

## Supplementation with mineral mix or concentrate booster restored fertility in cows following foot-and-mouth disease infection

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### ABSTRACT

This study aimed to evaluate the effects of supplementing mineral mix and concentrate booster on the fertility of cows recovering from foot-and-mouth disease (FMD). Eighteen post-FMD cows were assigned to three treatment groups. Cows in the T0 group received a standard feed consisting of elephant grass and a local concentrate. In the T1 group, cows were provided the standard feed supplemented with 40 g/day of mineral mix, while cows in the T2 group received the standard feed supplemented with 600 g/day of concentrate booster. The feeding treatments were applied for 42 days, and fresh drinking water was provided ad libitum. The results showed that the onset of estrus occurred significantly earlier ( $p < 0.05$ ) compared to the anestrus period experienced during FMD infection. Both supplemented groups exhibited higher ( $p < 0.05$ ) estrus rates, pregnancy rates, and calving rates than the T0 group. The interval between the last estrus and the initiation of additional feeding was considerably longer than the onset of estrus observed after treatment. The pH and ferning scores of cervical mucus in cows from the T0 group were lower than those in the T1 and T2 groups. However, no significant differences ( $p > 0.05$ ) were found in cervical mucus pH or ferning scores between the T1 and T2 groups, between pregnant and non-pregnant cows, or between offspring sex categories. In conclusion, supplementation with either a mineral mix or a concentrate booster improved fertility parameter, including estrus rate, pregnancy rate, and calving rate, in cows recovering from FMD.

**Keywords:** calf sex, cervical mucus, ferning score, onset of estrus, pregnancy rate

### INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed livestock, including cattle, pigs, and sheep, and remains a major global animal health concern

(Blacksell *et al.*, 2019). Although morbidity is high, the mortality rate is typically low (Brown *et al.*, 2022). Indonesia was declared FMD-free in 1986, and without vaccination in 1990 (Susila *et al.*, 2022). However, an FMD outbreak occurred on April 27, 2022, in Gresik Regency,

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East Java, affecting 402 beef cattle (Sutawi *et al.*, 2023). The disease subsequently spread to major cattle-producing regions across Indonesia (Susila *et al.*, 2022), including Lamongan and Madiun Regencies (Zainuddin *et al.*, 2023). By November 18, 2022, FMD had affected 35.18% (146/415) of regencies/cities across 50% (17/34) of provinces, with 578,060 infected cattle, 508,494 recoveries (87.97%), 13,177 conditionally culled cattle (2.28%), and 56,389 deaths (9.75%) (Sutawi *et al.*, 2023).

Foot-and-mouth disease results in reduced livestock productivity, impaired reproduction, decreased labor efficiency, and substantial economic losses (Triatmojo *et al.*, 2013). Economic impacts include reduced milk yield (25% annually), decreased growth rate of beef cattle (10-20%), loss of draft power (60-70%), reduced fertility (10%), delayed estrus, delayed conception, calf mortality (20-40%), and culling of chronically infected animals (Sumartono *et al.*, 2023). Control measures consist of strict biosecurity, treatment of affected cattle, and vaccination of healthy individuals (Syihabuddin *et al.*, 2025). Improved nutrition, including mineral supplementation, may accelerate recovery in cattle post-FMD (Sutaryono *et al.*, 2025).

Concentrates rich in protein, crude fat, and fiber enhance nutritional intake; however, most smallholder farmers provide insufficient concentrates and minerals, leading to suboptimal reproductive performance (Agus and Mastuti Widi, 2018). Minerals, both macro and trace minerals, play a crucial role in regulating livestock reproduction and production. Deficiencies can lead to reproductive disorders. Trace elements support key metabolic and physiological functions, and supplementation with Cu, Fe, Mg, Na, P, Mn, Zn, K, Ca, S, Se, and Co has been shown to improve reproductive performance and immune status in cattle (Anam *et al.*, 2021, 2022).

Inadequate feed intake suppresses weight gain, productivity, and reproductive function (Anam *et al.*, 2022). High-quality feed enhances estrus indicators such as ferning, vulvar swelling, vaginal temperature, cervical mucus

pH and ferning score, mucosal color, and mucus quantity (Ferdiansyah *et al.*, 2021). Estrus expression is influenced by breed, age, and physiological condition, and cervical mucus characteristics (appearance, consistency, elasticity, ferning pattern, and pH) may be altered in repeat breeders (Hayati *et al.*, 2021). Mineral supplementation has been associated with improved pregnancy rates in repeat-breeding animals (Molefe and Mwanza, 2020). Mineral mixtures have also been shown to enhance growth, feed efficiency, milk production, reproductive performance, immunity, and reduce calving intervals (Anam *et al.*, 2022).

