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Reproductive Characteristics of Bali Polled Bull: Study on Libido, Semen Quality, and Sperm Kinematics

Wildayanti Wildayanti [1], Tulus Maulana [2], Muhammad Gunawan [2], Ekayanti Mulyawati Kaiin [2], Sri Gustina [3], Sudirman Baco [3], Herry Sonjaya [3] and Hasbi Hasbi [3,*]

[1]Postgraduate student of Animal Science and Technology Study Program, Faculty of Animal Science Hasanuddin University, South Sulawesi, Indonesia
[2]Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Bogor 16911, Indonesia
[3]Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, 90245, Indonesia
***Corresponding author:** hasbi_fapetunhas@yahoo.com

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ABSTRACT

This study aimed to identify the reproductive characteristics of Bali Polled bulls (libido, semen quality, and sperm kinematics) to enhance breeding and conservation strategies. Semen samples were from three Bali Polled Bulls and one Bali Horned bull. Libido was measured from the bull's approach to the teaser until ejaculation. Semen quality was assessed macroscopically and microscopically, while spermatozoa kinematics were analyzed using Computer-Assisted Sperm Analysis. The study found significant differences in libido, with the horned exhibiting a significantly ($P<0.01$) faster rate than Polled 3. Still, there was no significant difference between the Horned, Polled 1, and Polled 2. Polled 1 was significantly ($P<0.05$) faster than Polled 3. In fresh semen quality of Bali cattle bulls, Horned bulls exhibited a significantly ($P<0.01$) greater semen volume and sperm concentration ($P<0.05$) compared to Polled 3, with no significant differences between Polled 1 and Polled 2. Semen color, mass motility, consistency, and motility were similar across all bulls ($P>0.05$). In frozen semen, the Horned bull showed higher progressive motility ($P<0.05$) than Polled 3, with no significant differences between Polled 1 and 2. Polled 1 and 2 had fewer sperm abnormalities ($P<0.05$) than the Horned bull, but did not differ significantly ($P>0.05$) from Polled 3. Sperm kinematics in the Horned bull showed higher VAP and VSL compared to Polled 2 and higher BCF compared to Polled 2 and Polled 3 ($P<0.05$). In conclusion, Polled Bali bulls exhibit a longer duration of libido, greater variability in fresh semen quality, and lower abnormality rates in frozen semen.

Key words: Libido, Sperm Quality, Sperm Kinematics, Bali Polled Bulls

INTRODUCTION

The Bali Polled cattle, first identified on commercial farms in South Sulawesi in the early 1980s (Baco et al. 2020), are a hornless variant of Bali cattle with genetic characteristics similar to those of the Horned Bali cattle (Zulkharnaim et al. 2023). This breed exhibits a more docile temperament (Qayyum et al. 2020), larger morphometric dimensions (Zulkharnaim et al. 2020), a higher number of private alleles (Dagong et al. 2023) and taller body dimensions in male calves (Fesrianti et al. 2023). However, the size of female calves is almost identical to that of Horned Bali cattle females (Parmadi et al. 2023). Regarding management, Bali Polled cattle are easier to maintain, exhibit high meat productivity, and pose less risk of injury to livestock and workers (Hasbi et al. 2021). Additionally, they cause less damage to facilities and experience fewer carcass injuries during transport (Grobler et al. 2021).

The development of Bali Polled bulls, especially in South Sulawesi, is being prioritized due to their various advantages. Research has shown that Bali Polled bulls have superior sperm quality and sperm kinematics compared to other breeds (Diansyah et al. 2022). Furthermore, the viability of frozen semen from Bali Polled bulls is higher than that from Bali Horned bulls (Uswa et al. 2022). Hasbi et al. (2023) also noted is that the spermatozoa of Bali Polled bulls have superior freezing capability and tolerance, which are key factors in selecting breeding. Both Bali Horned and Bali Polled bulls exhibit similar fertility capabilities (Gustina et al. 2024).

