

Zinc, calcium, and magnesium levels in seminal plasma as predictive markers of sperm quality in Bali bulls (*Bos javanicus domesticus*)

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ABSTRACT

Seminal plasma contains essential minerals that influence sperm performance and could act as reliable indicators of reproductive potential in bulls. Despite their importance, the role of these minerals in determining sperm quality has been rarely examined in indigenous breeds, such as Bali cattle (*Bos javanicus*), particularly across different phenotypes. This research aimed to evaluate the concentrations of magnesium (Mg), zinc (Zn), and calcium (Ca) in the seminal plasma of Bali bulls, and investigate how these minerals relate to key semen quality parameters. Fresh semen was collected from three apparently healthy horned and three polled Bali bulls. Semen quality parameters, including motility, viability, morphology, and membrane integrity, were assessed following standard protocols. Seminal plasma was isolated, and mineral concentrations were determined using atomic absorption spectrophotometry (AAS). Statistical evaluation involved independent t-tests, Pearson correlation, and linear regression. The results revealed that horned Bali bulls had significantly higher sperm concentration ($P < 0.01$), while polled bulls showed a lower percentage of abnormal spermatozoa ($P < 0.05$). Seminal plasma of polled bulls contained higher zinc (2.55 ± 0.37 ppm) and calcium ($0.11 \pm 0.02\%$) levels, whereas horned bulls had higher magnesium concentrations (57.93 ± 14.22 ppm). Magnesium showed a highly significant association with increased sperm concentration ($r = 0.79$; $B = 14.78$; $R^2 = 0.864$; $p < 0.001$), showing it to be the most influential mineral factor. These findings support the use of zinc, calcium, and magnesium as predictive biomarkers for evaluating bull fertility and selection in Bali cattle breeding programs.

Introduction

Reproductive efficiency is central to the success of livestock production, especially in bovine breeding systems where fertility directly affects genetic improvement, herd productivity, and economic sustainability. Among the critical determinants of male fertility in bulls, semen quality stands as a vital indicator, encompassing parameters such as sperm concentration, motility, morphology, viability, and membrane integrity (Hallap *et al.*, 2006; Jahan *et al.*, 2016; Morrell *et al.*, 2017). In recent years, studies have drawn attention to the role of seminal plasma—the fluid component of semen, excluding spermatozoa—as a key modulator of sperm function, primarily through its biochemical composition, which includes minerals, enzymes, proteins, and hormones (Asadpour, 2012; Xu *et al.*, 2021; Mappanganro *et al.*, 2025b).

Seminal plasma not only acts as a transport medium for sperm cells but also creates a biochemical environment crucial for sperm metabolism, capacitation, and protection against oxidative stress. Among its many components, minerals such as magnesium (Mg), calcium (Ca), and zinc (Zn) (Sorensen, 1999), have been shown to affect sperm quality significantly (Gür and Demirci, 2000; Eghbali *et al.*, 2010; Elango *et al.*, 2020; Khaki *et al.*, 2021). These elements serve a variety of functions, including maintaining osmotic balance, stabilizing cell membranes, regulating enzymatic activity, and oxidative defense mechanisms (Wong *et al.*, 2001; Horvat *et al.*, 2009). Zinc, for example, is widely recognized for its role in enhancing sperm motility, membrane integrity, and antioxidant defense. It is involved in the stabilization of cell membranes and chromatin structures and participates in numerous enzymatic reactions crucial for spermatogenesis and sperm maturation (Aydemir *et al.*, 2006; Alavi-Shoushtari *et al.*, 2009). Calcium plays a pivotal role in sperm capacitation and acrosomal reaction, both of which are essential for successful fertilization (Liang *et al.*, 2016; Sachan *et al.*, 2025). Magnesium, in turn, is required for ATPase activity and mitochondrial function, thereby influencing the motility and viability of sperm (Wong *et al.*, 2001; Liang *et al.*, 2016).

