compared to cattle with poor fertility. Our study showed that the mucus fern pattern score was positively correlated with the probability of conception. In this study, heifers with good fern pattern scores had better conception rates at good reproductive track. The appearance of the ferning pattern indicated the cattle estrus level and is related to the ovarian activity (Ferdiansyah *et al.*, 2022). Similar findings are reported by Ningwal *et al.* (2018) who reported increased pregnancy rate in cattle with typical arborisation pattern compared to those atypical patterns. Cervical mucus with an optimum pH could help the survival of spermatozoa (García-Galán *et al.*, 2020), but if the cervical mucus pH exceeds the optimum level, it will reduce the sperm fertility.

To further explore the interplay between RTS and cervical mucus characteristics, a correlation analysis was conducted. The correlation indicates a weak positive correlation between RTS and cervical mucus characteristics (Table 4). This suggests that while there is a slight tendency for higher RTS to be associated with better cervical mucus quality, the relationship is not strong. The coefficient of determination implies that only 0.3% of the variability in cervical mucus characteristics can be explained by changes in RTS. This weak correlation highlights that although RTS and cervical mucus characteristics are crucial for reproductive success, they do not heavily influence each other. Studies have found similar weak correlations, indicating that while RTS is a valuable predictor of reproductive organ development, cervical mucus characteristics may be influenced by a broader range of factors, including hormonal levels and environmental conditions. Characteristics of the mucus depend on the hormones produced in the estrus phase (Rizki *et al.*, 2019). Cervical mucus characteristics which depended on the high or low estrogen produced by mature follicles (Crowe, 2016). Furthermore, it is notified that heat stress leads to changes in the physical and biochemical properties of cervical mucus at the time of insemination (Abd-El-Hafeez *et al.*, 2020). These findings suggest the need for a multifaceted approach to evaluating reproductive potential.

The findings of this study underscore the importance of both RTS and cervical mucus characteristics in evaluating the reproductive potential of Bali heifers. Higher RTS values correlate with better-developed reproductive organs, which is a critical factor for successful breeding. Simultaneously, optimal cervical mucus characteristics are essential for facilitating sperm transport and enhancing fertility. However, the weak correlation between RTS and cervical mucus characteristics suggests that other factors also significantly impact reproductive success. Assessment of the Reproductive Tract Score (RTS) and cervical mucus characteristics is a very effective and accurate method in cattle breeding and reproductive management programs because it provides a comprehensive evaluation of the reproductive health of heifers. RTS provides the condition and development of reproductive organs such as the ovaries and uterus, helping to identify Bali heifers that have high reproductive potential and those that hinder higher levels of fertility and pregnancy success. Meanwhile, cervical mucus analysis provides additional information regarding the heifer's physiological readiness for fertilization that reflects optimal hormonal conditions. The combination of these two methods enables better prediction of reproductive and artificial insemination rates. It helps farmers focus on heifers with high reproductive potential, thereby increasing the efficiency and accuracy of breeding and reproductive management programs.

Therefore, a holistic approach that includes comprehensive assessments of both RTS and cervical mucus character-

istics, along with other relevant factors, is necessary for a more accurate evaluation of reproductive potential. Future research should investigate additional variables that may influence reproductive performance, such as hormonal profiles, genetic factors, and environmental conditions. Larger sample sizes and longitudinal studies would provide more robust data, enabling a deeper understanding of the complex interactions that govern reproductive success in Bali heifers.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, while both RTS and cervical mucus characteristics are individually important for predicting reproductive performance in Bali heifers, their interdependence is minimal. Therefore, integrating multiple evaluative criteria is essential for a comprehensive assessment of reproductive potential. This approach will help optimize breeding strategies and enhance the overall productivity and sustainability of Bali heifer populations.

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NOVELTY STATEMENT

This study is predicting fertility ability through reproductive tract scores (RTS) and quality of cervical mucus in Bali heifers.

AUTHOR'S CONTRIBUTIONS

All authors equally contributed and approved the manuscript.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interests.

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