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Reproductive traits, including libido, semen quality, and sperm motility, are crucial for successful breeding programs. The Indonesian National Standard (7651-4:2020) requires Bali bulls to have a strong libido and high semen quality (BSN, 2020). Semen quality is measured by volume, concentration, motility, membrane integrity, morphology, viability, and sperm kinematics (Maulana and Said 2022). This study focuses on these traits in Polled Bali bulls, particularly in relation to libido, semen quality, and sperm kinematics. The findings will help develop more effective breeding and conservation strategies, thereby enhancing productivity and genetic quality. This research also creates opportunities to improve the reproductive performance of Polled Bali bulls.

MATERIALS AND METHODS

Animals

This study utilized three Polled bulls and one Horned bull, aged 6 to 8 years, with an average body weight of approximately 480kg.

Libido assessment

Bull libido was evaluated based on the observation procedures outlined by García-Paloma et al. (2022), adapted from those described by Hoflack et al. (2006). This observation involved recording reaction times and collecting five ejaculates from each bull during each collection period.

Evaluation of semen quality and sperm kinematics

Evaluation of semen quality in fresh semen includes volume, color, mass motility, consistency, concentration, and motility. In contrast, frozen semen is assessed for motility, progressive motility, viability, abnormality, plasma membrane integrity, acrosome integrity and protamine deficiency using an Olympus CX23 microscope (Olympus, Tokyo, Japan). Sperm concentration is measured with a Photometer SDM 6 (Minitube, Germany), while acrosome integrity and protamine deficiency are examined using an Axio Imager Z2 microscope (Carl Zeiss, Germany). Sperm motility, progressive motility, and kinematics were evaluated using Computer-Assisted Sperm Analysis (CASA Sperm Vision™ 3.7, Minitube, Germany) connected to a Zeiss Axio Scope A1 microscope (Maulana et al. 2022). Semen evaluation was performed by placing 3µL of the semen sample onto a glass slide, covering it, and analyzing it at a magnification of 200x using Sperm Vision software (Maulana and Said, 2019).

The eosin-nigrosin staining method was used to evaluate sperm viability and abnormalities, following the procedure described by Moskovtsev and Librach (2013), which involved dripping 10µL of semen into 20µL of eosin-nigrosin solution. Another glass slide was attached at a 45° angle to the sample glass slide and then flattened to form a thin preparation. It was dried on a heated plate and observed under a microscope with a magnification of 400x. The integrity of the plasma membrane was assessed using the hypoosmotic swelling test (HOST) (Gustina et al. 2024). Thirty microliters of semen were homogenized with 300µL of HOST solution for 30min at 37°C. The solution was then placed on a glass slide, covered with a cover slip, and observed under a microscope at 400x magnification.

Acrosome integrity was evaluated using FITC-PNA and Propidium Iodide (PI) staining. Semen samples were prepared as smear slides and allowed to air-dry. The slides were then fixed in 96% ethanol for 10min and air-dried (Rajabi-Toustani et al. 2019). A solution of FITC-PNA (25–50µL) was applied to the slides, which were then incubated at 37°C for 30min. Subsequently, 5µL of PI was added, and the samples were incubated again at 37°C for 5min; then, the slides were washed with PBS and air-dried

Protamine deficiency was evaluated using a modification of the Chromomycin A3 (CMA3) method as described by Simões et al. (2009). Semen samples were prepared as smear slides, air-dried, and fixed in Carnoy's solution at -4°C for 10min. CMA3 solution (30–50µL) was applied to the slides and incubated for 20min at 37°C. The slides were then rinsed with McIlvaine buffer and air-dried.

Statistical Analysis

The data are presented as mean ± SD and were analyzed using the Shapiro-Wilk test for normality and Levene's test for homogeneity. Parametric analyses were performed on normally distributed and homogeneous data using ANOVA, followed by Tukey's post-hoc test. Categorical data were examined using the chi-square test. All analyses were conducted with SPSS software (version 25; IBM® Corp. Armonk, NY, USA).

RESULTS

Libido

The study revealed a highly significant difference ($P<0.01$) between Horned bulls and Polled 3 in libido characteristics. However, no significant differences between Horned bulls and Polled 1 and 2 were found. Polled 1 and Polled 3 were significantly different ($P<0.05$), while no significant differences were found between Polled 1 and Polled 2 or between Polled 2 and Polled 3 (Fig. 1). The libido durations were 11.43 seconds for the Horned bull, 17.78s for Polled 1, 20.33s for Polled 2, and 37.66s for Polled 3.