Despite the recognized importance of seminal plasma minerals in bull fertility, relatively few studies have examined their specific relationships with sperm quality traits across distinct cattle phenotypes, particularly in indigenous breeds such as Bali cattle (*Bos javanicus*). Bali cattle are a genetically and economically significant breed in Indonesia, known for their adaptability, high carcass yield, and unique morphology, including the presence or absence of horns. The horned and polled phenotypes of Bali cattle may reflect underlying genetic and physiological differences, including reproductive traits. Yet, these differences remain underexplored in the context of seminal plasma composition and sperm performance. Emerging evidence suggests that the mineral profile of seminal plasma may differ significantly among breeds, ages, and even individual animals, potentially reflecting genetic variability, environmental influences, and management practices (Nongbua, 2017; Baco *et al.*, 2020).

In light of this, the purpose of this study was to investigate the potential of seminal plasma mineral concentrations as indicators of sperm quality in horned and polled Bali bulls. By analyzing the levels of key minerals and correlating them with sperm motility, viability, and morphology, this research aims to identify reliable biomarkers for evaluating bull fertility and provide a scientific basis for selection criteria in Bali cattle breeding programs. Furthermore, this research contributes to the broader understanding of how biochemical parameters in semen influence reproductive performance across livestock species. It also underscores the importance of integrating biochemical, physiological, and genetic approaches in animal reproductive science, particularly in indigenous breeds that have received limited scientific attention.

Materials and methods

Animals and experimental design

This study was conducted on a total of six apparently healthy, sexually mature Bali bulls (*Bos javanicus*) maintained at the Artificial Insemination Technical Implementation Unit, Animal Husbandry and Animal

Health Service of South Sulawesi Province, Indonesia. The bulls were classified into two groups based on their phenotype: horned ($n=3$) and polled ($n=3$). All animals were kept under similar management, feeding, and environmental conditions throughout the study. All animal-related procedures adhered to established ethical guidelines and received approval from the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University, Bali (Number: B/143/UN14.2.9/PT.01.04/2024).

Semen collection and evaluation

Fresh semen was obtained from each bull using an artificial vagina once per week for seven consecutive weeks to minimize ejaculatory variation. Immediately after collection, the samples were transported to the laboratory in a water bath at 37 °C for analysis. The semen characteristics assessed included volume, sperm concentration, motility, morphology, intact membrane, and acrosome. These data were previously reported in our earlier study, which compared horned and polled Bali bulls (Mappanganro *et al.*, 2025a). In the present study, these semen quality parameters were re-utilized for correlation with seminal plasma minerals.

Seminal plasma collection and mineral analysis

Fresh semen from both types of bulls was centrifuged at $800 \times g$ for 15 minutes at 4°C. The resulting supernatant was transferred to a new tube and further centrifuged at $10,000 \times g$ for 30 minutes at 4°C (Fu *et al.*, 2019). Following the second centrifugation, the supernatant was carefully pipetted into a cryotube, sealed with Parafilm M (Sigma-Aldrich, Darmstadt, Germany), and then frozen in liquid nitrogen. The cryotube was stored at -80°C for future analysis (Viana *et al.*, 2018). The concentrations of the following minerals in the seminal plasma were measured: calcium (Ca), zinc (Zn), and magnesium (Mg). Seminal plasma samples were analyzed using Atomic Absorption Spectrophotometry (AAS), following established procedures recommended by the Association of Official Analytical Chemists (AOAC, 2005). A 10 mL seminal plasma sample was transferred into a digestion block tube, followed by the addition of 0.5 mL distilled water to prevent splashing and facilitate reaction with the acid. The sample was digested with 10 mL concentrated nitric acid (HNO_3) at ~100°C for 2 h, cooled for 15 min, and subsequently mixed with 0.5 mL perchloric acid (HClO_4). The mixture was reheated in the digestion block for 1 h before further analysis.

The digested solution was then rinsed with distilled water up to the calibration line and mixed thoroughly until homogeneous, making it ready for mineral determination. Next, 1 mL of the prepared solution was pipetted into a 50 mL volumetric flask, followed by the addition of 3 mL of ammonium molybdate solution and 2.5 mL of ascorbic acid solution. Distilled water was added to the flask until the calibration line was reached, and the solution was mixed thoroughly until it became homogeneous. After standing for 30 minutes, the solution was transferred into a cuvette and placed in a spectrophotometer set to a wavelength of 570 nm. The spectrophotometer reading was then recorded. A standard curve for the mineral was also prepared.