To date, no reports have assessed the use of mineral mixtures to restore reproductive function in cattle after FMD infection. Therefore, this study aimed to determine the effects of mineral mix or concentrate booster supplementation on estrus quality, pregnancy, and calving outcomes in cows following FMD infection.

## MATERIALS AND METHODS

This study was conducted in Kebonsari District, Madiun Regency, East Java, Indonesia. Cattle were diagnosed with FMD in May 2022, and the study was conducted from October 2022 to November 2023. This study protocol has been approved by the Study Ethics Approval Committee of Airlangga University, Certificate Number: 2.KEH.135.10.2022.

Eighteen post-FMD cows were administered 10 mL of vitamin A-D-E and 20 mL of long-acting antibiotics intramuscularly, and then randomly allocated into three treatment groups for a 42-day feeding trial. Group T0 received standard feed (elephant grass at 10% of body weight and commercial concentrate containing 20-22% crude protein at 1% of body weight). Group T1 received standard feed plus 40 g/day of a mineral mix, and Group T2 received standard feed plus 600 g/day of a concentrate booster. Feed was provided twice daily at 08:00 and 16:00, and water was provided *ad libitum*. The concentrate booster was

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dissolved in 450 mL of water and administered in the morning, while the mineral mix was diluted in 600 mL of water and given in the afternoon. The nutrient content of elephant grass and the concentrate booster is presented in Table 1, and the mineral composition of the mineral mix is shown in Table 2.

**Table 1** Composition (%) of elephant grass and concentrate booster

| nutrient content | elephant grass | concentrate booster |
|------------------|----------------|---------------------|
| ash              | 2.08 ± 1.61    | 10.80               |
| crude fat        | 0.69 ± 0.14    | 3.37                |
| crude fiber      | 32.60 ± 4.97   | 20.92               |
| crude protein    | 6.26 ± 0.58    | 18.03               |
| dry matter       | 20.29 ± 3.24   | 61.94               |

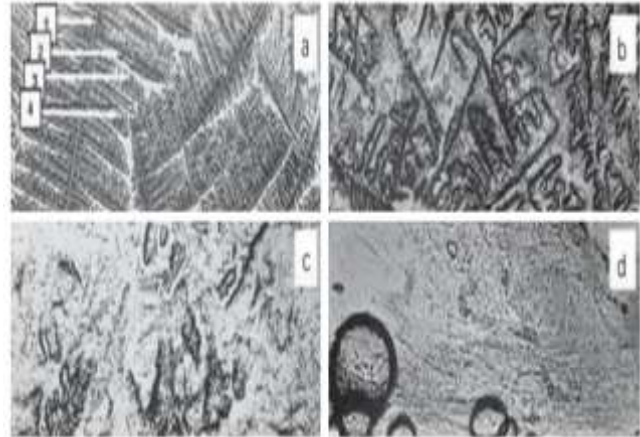
**Table 2** Composition of 100 g mineral mix

| minerals       | amount |
|----------------|--------|
| Calcium (Ca)   | 50 g   |
| Cobalt (Co)    | 3 mg   |
| Copper (Cu)    | 4 mg   |
| Iodine (I)     | 0.3 mg |
| Iron (Fe)      | 11 g   |
| Potassium (K)  | 2.4 g  |
| Magnesium (Mg) | 6 g    |
| Manganese (Mn) | 5.4 mg |
| Phosphorus (P) | 17 g   |
| Selenium (Se)  | 2 mg   |
| Sodium (Na)    | 3.6 g  |
| Sulfur (S)     | 0.7 g  |

Observations were conducted twice daily, alongside routine pen cleaning, feeding, and milking. When cows exhibited estrus, cervical mucus discharged from the vulva was collected in sterile containers and labeled. Cervical mucus samples were evaluated for pH, consistency, and fern pattern formation. The pH was measured using a digital pH meter (Sartorius PB-10). The mucus samples were homogenized and placed onto previously cleaned, flattened, and air-dried glass slides. The slides were then examined under a light microscope (Nikon E100) at 100× and 400× magnification.