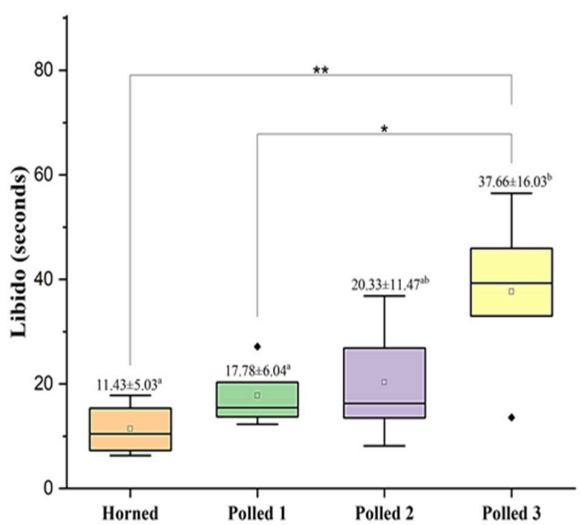

Fig. 1: The box plot analysis compares libido characteristics between Horned and Polled Bali bulls, with significance levels indicated by asterisks, * for $P<0.05$ and ** for $P<0.01$.

Quality of fresh semen

The quality of fresh semen in Bali bulls, including Bali Polled 1, Polled 2, Polled 3, and Horned Bali, was assessed based on volume, color, mass motility, consistency, concentration, and motility. Significant differences were observed in semen volume and sperm concentration. The Horned Bali bull exhibited a semen volume of 8.64±2.03mL ($P<0.01$) and a sperm concentration of 0.958×10^9/mL ($P<0.05$), both of which were higher than those of Polled 3 but not significantly different from Polled 1 and Polled 2. Similarly, there were no significant differences among Polled 1, Polled 2, and Polled 3. Polled 3 showed the lowest semen volume and concentration, at 2.80±1.15mL and 0.458×10^9/mL, respectively. Semen color was consistently creamy, mass motility was uniform, consistency was moderate, and sperm motility ranged from 64 to 70%, with no significant differences among the bulls.

Frozen semen quality and sperm kinematics

The quality of frozen semen was evaluated using various parameters, including total motility, progressive motility, viability, abnormality, plasma membrane integrity, acrosome integrity, and protamine deficiency, as shown in Table 2. Significant differences ($P<0.05$) were observed in progressive motility and sperm abnormality among Bali Polled bulls (Polled 1, Polled 2 and Polled 3) and the Horned Bali bull. The Horned Bali bull exhibited significantly higher progressive motility than Polled 3 but was similar to Polled 1 and 2. No significant differences were observed among Polled 1, Polled 2, and Polled 3. Sperm abnormality was significantly higher in the horned Bali bull compared to Polled 1 and Polled 2 but was not different from Polled 3. No significant differences were observed in total motility, viability, plasma membrane integrity, acrosome integrity, and protamine deficiency among the Horned Bali and Bali Polled bulls.

The analysis results presented in Fig. 2 reveal significant differences ($P<0.05$) in the average path velocity (VAP) and straight-line velocity (VSL) of the Horned bull compared to Polled 2. However, these metrics were similar to those observed in Polled 1 and 3. No significant differences were found among Polled 1, Polled 2, and Polled 3, indicating more efficient sperm movement toward the egg and potentially enhanced fertilization chances. Additionally, beat cross frequency (BCF) values for Horned bulls were higher than those for Polled 2 and 3 but not significantly different from those for Polled 1. There were no significant differences between Polled 1, Polled 2, and Polled 3, suggesting that the flagellar beat frequency in horned bulls is more significant than in Polled bulls. The parameters of curvilinear velocity (VCL), straightness (STR), linearity (LIN), and amplitude of lateral head displacement (ALH) did not show significant ($P>0.05$) differences among the bulls.