Data analysis

Differences in semen quality parameters and seminal plasma mineral concentrations between horned and polled Bali bulls were analyzed using an independent samples t-test. A significance level of $P < 0.05$ was considered statistically significant. Variables, including sperm motility, abnormality, viability, membrane integrity, and intact acrosome, were analyzed using Pearson's correlation coefficient to examine their relationship with seminal plasma concentrations of zinc (Zn), calcium (Ca), and magnesium (Mg). In contrast, the percentage of abnormal spermatozoa was not normally distributed ($P < 0.05$). Subsequently, multiple linear regression analysis was performed to assess the combined and individual

effects of Zn, Ca, and Mg on each sperm quality trait. Statistical significance was considered at $P < 0.05$. Correlation coefficients (r -values) were interpreted according to conventional criteria: weak (0.10–0.30), moderate (0.31–0.70), and strong (>0.71 –1.00). Data were analyzed using SPSS version 27.0.

Results

Semen quality of horned and polled Bali bulls

Table 1 presents a comparison of semen quality parameters between horned and polled Bali bulls. Horned bulls exhibited significantly higher semen concentration than polled bulls ($P < 0.01$, t-test). In contrast, polled bulls showed a notably lower percentage of abnormal spermatozoa ($P < 0.05$). There was no statistically significant difference between the two groups in sperm motility, viability, membrane integrity, or the percentage of intact acrosomes ($P > 0.05$).

Table 1. Summary of semen quality traits in horned and polled Bali bulls.

Parameter	Horned bulls (42 ejaculates)	Polled bulls (42 ejaculates)	sig.
Concentration ($\times 10^6$ cells/mL)	1,053.38 $\pm$ 195.38	667.29 $\pm$ 300.70	**
Motility (%)	77.62 $\pm$ 4.36	77.14 $\pm$ 4.35	ns ¹
Viability (%)	88.23 $\pm$ 7.62	87.35 $\pm$ 7.09	ns
Abnormality (%)	8.96 $\pm$ 7.19	4.96 $\pm$ 1.68	*
Membrane integrity (%)	81.13 $\pm$ 3.02	83.00 $\pm$ 4.79	ns
Intact acrosome (%)	94.80 $\pm$ 3.37	95.85 $\pm$ 3.00	ns

Ins= no significant; *= significant at level $P < 0.05$; **= significant at level $P < 0.01$

Zinc, calcium, and magnesium concentration

The concentrations of Zn, Ca, and Mg in the seminal plasma of Bali bulls differed between horned and polled phenotypes. As shown in Table 2, polled bulls had higher levels of Zn (2.55 ± 0.37 ppm) and Ca ($0.11 \pm 0.02\%$) compared to horned bulls, which showed Zn and Ca concentrations of 1.55 ± 0.30 ppm and $0.09 \pm 0.02\%$, respectively. Conversely, the concentration of Mg was markedly higher in horned bulls (57.93 ± 14.22 ppm) than in polled bulls (22.83 ± 11.20 ppm). These differences indicate phenotypic variations in the mineral composition of seminal plasma that may influence sperm quality.

Table 2. Concentration of Zn, Ca, and Mg in the seminal plasma of Bali bulls (Mean and SD)

Mineral	Bali Bull				Sig.
	Horned	SD	Polled	SD	
Zn (ppm)	1.55	0.3	2.55	0.37	**
Ca (%)	0.09	0.02	0.11	0.02	*
Mg (ppm)	57.93	14.22	22.83	11.2	**

Ins= no significant; *= significant at level $P < 0.05$; **= significant at level $P < 0.01$

Correlation between mineral levels and sperm quality traits

Heatmap illustration of Pearson correlation coefficient between seminal plasma minerals (Zn, Ca, and Mg) with sperm quality parameters in horned and polled Bali bull is presented in Figure 1. In horned bulls, Mg exhibited strong positive correlations with sperm concentration ($r = 0.79$, $p < 0.05$), motility ($r = -0.96$, $p < 0.01$), viability ($r = -0.98$, $p < 0.01$), and membrane integrity ($r = -1.00$, $p < 0.01$), but a strong negative correlation with abnormal morphology ($r = 0.94$, $p < 0.01$). Similarly, Zn and Ca showed significant associations with several sperm parameters. In polled bulls, Zn was highly correlated with concentration ($r = 0.98$, $p < 0.01$), motility ($r = 0.91$, $p < 0.01$), and intact acrosome ($r = 0.98$, $p < 0.01$), while Mg

displayed strong negative correlation with sperm abnormalities ($r = -0.80$, $p < 0.01$). These findings suggest that mineral concentrations in seminal plasma are closely linked to key sperm quality traits in Bali bulls, with notable differences between horned and polled phenotypes.

