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The Quality of Fresh and Frozen Semen and its Correlation with Molecular Weight of Seminal Plasma Protein in Bali Cattle

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ABSTRACT

This study evaluated the quality of fresh and frozen semen of Bali cattle and its correlation with the molecular weight (MW) of seminal plasma protein. This study collected semen from 10 bulls aged 5–10 years using an artificial vagina and evaluated the samples macroscopically and microscopically. Two batches of frozen semen obtained in 2020 and 2021 were also analyzed. The frozen semen samples were thawed at 37 °C for 30 seconds. The sperm motility, viability, intact plasma membrane (IPM), and sperm abnormalities were investigated. The concentration of the seminal plasma proteins was determined using the Bradford method, and the proteins were characterized using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1D-SDS-PAGE). Additionally, the gels were stained with Coomassie brilliant blue, and the MWs of the proteins were determined using MW markers. The sperm motility, viability, and abnormalities of fresh semen varied significantly among the bulls ($p<0.05$); however, sperm IPMs among the bulls were similar ($p>0.05$). No differences in sperm motility after freezing were found among the bulls. However, the sperm viability, abnormality, and IPM varied among the bulls. Meanwhile, the seminal plasma proteins contained bands with different MWs. No difference in the expression of protein bands between bulls. Linearity analysis showed that sperm motility ($r= 0.281$), viability ($r= 0.189$), abnormalities ($r= 0.141$), and IPM ($r= 0.173$) were positively correlated with the protein bands at each MW ($p<0.05$). The results conclude there was a positive correlation between the MW of the protein marker and the same protein expression levels in Bali bulls. Therefore, the band intensity of Bali cattle seminal plasma proteins can be used as a biomarker for selecting superior Bali bulls.

Keywords: Bali cattle; semen quality; seminal plasma protein; superior bull

INTRODUCTION

Bali cattle, one of the native Indonesian cattle, is the most preferred breed in the smallholding systems in Indonesia. These cattle have high resistance against diseases with a remarkable ability to grow on low-quality fodders and a high fertility rate (Purwantara *et al.*, 2012). As a result, Bali cattle are essential for the smallholder farming enterprises in eastern Indonesia, making up 25% of the cattle population in Indonesia (Lisson *et al.*, 2010; Tahuk *et al.*, 2018).

Bali cattle has spread to almost all of Indonesia, including South Sulawesi, where Bali cattle breeding is mainly done by artificial insemination (AI). According to Sutarno & Setyawan (2016), the AI of heifers and cows with frozen-thawed sperms has been applied in Indonesia since the 1970s. Since cryopreserved semen is primarily used in cattle breeding (Zhang *et al.*, 2015), its quality plays an essential role in fertilization success (Beran *et al.*, 2013; De Lazari *et al.*, 2018). Semen quality can also predict male fertility by measuring parameters including sperm motility, viability, and morphology (Zubair *et al.*, 2014). However, the success rate of embryo transfer using fresh and frozen Bali cattle embryos produced *in vitro* is still low, with 40% for fresh embryos and 12.5% for frozen embryos (Ismirandy *et al.*, 2021).

Semen comprises sperms and seminal plasma. Seminal plasma contains protein from the testicles, epididymis, and accessory glands (De Lazari *et al.*, 2018). According to Chacur (2012), seminal plasma contains proteins and several components, such as enzymes, lipids, organic acids, and minerals; each component plays a role in sperm metabolism. The protein composition of seminal plasma of mammalian animals varies between species. In addition, Govindaraju *et al.* (2012) have observed that the molecular and cellular integrity

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of sperms is essential for fertilization, embryo development, and fetal development.

Parameters of semen quality, such as motility, viability, intact plasma membrane, and sperm abnormalities, are insufficient for predicting male fertility. Proteins in seminal plasma have been identified as fertility markers in Zebu bulls (Chacur, 2012), Hanwoo bulls (Park *et al.,* 2012), Holstein bulls (Rosyada *et al.,* 2020), and Simmental bulls (Baharun *et al.,* 2021). In addition, various proteins in seminal plasma can maintain sperm viability in the male and female reproductive tracts (Samanta *et al.,* 2018). Moreover, the binder of sperm proteins 1 (BSP1) is associated with the binding of sperms to eggs, fertilization, and initiation of embryonic development (Rodriguez-Villamil *et al.,* 2016).

Seminal plasma proteins exert positive or negative effects on sperm fertility. The function of a protein related to male fertility can be explored by studying sperm cells and seminal plasma (Druart & de Graaf, 2019). For example, the plasma protein bands of male and female Bali cattle (aged 0 to 1.5 years), puberty (aged 2 to 2.5 years), and adults (age 3 to 5 years) are different (Wahyu *et al.,* 2017). For example, there were 14 protein bands with varying thickness from 5 fractions, namely albumin, globulin α1, α2, β, and γ (Wahyu *et al.,* 2017). Therefore, the protein study of Bali bull which correlated with fertility is necessary to be explored further. This study aimed to evaluate the quality of fresh and frozen semen and its correlation with the molecular weight (MW) of the seminal plasma proteins used as indicators for selecting superior Bali bull.