Table 1: Fresh Semen Quality of Bali Polled Bulls

Parameters	Horned	Polled 1	Polled 2	Polled 3	Level Sig.
Volume (mL)	8.64±2.03^{a}	5.28±3.43^{ab}	5.92±1.66^{ab}	2.80±1.15^{b}	**
Color	Creamy	Creamy	Creamy	Creamy	ns
Mass Motility	++	++	++	+	ns
Consistency	Moderate	Moderate	Moderate	Moderate	ns
Concentration ($\times10^9$)	0.958±0.14^{a}	0.741±0.15^{ab}	0.689±0.30^{ab}	0.458±0.22^{b}	*
Motility (%)	70.00±0.00	68.00±4.47	67.00±4.47	64.00±4.18	ns

Values (mean±SD) bearing different superscripts in a row indicate significant differences at P<0.05*, P<0.01**, and ns = non-significant.

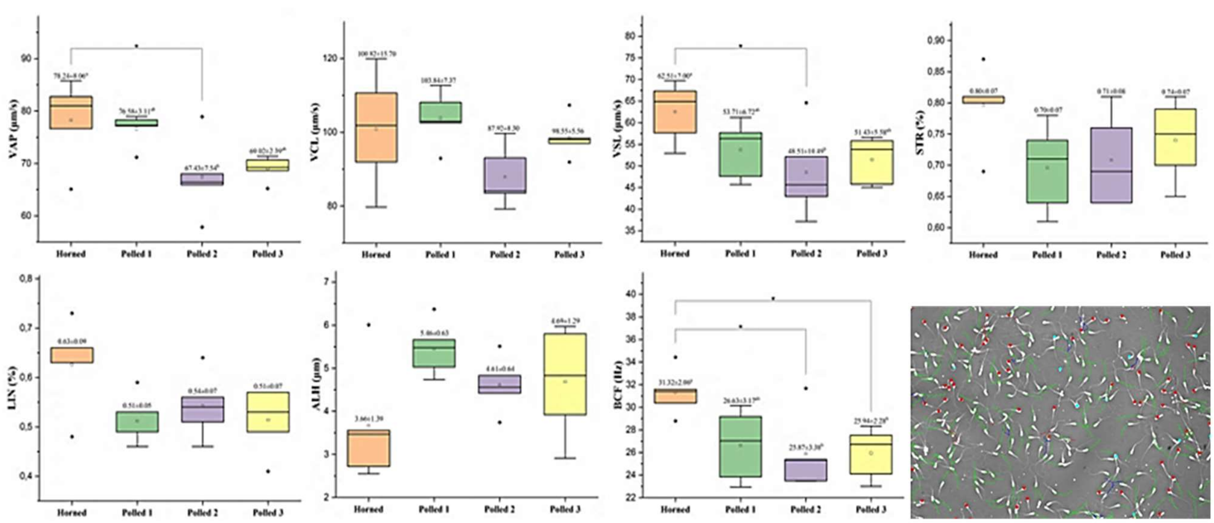

Fig. 2: Box plot analysis of kinematic sperm parameters, including VAP (Velocity Average Path), VCL (Velocity Curvilinear), VSL (Velocity Straight Line), STR (Straightness), LIN (linearity), ALH (Amplitude of Lateral Head Displacement), and BCF (Beat Cross Frequency), compares horned and polled Bali cattle. In the sperm motion analysis in Bali Polled bulls, the green line represents progressive motility, the cyan line denotes local motility, the red line indicates immotile sperm, and the blue line signifies hyperactive sperm. Asterisks denote significance levels at P<0.05.

Table 2: Frozen Semen Quality of Bali Polled Bulls

Parameters	Horned	Polled 1	Polled 2	Polled 3	Level Sig.
Motility (%)	66.30±2.77	66.38±3.85	64.40±4.13	59.21±9.31	ns
Progressive Motility (%)	62.46±4.65[a]	58.94±4.61[ab]	55.54±3.15[ab]	52.48±7.35[b]	*
Viability (%)	61.01±4.18	61.55±7.00	61.53±4.69	59.58±3.95	ns
Abnormality (%)	13.27±5.73[a]	5.66±1.25[b]	6.09±1.12[b]	9.22±3.92[ab]	*
Plasma Membrane Integrity (%)	69.42±2.90	71.29±4.99	65.97±4.72	65.05±3.30	ns
Acrosome integrity (%)	96.87±0.99	95.95±2.20	95.58±2.24	95.27±0.72	ns
Protamine Deficiency (%)	1.38±0.75	3.00±0.71	3.25±0.35	2.08±0.37	ns

Values (mean±SD) bearing different superscripts in a row indicate significant differences at P<0.05* and ns = non-significant

DISCUSSION

The study revealed that libido differed between horned and Polled Bali bulls, with the horned bull exhibiting a faster response and Polled 1 showing significantly faster libido than Polled 3. The libido characteristics of Polled Bali bulls observed in this study are consistent with the findings of Hasbi et al. (2021), which indicates that Polled Bali bulls require more time than Horned bulls from the initial fondling stage with the female or teaser to ejaculation. According to Parkinson (2004), libido has a significant impact on fertility levels in bulls.