Cervical mucus was scored using a fern

pattern formation scoring system based on the complexity of the fern-like crystalline pattern and the density of its coverage (Figure 1). The scoring criteria included the absence of crystallization, atypical fern formation, primary and secondary fern formation, and tertiary and quaternary fern formation (scores 0-3) (Atrio *et al.*, 2015; Najmabadi *et al.*, 2021; Ferdiansyah *et al.*, 2022).



**Figure 1** Microscopic views of cervical mucus fern pattern formation; (a) ferning score 3: complete ferning, showing primary [1], secondary [2], tertiary [3], and quaternary branches [4]; (b) ferning score 2: predominance of primary and secondary branches, with some tertiary branching; (c) ferning score 1: incomplete or abnormal crystallization; (d) ferning score 0: no crystallization (air bubble formation); (Bernardi *et al.*, 2016).

Artificial insemination was performed by a certified inseminator 6-12 hours after estrus detection. Pregnancy diagnosis was conducted by a veterinarian via rectal palpation two months after insemination. Pregnant cows were monitored until calving to determine calf sex.

### Data analysis

The onset of estrus (interval from last estrus to treatment, and treatment to estrus) was analyzed using Analysis of Variance (ANOVA) followed by Duncan's multiple range test. Ferning score data were analyzed using the Kruskal-Wallis test, with Mann-Whitney tests

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applied when significant differences were detected. Pregnancy and calf sex data were analyzed using Chi-square tests. All statistical analyses were performed at a 95% confidence level using SPSS version 20 for Windows.

## RESULTS

Cows in groups T1 and T2 had a significantly higher percentage of estrus ( $p < 0.05$ ) compared with group T0. The onset of

estrus (21-30 days after treatment) was significantly shorter ( $p < 0.05$ ) than the prolonged anestrus period experienced before treatment (6-8 months in all groups). Only one cow in T0 exhibited estrus; therefore, estrus-related variables could not be statistically compared with T1 and T2. No significant differences ( $p > 0.05$ ) were observed between T1 and T2 in the duration of anestrus before treatment or the interval from treatment to estrus (Table 3).

**Table 3** Onset of estrus (days) in cows post-FMD infection and after treatment (administered mineral mix or concentrate booster)

|    | estrus rate               | last estrus to treatment     | treatment to estrus         |
|----|---------------------------|------------------------------|-----------------------------|
| T0 | 16.67% (1/6) <sup>a</sup> | 180.33 ± 35.85               | 23                          |
| T1 | 100% (6/6) <sup>b</sup>   | 175.67 ± 45.02 <sup>Ba</sup> | 30.00 ± 7.95 <sup>Aa</sup>  |
| T2 | 83.33% (5/6) <sup>b</sup> | 178.00 ± 20.07 <sup>Ba</sup> | 21.33 ± 10.65 <sup>Aa</sup> |

T0: standard feed; T1: standard feed + 40 g/day mineral mix; T2: standard feed + 600 g/day concentrate booster; different lowercase superscripts within a column and uppercase superscripts within a row indicate significant differences ( $p < 0.05$ )

Cows in T0 had lower cervical mucus pH and ferning scores compared with T1 and T2. However, because only one cow in T0 exhibited estrus, these data could not be statistically

compared with the other treatment groups. No significant differences ( $p > 0.05$ ) in mucus pH or ferning score were found between T1 and T2 (Table 4).

**Table 4** Estrus rate, cervical mucus characteristics, pregnancy rate, and calving rate

|    | cervical mucus           |                          | pregnancy rate            | calving rate              |
|----|--------------------------|--------------------------|---------------------------|---------------------------|
|    | pH                       | ferning score            |                           |                           |
| T0 | 6.90 ± 0.00 <sup>*</sup> | 1.00 ± 0.00 <sup>*</sup> | 0% (0/6) <sup>a</sup>     | 0% (0/6) <sup>a</sup>     |
| T1 | 7.70 ± 0.29 <sup>a</sup> | 2.40 ± 0.89 <sup>a</sup> | 83.33% (5/6) <sup>b</sup> | 83.33% (5/6) <sup>b</sup> |
| T2 | 7.92 ± 0.55 <sup>a</sup> | 1.87 ± 1.17 <sup>a</sup> | 50% (3/6) <sup>b</sup>    | 50% (3/6) <sup>b</sup>    |

T0: standard feed; T1: standard feed + 40 g/day mineral mix; T2: standard feed + 600 g/day concentrate booster; different superscript within a column indicates significant difference ( $p < 0.0$ ); \*: only one estrus cow in T0.