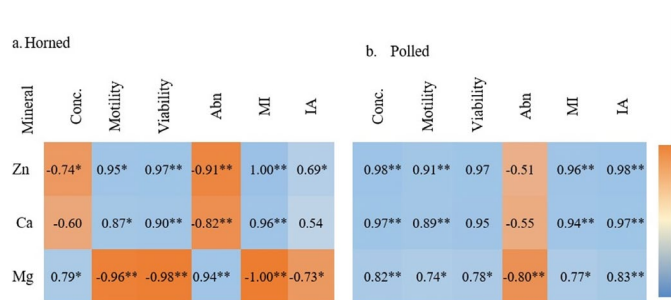


Fig 1. Heatmap of Pearson correlation coefficients between seminal plasma minerals and sperm quality parameters in Bali bulls. Positive correlations are shown in blue, where values closer to +1 indicate strong positive relationships. Negative correlations are shown in orange, where values closer to -1 indicate strong negative relationships. Darker shades reflect stronger correlations. Asterisks denote statistical significance: $P < 0.05$ (*), $P < 0.01$ (**).

Relationship between seminal plasma minerals (Zn, Ca, and Mg) and various sperm quality parameters

The results of multiple linear regression analyses revealed that among the seminal plasma minerals assessed (Zn, Ca, and Mg), only magnesium (Mg) exhibited a statistically significant relationship with sperm concentration in Bali bulls ($B = 14.78$, $p < 0.001$), with a high standardized coefficient ($\beta = 1.16$). This indicates a strong and positive contribution of Mg to sperm concentration. The model demonstrated excellent explanatory power, with an R^2 of 0.864 ($p < 0.001$), indicating that 86.4% of the

variation in sperm concentration could be attributed to the combined influence of zinc, Calcium, and magnesium (Table 3). The scatter plot in Figure 2 further supports this finding, illustrating a strong positive linear association ($y = -136.95 + 14.78x$; $R^2 = 0.86$) between Mg concentration and sperm concentration in seminal plasma.

For other sperm parameters, viability, motility, membrane integrity, and intact acrosome, the regression models did not show statistical significance ($p > 0.05$), and none of the predictors had individually significant effects. Although Mg showed relatively higher beta coefficients for motility ($\beta = 0.53$) and viability ($\beta = -0.70$), the relationships were not statistically significant ($p = 0.31$ and $p = 0.16$, respectively).

In contrast, the regression model predicting sperm abnormality from Zn, Ca, and Mg was statistically significant overall ($R^2 = 0.611$, $p = 0.047$), suggesting that these minerals collectively accounted for 61.1% of the variance in abnormal morphology. However, none of the individual predictors reached statistical significance ($p > 0.05$), likely due to overlapping contributions or limited statistical power from the small sample size. This may reflect multivariate effects rather than the independent influence of each predictor (Figure 2).

Variance inflation factor (VIF) values for all predictors were below 3, indicating no concerns about multicollinearity across the models. Collectively, these results highlight the significant role of Mg in regulating sperm concentration. In contrast, its effects on other sperm parameters and the combined influence of mineral profiles on abnormality warrant further exploration using larger sample sizes.

Discussion

This research examined how horn phenotype relates to semen quality in Bali bulls, along with the mineral composition of seminal plasma

Table 3. Interpretation of multiple linear regression between Zn, Ca, and Mg in seminal plasma on Bali bulls' sperm motility.