These findings indicate that the sexual behavior of Bali Polled cattle is influenced by genetic factors, hormone levels, heat stress, and physiological factors (Sukandi et al. 2023). Testosterone is essential for good semen quality, the proper functioning of accessory sex glands, the development of secondary sexual traits, libido, and sperm production (Hafez et al. 2000). High levels of testosterone in the blood boost libido, increase mating desire in bulls, and shorten mating duration (Monaco et al. 2015). Dick et al. (2020) state that testosterone enhances sexual activity by increasing dopamine release in the medial preoptic area, which facilitates the somatomotor components of copulation and directs sexual drive towards sexual stimuli. Hasbi et al. (2021) found a positive link between libido and testosterone levels in these cattle. Factors such as feed quality (Chacón et al. 2022) and the temperature-humidity index affect libido (Korkmaz et al. 2023).

Fresh semen quality in Bali bulls differs (Table 1), with the Horned bulls showing higher semen volume and concentration than the Polled bulls. These results are consistent with the findings of Diansyah et al. (2022). A study by Mappanganro et al. (2025) found that the semen volume of Polled Bali bulls was higher than that of horned bulls (4.89±2.61 vs 4.82±2.60mL). Drabovich et al. (2014) explain that the high semen volume is correlated with bull performance (Sonjaya et al. 2021). Maulana et al. (2022) stated that spermatozoa concentration and semen volume significantly impact the total spermatozoa count per ejaculate and frozen semen production. Evaluating spermatozoa concentration is crucial for optimizing dilution and enhancing fertility performance (Hasbi et al. 2023).

The color, motility and consistency of fresh semen from Bali bulls, both polled and horned, are uniform. They are characterized by a creamy color, sperm motility of 64 to 70%, and moderate consistency. The creamy color is attributed to riboflavin pigments in the feed. High-quality semen is thicker than milk, whereas poor-quality semen is comparable to coconut water (Ax et al. 2000). Sperm motility in tropical cattle generally ranges between 40 and 75% (Garner and Hafez 2000). Semen freezing can reduce sperm motility by approximately 20-30% (Hapsari et al. 2018). Normal spermatozoa movement ranges from fast (++) to very fast (+++), which improves fertilization potential, while slow movement (+) is considered abnormal (Komariah et al. 2020). According to Wijayanti et al. (2023), the semen consistency of Bali bulls varies from medium to thick.

The analysis of frozen semen quality in Bali polled bulls revealed significant differences in progressive motility and sperm abnormalities. Bali horned bulls showed higher progressive motility but also had more abnormalities. In contrast, Bali polled bulls had lower progressive motility than the horned bulls. However, their motility values were similar to those previously reported by Indriastuti et al. (2020), with motility ranging from 59.21 to 66.38%. The decreases in motility were attributed to variability in mitochondrial function and ATP synthesis. Additionally, Handayani et al. (2021) used SNI-certified semen (60.31±14.65%) and non-SNI semen (42.66±18.03%). Furthermore, Agil et al. (2025) reported that the total frozen semen motility of Bali bulls was 52.29±0.21 in the low category and 68.93±0.16 in the high category, with progressive motility ranging from 42.61±0.16 to 63.88±0.32.

Sperm abnormalities are higher in the Horned Bali bull than in Polled Bali bulls (Table 2). However, as long as abnormalities remain below 20%, as per the Indonesian National Standards (Directorate General of Livestock and Animal Health 2022), their impact on fertility is considered insignificant. Environmental conditions and heat stress, particularly during dry seasons, affect sperm abnormalities and fertility in Horned and Polled Bali bulls (Kaiin et al. 2017). Sperm with coiled tails are highly inefficient at fertilizing eggs due to their non-progressive, circular movement (Hernández-Avilés et al. 2020).