There were no significant differences ( $p > 0.05$ ) in cervical mucus pH or ferning score between pregnant and non-pregnant cows (Table 5). Similarly, there were no significant

differences ( $p > 0.05$ ) in these parameters between cows giving birth to male or female calves (Table 6).

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**Table 5** Cervical mucus characteristics based on pregnancy status

|                      | pH          | ferning score |
|----------------------|-------------|---------------|
| pregnant (n= 8)      | 7.75 ± 0.35 | 2.50 ± 0.76   |
| non-pregnant (n= 10) | 7.73 ± 0.79 | 1.00 ± 0.82   |

**Table 6** Cervical mucus characteristics based on calf sex

| calf sex      | pH          | ferning score |
|---------------|-------------|---------------|
| female (n= 2) | 7.75 ± 0.40 | 2.33 ± 0.82   |
| male (n= 6)   | 7.75 ± 0.21 | 3.00 ± 0.00   |

## DISCUSSION

Foot-and-mouth disease is a highly contagious epizootic disease that causes considerable losses for livestock farmers due to its high morbidity. Vesicles and bullae develop rapidly on the muzzle, nostrils, lips, and oral cavity, reducing feed intake and leading to a marked decline in nutrient availability (Underwood *et al.*, 2015). In cows, FMD can cause delayed estrus, miscarriage, and infertility. Nutritional deficiencies greatly affect the reproductive system, and inadequate intake of carbohydrates, lipids, proteins, minerals, and vitamins negatively impacts physiological well-being and reproductive capacity (Asín *et al.*, 2021). Minerals, in particular, are essential for growth, health, reproduction, and immunity in cows (Velladurai *et al.*, 2016).

### Estrus rate

In this study, cows supplemented with concentrate or mineral mix after FMD infection exhibited a higher estrus rate than cows receiving standard feed alone. Prior to treatment, cows had remained in anestrus for 6-8 months, but provision of concentrate or mineral mix restored estrus within 20-30 days. Foot-and-mouth disease significantly affects the health and productivity of cows, including their reproductive performance. Dietary nutrient content, such as crude fat, crude fiber, crude protein, and dry matter, plays a crucial role in

recovery and in the re-establishment of normal estrus cycles following FMD infection. Crude protein is essential for maintaining body condition and supporting immune function. Post-FMD, cows often experience reduced serum protein levels, which can impair overall health and reproductive efficiency (Sutaryono *et al.*, 2025). Supplementation with higher crude protein levels can improve body weight recovery and shorten the interval to first estrus and days open (Son *et al.*, 2024). Protein-rich diets also contribute to restoring body condition scores, which are critical for the resumption of normal estrous activity (Sutaryono *et al.*, 2025).

Crude fat serves as a concentrated energy source, which is essential for cows recovering from the energy deficit associated with FMD. Diets containing adequate fat can help restore energy balance and improve milk yield, thereby indirectly supporting reproductive performance by ensuring that cows have sufficient energy reserves for estrus and conception (Moallem *et al.*, 2020). However, excessive dietary fat may lead to metabolic disturbances, making proper balance critical (Zhang *et al.*, 2025). Crude fiber is crucial for optimal rumen function and overall digestive health. High-fiber diets help prevent excessive loss of body condition and support stable energy levels, both of which are necessary for maintaining regular estrous cycles. Fiber also contributes to a healthy gut microbiome, which can influence overall health and reproductive efficiency (Zhang *et al.*, 2025; Little *et al.*, 2016). Overall dry matter intake (DMI) is vital for meeting the nutritional requirements of cows, particularly in the post-FMD period when appetite may be reduced. Ensuring adequate DMI through palatable and nutrient-dense feeds can facilitate faster recovery and a return to normal feeding behavior, which is essential for the resumption of regular estrus cycles (Somagond *et*

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*al.*, 2023). Diets that enhance DMI have been shown to promote body weight recovery and support reproductive health (Somagond *et al.*, 2024).