Predictor Variable	Unstandardized Coefficient (B)	Standard Error	Standardized Coefficient (Beta)	t-value	Significance (p)	VIF
Sperm Concentration						
Zn	104.24	86.33	0.27	1.21	0.26	2.86
Ca	19.55	39.05	0.1	0.5	0.63	2.35
Mg	14.78	2.5	1.16	5.91	< 0.001	2.26
Motility						
Zn	-0.08	2.07	-0.02	-0.04	0.97	2.86
Ca	83.59	93.78	0.44	0.89	0.4	2.35
Mg	0.07	0.06	0.53	1.08	0.31	2.26
Viability						
Zn	-1.55	4.38	-0.18	-0.35	0.73	2.86
Ca	-217.93	198.08	-0.51	-1.1	0.3	2.35
Mg	-1.2	0.77	-0.7	-1.55	0.16	2.26
Abnormality						
Zn	0.36	2.27	0.06	0.16	0.88	2.86
Ca	-108.58	102.65	-0.36	-1.06	0.32	2.35
Mg	0.11	0.07	0.55	1.66	0.14	2.26
Membrane integrity						
Zn	1.25	2.76	0.22	0.45	0.66	2.86
Ca	-55.9	124.88	-0.45	-0.45	0.67	2.35
Mg	-0.05	0.08	-0.28	-0.56	0.59	2.26
Intact acrosome						
Zn	-0.82	2.2	-0.2	-0.37	0.72	2.86
Ca	124.27	99.5	0.6	1.25	0.25	2.35
Mg	0.01	0.06	0.05	0.1	0.93	2.26

VIF (Variance Inflation Factor): assesses multicollinearity. VIF > 5 indicates potential multicollinearity issues.

and its possible impact on sperm traits. Results showed clear differences

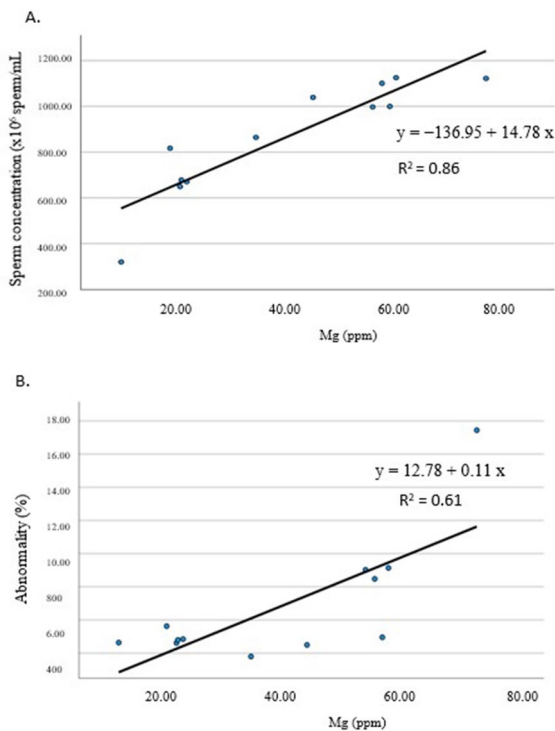


Fig. 2. Relationship between seminal plasma magnesium concentration (ppm) to (A) Sperm Concentration (B) Abnormality, in Bali bulls.

in both semen characteristics and mineral composition between horned and polled bulls, implying that horn phenotype may be linked to physiological factors influencing reproductive efficiency. This connection, which is likely influenced by multiple factors, highlights the need to account for phenotypic variation when planning breeding and reproductive management. A key outcome of the study was the variation in semen quality profiles between the two phenotypes: horned bulls had higher semen concentrations, while polled bulls displayed fewer abnormal sperm forms. These differences suggest that although horned bulls may yield more sperm, polled bulls may possess better overall sperm quality. This is an important consideration for breeding assessments, as a high sperm count alone does not guarantee improved fertility if it coincides with elevated morphological defects.

The variations in seminal plasma mineral composition observed between horned and polled bulls may help explain these differences in semen quality. Essential minerals such as zinc (Zn), calcium (Ca), and magnesium (Mg) are known to be crucial for the process of spermatogenesis, sperm motility, and cellular integrity (Liang *et al.*, 2016; Wong *et al.*, 2001). The elevated levels of certain minerals in one phenotype over another suggest a link between mineral metabolism and genetic or phenotypic traits. Such physiological variation may reflect underlying genetic regulation affecting both horn development and reproductive endocrinology, although further genetic analysis would be required to confirm this.