The total motility, viability, plasma membrane integrity, acrosome integrity, and protamine deficiency parameters did not differ. This suggests that semen quality is similar in horned and polled Bali bulls for these measures, meaning that genetic or phenotypic differences do not significantly affect semen quality. Both horned and polled Bali bulls appear to have similar abilities to protect against damage, as evidenced by their ability to prevent the formation of free radicals (Uswa et al. 2022). Freezing can cause temperature changes and osmotic stress, resulting in the production of reactive oxygen species (ROS), which harm sperm membranes, motility, and viability (Ribas-Maynou et al. 2022). Despite this, polled Bali spermatozoa show better freezing tolerance and membrane integrity, which are important for motility and capacitation (Hasbi et al. 2023).

Acrosome quality and protamine are important for fertility. Rajabi-Toustani et al. (2019) explain that sperm entering the female reproductive tract need an intact acrosome to react quickly at the fertilization site. The acrosome integrity obtained in this study was higher than that previously reported by Nurgina et al. (2025), who found that the acrosome integrity of sexed-frozen semen from Polled Bali bulls ranged from 71.74±13.24 to 87.45±10.27. Protamine is linked to DNA damage (Fortes et al. 2014) and a key protein that binds to DNA in sperm also plays a vital role (Dogan et al. 2015). Low protamine deficiency in Bali Polled bull suggests stable sperm DNA (Hasbi et al. 2023). Protamine deficiency can impair sperm quality by causing chromatin instability, DNA damage, and alterations in sperm head morphology (Kusumawati et al. 2023). Protamine 1 is vital for maintaining sperm structure, motility, capacitation, and fertilization (Pardede et al. 2021). Sperm with abnormalities, such as narrow heads, may show increased progressive motility due to their streamlined shape (Enciso et al. 2011).

Specific kinematic parameters were superior in horned Bali bulls, while others showed no significant differences. These findings are consistent with those of Diansyah et al. (2022), who reported higher kinematic values in horned Bali than in polled Bali bulls. In contrast, Hasbi et al. (2023) observed higher kinematic values in polled Bali bulls. Increased progressive sperm motility and elevated VAP, VSL, and VCL values are associated with higher fertilization capacity (Piddubna et al. 2022). Furthermore, Diansyah et al. (2025) reported that VAP 64.80±3.54μm/s, VSL 37.86±1.07μm/s and VCL 111.70±2.93μm/s showed a service/conception figure of 1.52±0.08. According to Khezri et al. (2020), spermatozoa are classified as hyperactive if VCL>80μm/s, ALH>6.5μm and LIN<0.65%, progressive if VAP>55μm/s, STR>0.50%, and LIN>0.35%, and immotile if BCF<20 and VSL<20 (Hurri et al. 2022).

Conclusion

This study concluded that Polled Bali bulls have a longer duration of libido and more variable fresh semen quality than Horned Bali bulls. Additionally, they show lower abnormality rates in frozen semen. These findings underscore the importance of targeted selection and management practices in enhancing breeding outcomes in Polled Bali cattle.

DECLARATIONS

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Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability: All data supporting the findings of this research are available in this article.

Ethics Statement: The Animal Ethics Commission of Hasanuddin University approved all research procedures (approval number: 302/UN4.6.4.5.31/PP36/2021).

Author's Contribution: Wildayanti Wildayanti, Hasbi Hasbi, Herry Sonjaya, and Ekayanti Mulyawati Kaiin: Conceived and designed the study. Wildayanti Wildayanti, Tulus Maulana, and Muhammad Gunawan: Performed the study. Hasbi Hasbi, Herry Sonjaya, and Ekayanti Mulyawati Kaiin: Supervision. Wildayanti Wildayanti, Sudirman Baco, Sri Gustina, and Tulus Maulana: Data curation, validation, formal analysis, visualization. Wildayanti Wildayanti: writing – original draft. Wildayanti Wildayanti, Hasbi Hasbi, Ekayanti Mulyawati Kaiin, Herry Sonjaya, Sudirman Baco, Sri Gustina, Tulus Maulana, and Muhammad Gunawan: reviewed and edited the manuscript. All authors have read, reviewed, and approved the final manuscript.

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