Foot-and-mouth disease infection leads to significant biochemical alterations in cows, including reduced levels of essential minerals such as magnesium and iron, as well as vitamins such as vitamin C and D3 (Yörük *et al.*, 2014). These deficiencies impair immune function and overall health, both of which are critical for reproductive performance (Van Emon *et al.*, 2020). Minerals are essential for muscle function, nerve signaling, and metabolic regulation; therefore, deficiencies can result in metabolic disorders and reduced reproductive efficiency (Molefe and Mwanza, 2023). Magnesium (Mg) plays an important role in enzyme activity and energy metabolism, and its deficiency can cause immunosuppression and disrupt estrus cycles (Libera *et al.*, 2021). Copper (Cu) and zinc (Zn) are crucial for immune function and antioxidant defense, and deficiencies in these trace elements may lead to oxidative stress and impaired reproductive health (Nazari *et al.*, 2019). Phosphorus (P), which is vital for energy metabolism and skeletal integrity, is also essential for optimal reproductive outcomes (Wächter *et al.*, 2022).

Supplementation with minerals such as Cu, Zn, Mg, and P has been shown to enhance reproductive performance in cows, including improved estrus synchronization and follicular development, both key factors for successful breeding post-FMD infection (Susilowati *et al.*, 2025; Sharma *et al.*, 2020). Minerals such as selenium and zinc further strengthen the immune system, reduce the incidence of secondary infections, and improve overall health, thereby indirectly supporting reproductive function (Libera *et al.*, 2021; Nazari *et al.*, 2019). Mineral supplementation also contributes to normalization of hematological parameters, including increases in hemoglobin and packed cell volume, indicating improved tissue oxygenation and systemic health—conditions essential for restoring the estrus cycle (Yarmots *et al.*, 2023).

Micronutrients such as copper and zinc serve as active centers of antioxidant enzymes, reducing oxidative stress and supporting cellular integrity (Nazari *et al.*, 2019). Minerals also act as cofactors for key enzymes involved in metabolism, neuromuscular function, and endocrine regulation, all of which are necessary for maintaining normal estrous activity (Molefe and Mwanza, 2020). Adequate mineral levels help maintain a robust immune system, preventing immunosuppression and secondary infections that can disrupt the estrus cycle (Libera *et al.*, 2021). Mineral supplementation post-FMD infection is crucial for restoring and maintaining the estrus cycle in cows. By correcting deficiencies in essential minerals such as calcium, magnesium, copper, and zinc, supplementation after FMD infection supports immune competence, reduces oxidative stress, and improves overall reproductive performance (Sutaryono *et al.*, 2025). This integrated approach promotes effective recovery from FMD and helps ensure the re-establishment of normal reproductive function in cows.

### pH and ferning score of cervical mucus

In this study, post-FMD cows receiving additional mineral mix or concentrate booster exhibited higher cervical mucus pH and ferning scores than cows on standard feed alone. Cervical mucus pH influences spermatozoa motility, viability, and transport. A neutral to slightly alkaline pH creates an optimal environment for spermatozoa survival and fertilization (Bernardi *et al.*, 2016).

The pH of cervical mucus affects its viscoelastic properties, which are crucial for spermatozoa passage through the reproductive tract. Optimal consistency, typically observed at neutral pH, facilitates spermatozoa transport. Mucins, the structural components of cervical mucus, undergo pH-dependent conformational changes that alter the gel-like structure of the mucus (Huang *et al.*, 2024). Spermatozoa motility is highest at a neutral to slightly alkaline pH (approximately 7.2-8.2), whereas acidic conditions (pH 5.2-6.2) markedly reduce motility and viability (Ferdiansyah *et al.*, 2022).

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Enzymes essential for spermatozoa motility, such as Na<sup>+</sup>/K<sup>+</sup>-ATPase, are also sensitive to pH; reduced activity under acidic conditions further impairs spermatozoa movement and capacitation (Zhou *et al.*, 2015).

Cows with cervical mucus pH values of 7-8 generally have higher pregnancy rates than those with pH 6 or 9, indicating that neutral to slightly alkaline conditions are favorable for fertilization (Siregar *et al.*, 2019). Spermatozoa penetration is significantly enhanced when cervical mucus is at an optimal pH, corresponding to the estrus phase, when mucus becomes more conducive to spermatozoa transport (Huang *et al.*, 2024). The pH and composition of cervical mucus are regulated by steroid hormones, particularly estrogen and progesterone, which modulate mucus secretion and fertility potential (Bernardi *et al.*, 2016). The presence of polymorphonuclear leukocytes (PMNs), indicative of inflammation, is associated with reduced fertility. Elevated PMN levels can alter mucus characteristics, including pH, thereby creating a less favorable environment for spermatozoa (Oropeza *et al.*, 2024).