The mineral profile of polled bulls, which showed elevated levels of zinc and calcium, may offer insight into their lower rates of sperm abnormalities and higher integrity traits. Zinc has well-established roles in stabilizing sperm membranes, reducing oxidative stress, and supporting DNA integrity (Mogielnicka-Brzozowska *et al.*, 2022; Faggi *et al.*, 2024; Zečević *et al.*, 2025). Zinc functions as a membrane stabilizer by helping to maintain the structure of the phospholipid bilayer and membrane proteins (Baltaci *et al.*, 2017). In the presence of Zn, the sperm membrane becomes more resistant to mechanical and oxidative damage, thereby preserving its integrity (Wu *et al.*, 2015). Zinc also acts as a cofactor for antioxidant enzymes, particularly superoxide dismutase (SOD), which protects sperm cells from oxidative stress caused by free radicals (Badade *et al.*, 2011; Liu *et al.*, 2019). These radicals can trigger lipid peroxida-

tion, leading to membrane damage (Colagar *et al.*, 2009; Narasimhaiah *et al.*, 2018). Consequently, zinc helps preserve membrane fluidity and functionality, both of which are fundamental to achieving fertilization. Without sufficient fluidity, sperm cells are unable to penetrate the zona pellucida (Green, 1987; Drobnis *et al.*, 1988).

Calcium is essential for sperm motility and capacitation processes (Holm *et al.*, 2000; Srivastav *et al.*, 2018; Finkelstein *et al.*, 2020; Antonouli *et al.*, 2024). Intracellular calcium (Ca^{2+}) has a regulatory role in sperm motility, capacitation, and acrosome reaction (AR) (Akter *et al.*, 2017; Yang *et al.*, 2024). During capacitation, sperm undergo changes in membrane lipids, surface properties, fluidity, calcium ion permeability, and cholesterol content. The altered calcium permeability of the sperm membrane after capacitation serves as the primary trigger for the acrosome reaction (Marín-Briggiler *et al.*, 2003; Aleissa *et al.*, 2024). Calcium (Ca) is involved in the activation of cellular signaling pathways, such as protein kinase A (PKA), which is essential for the regulation of sperm flagellar activity. Calcium also mediates the release of enzymes during the acrosome reaction, a crucial step for successful fertilization (Girsh, 2021). The stronger correlations between these minerals and sperm quality traits in polled bulls suggest a synergistic role, wherein optimal mineral balance supports not only the structural but also the functional aspects of spermatozoa (Cardoso *et al.*, 2024). The differences in how mineral concentrations correlate with sperm quality between horned and polled bulls also point to potential phenotype-specific physiological responses. For example, the positive correlation between magnesium and sperm concentration in horned bulls was notably robust, while in polled bulls, zinc appeared more strongly associated with several sperm quality parameters. These contrasting patterns may reflect differences in mineral absorption, metabolism, or regulation of seminal plasma linked to the horn phenotype. It is conceivable that the horned and polled variants possess distinct reproductive endocrine profiles, possibly influenced by differential expression of genes involved in mineral transport or steroidogenesis (Elango *et al.*, 2020). Minerals such as Zn, Mg, and Ca play a direct role in sperm function and are regulated through various transporter genes and metabolic/steroidogenic regulators, the expression of which may vary according to race, phenotype, or nutritional status. TRPM7 (Mg transporter gene) expression correlates with metabolic and hormonal parameters, indicating involvement in metabolism and possibly fertility (Lee *et al.*, 2021). Zn supplementation in bulls affects Zn transporter expression and muscle growth metabolism, indicating a direct interaction between Zn homeostasis and genetic regulation in reproductive (Rients *et al.*, 2023).

Magnesium (Mg) exhibited a markedly opposite correlation pattern between groups, negatively associated with sperm quality in horned bulls but positively associated with polled bulls. The seminal plasma Mg concentration was 57.93 $\mu\text{g/mL}$ in horned bulls, compared to 22.83 $\mu\text{g/mL}$ in polled bulls. Magnesium is an essential ion that plays a key role in regulating enzymatic activity, maintaining cell membrane stability, and supporting sperm metabolism, particularly ATPase activity (Hartwig, 2001), which is critical for preserving sperm motility and membrane integrity (Vignini *et al.*, 2009). However, excessive Mg levels may disrupt ionic homeostasis within sperm cells. Mg regulates the activity of ATPases, including Na^+/K^+ -ATPase and $\text{Ca}^{2+}/\text{Mg}^{2+}$ -ATPase, which are vital for maintaining ion balance. Magnesium imbalance can interfere with sodium, potassium, and calcium transport, all of which are essential for sperm function (Okorodudu *et al.*, 2001). The Mg concentrations observed in this study were lower than those reported in previous studies: 11.94 $\pm$ 0.36 mg/dL in buffalo (Eghbali *et al.*, 2010) and 8-12 mg/dL in bulls (Cragle *et al.*, 1958). Magnesium emerged as a key element strongly associated with sperm concentration, as indicated by both correlation and regression analyses. Its significant positive contribution suggests that Mg plays a central role in enhancing spermatogenesis or sperm release. Magnesium serves as a vital cofactor for numerous enzymes, playing a role in cellular energy production and helping maintain membrane stability (Mirnamniha *et al.*, 2019; Chandra *et al.*, 2013). Its association with higher sperm concentra-

tion supports the hypothesis that adequate magnesium levels are necessary for optimal sperm production and testicular function.

Interestingly, the relationship between magnesium and other sperm quality traits-such as motility, viability, and membrane integrity-was less clear. Although correlation coefficients were sometimes strong, regression analyses did not support significant predictive value. This discrepancy may reflect complex biological interactions where magnesium influences certain sperm parameters under specific physiological or environmental conditions (Kasperczyk *et al.*, 2015; Valsa *et al.*, 2016). Additionally, the sample size may have limited the statistical power to detect these associations with higher confidence. It is also plausible that threshold effects exist, where only magnesium levels above or below a certain range exert significant functional impacts.

The regression findings further support the notion that magnesium exerts a unique and dominant influence on sperm concentration across phenotypes. The high proportion of explained variance in the regression model suggests that magnesium may serve as a reliable biochemical marker for sperm production capacity in Bali bulls. However, the lack of statistically significant individual effects of zinc and calcium, despite a significant overall model for sperm abnormality, points to possible multicollinearity or shared physiological pathways. This supports the view that mineral elements often work in concert rather than in isolation to influence reproductive outcomes.

From a practical perspective, these results highlight the importance of nutritional and physiological monitoring in bull fertility management. Mineral supplementation strategies tailored to the specific needs of horned and polled bulls may help optimize reproductive efficiency. For example, ensuring adequate magnesium levels in horned bulls could support higher sperm output, while managing zinc and calcium intake in polled bulls might help maintain sperm integrity. However, caution should be exercised, as excessive supplementation may disrupt the delicate mineral balance and have unintended negative effects.

Further research is warranted to clarify the causal relationships and underlying mechanisms linking phenotype, mineral metabolism, and semen quality. Larger sample sizes, combined with hormonal profiling, gene expression analysis, and detailed reproductive performance data, could help disentangle the multifactorial influences at play. Additionally, longitudinal studies monitoring mineral levels and semen parameters over time could provide insights into the dynamics of mineral influence across reproductive seasons or life stages.

Conclusion

This study confirms that zinc, calcium, and magnesium levels in seminal plasma are closely linked to sperm quality in Bali bulls (*Bos javanicus domesticus*). Zinc and calcium also correlated with several quality parameters, such as morphology and acrosome integrity, though with less predictive strength. Magnesium showed the strongest and most consistent association, particularly with sperm concentration, indicating its potential as a key predictor of bull fertility. However, excessive levels can disrupt the delicate mineral balance and have undesirable negative effects. Differences observed between horned and polled bulls suggest that phenotype may influence how these minerals affect fertility traits. Overall, seminal plasma mineral profiling, particularly magnesium, presents a promising, non-invasive tool for assessing and potentially enhancing reproductive performance in Bali bulls.

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Conflict of interest

The authors have no conflict of interest to declare.

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