The ferning pattern of cervical mucus in cows is an important indicator of fertility. This crystallization pattern, resembling fern leaves, is influenced by hormonal fluctuations and can be used to predict optimal insemination times and pregnancy outcomes. The ferning pattern is closely associated with estrogen and progesterone levels; high estrogen and low progesterone concentrations produce a more pronounced ferning pattern, reflecting the fertile phase of the estrous cycle (Bernardi *et al.*, 2016). During estrus, the cervical mucus becomes copious, clear, and watery, similar to egg white, creating a favorable environment for spermatozoa motility and survival (Khanoria *et al.*, 2022).

The degree of crystallization varies throughout the estrous cycle. During estrus, higher arborization (ferning) is typically observed and correlates with improved fertility rates (Bernardi *et al.*, 2016). The presence of well-defined fern-like structures is positively associated with successful conception (Khanoria

*et al.*, 2022). Physical properties of cervical mucus, including spinnbarkeit (stretchability), pH, and viscosity, also play important roles in fertility. Higher spinnbarkeit and optimal pH levels (approximately 7-8) are linked to increased pregnancy rates, as thin and abundant mucus supports effective spermatozoa transport (Siregar *et al.*, 2019). The ferning pattern may also serve as a non-invasive tool for early pregnancy detection. Pregnant cows often display atypical fern structures accompanied by rosette formations and needle-like projections, changes attributed to shifts in hormonal profiles post-insemination. These patterns may be observed in both cervical mucus and saliva (Chavan *et al.*, 2023).

### Pregnancy rate and calf sex

In this study, cervical mucus pH values and ferning scores were similar between pregnant and non-pregnant cows, as well as between cows that produced male or female calves. Although cervical mucus pH influences pregnancy rates, with values of 7-8 being more conducive to conception than pH 6 or 9, it does not appear to directly correlate with offspring sex (Bernardi *et al.*, 2016). The relationship between cervical mucus and calf sex determination is complex and involves selective permeability of spermatozoa. Mucus from cows that consistently produce offspring of a single sex shows selectivity toward either X- or Y-bearing spermatozoa (Huang *et al.*, 2024b). However, pH differences among cows producing predominantly male calves, predominantly female calves, or alternating sexes were not significant, suggesting that pH is not the primary factor driving this selectivity.

Cervical mucus characteristics, such as viscosity, crystallization, and stretchable, are strongly influenced by hormonal activity and can affect spermatozoa permeability. During estrus, the mucus becomes thinner and more abundant, facilitating spermatozoa penetration (Huang *et al.*, 2024a). Elevated estrogen and reduced progesterone levels create conditions favorable for spermatozoa passage, and these hormonal effects may indirectly influence the

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ability of X- or Y-bearing spermatozoa to navigate the cervical environment (Bernardi *et al.*, 2016).

The reproductive tract mucus of cows that consistently produce offspring of a particular sex exhibits selective permeability toward either X- or Y-bearing spermatozoa (Huang *et al.*, 2024). Ferdiansyah *et al.* (2022) reported, although based on a very limited sample size, that acidic cervical mucus tended to reduce the likelihood of male offspring, and vice versa. Meles *et al.* (2022) also found that artificial insemination performed 18-20 hours after estrus using sexed semen resulted in a higher proportion of male calves. However, in vitro studies indicate that spermatozoa pH alone does not determine the sex of bovine offspring (Raval *et al.*, 2019). In this study, artificial insemination was performed approximately 12 hours after the onset of estrus signs, without any intervention to alter sex ratios. Under normal conditions, the likelihood of male versus female calf birth remains approximately 50:50 (López-Gatius, 2022).

## CONCLUSIONS

This study assessed the effects of supplementing mineral mix or concentrate booster on the reproductive performance of cows recovering from FMD infection. Both supplements had a positive impact on fertility parameters, including estrus rates, pregnancy rates, and calving rates. Moreover, cervical mucus pH and ferning scores did not differ significantly among treatments, nor were they associated with pregnancy outcomes or calf sex. These findings suggest that mineral mix or concentrate booster supplementation can serve as a practical approach to help restore fertility in cows following FMD infection.

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## AUTHOR CONTRIBUTIONS

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APN, AA, MSA conceived the study; APN, SS, DAP designed the manuscript framework; APN acquired, analyzed, and interpreted data and drafted the manuscript; MAA and HAH critically reviewed and revised the manuscript. All authors approved the final version.

## CONFLICTS OF INTEREST

The authors declare no competing interests.

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