MATERIALS AND METHODS

Ethical Approval

The Animal Ethics Commission of Hasanuddin University approved the animal models and experimental designs for this study (certificate number 302/UN4.6.4.5.31/PP36/2021). All procedures were conducted according to the standard operating procedure (SOP) for frozen semen production at the South Sulawesi Regional Artificial Insemination Center (RAIC). South Sulawesi RAIC was responsible for the use and the management of bulls.

Experimental Animals

Ten Bali bulls aged 5–10 years belonging to the South Sulawesi RAIC at Puncak-Maros, Indonesia, were used. This study maintained the bulls according to the SOP of the AI Center. The Bali bulls were individually kept in 2.5 × 2 m cages equipped with feed and drinks containers. All bulls were fed with 10% fresh forage and 2 kg concentrate of total body weight twice a day, once in the morning and the evening, and water was given *ad libitum*. Only semen ejaculates with sperm motility of >70%, a sperm concentration of more than 800 × 10^6/mL, and a sperm abnormality of <20% were used. Lastly, semen was collected from October to December 2020.

Fresh Semen Collection and Analysis

Semen of Bali cattle was collected twice a week in the morning using an artificial vagina. After collection, the samples of fresh semen were immediately delivered to the laboratory for evaluation using macroscopic and microscopic methods, according to Arifiantini (2012). Macroscopic evaluations included measuring the volume, color, consistency, and degree of acidity (pH) of a semen sample. Besides, microscopic evaluations included sperm motility, viability, IPM, concentration, and sperm abnormalities. All microscopic evaluations were performed under a binocular microscope (Olympus CX31) with a magnification of 400 ×.

Analysis of sperm motility was performed by first mixing 10 μL of fresh semen with 40 μL of saline solution (1:4 ratio), dripping 10 μL of the mixture solution on a warm object-glass, and covering with a cover glass. Then, sperm motility was observed using a binocular microscope. The proportion of the progressively moving sperms compared to the non-progressive ones was calculated and expressed in percentages. Meanwhile, sperm viability and morphology were measured using eosin-nigrosine staining. First, 10 μL of a semen sample was mixed with 40 μL of eosin-nigrosine (1:4 ratio). Next, the mixture of semen and eosin-nigrosine was smeared on a heating stage and dried for 10 seconds. Viable sperms do not absorb the color, staying transparent.

In contrast, the heads of non-viable (dead) sperms will be stained red-purple. Lastly, sperms were classified by their morphologies into (i) sperms with normal morphologies, (ii) sperms with abnormal heads, (iii) sperms with abnormal midpieces, and (iv) sperms with abnormal principles piece tail (Ntemka *et al.,* 2016). Observations were made using a microscope at 400 × magnification in 10 fields of view or 200 cells.

The IPM was measured using the hypo-osmotic swelling (HOS) test (Fonseca *et al.,* 2005). First, 10 μL of semen was mixed in 1 mL of HOS solution (1:100 ratio), homogenized, and incubated at 37 °C for 30–45 minutes. Then, the HOS solution with semen was dripped onto an object glass, covered with a cover glass, and evaluated using a microscope with a 400 × magnification. The sperm with IPM was marked with a circular or bulging tail, while a straight tail marked the damaged ones. Lastly, sperm concentration was calculated using a photometer (SDM 6, Minitube, Tiefenbach, Germany) and expressed in million per mL (Santoso *et al.,* 2021).

Frozen-Thawed Semen Analysis

The frozen semen from two batches of the production year 2020 and 2021 with three straws/bull/batches was used; the total frozen semen used in this study was 60 straws. The quality of the frozen semen was evaluated at the In Vitro Embryo Production Laboratory, Hasanuddin University. The samples of frozen semen were thawed in a 37 °C water bath for 30 seconds and kept at the same temperature during observation. The parameters observed, including sperm motility, viability, IPM, and sperm abnormalities, were measured

using the same approach for the fresh semen with a few modifications. For example, sperm motility was assessed without the saline solution. In addition, sperm viability and sperm abnormalities were measured using 10 μL of semen mixed with 10 μL of eosin-nigrosine (1:1 ratio). Lastly, the IPM was measured using 10 μL of semen mixed with 20 μL of HOS solution (1:2 ratio).

Determination of Seminal Plasma Protein Concentrations

First, the semen was centrifuged at 3000 g for 30 minutes. Then, the supernatant (seminal plasma) was put in a straw and stored in liquid nitrogen (Karunakan *et al.,* 2019). After that, seminal plasma proteins were characterized using 1D-SDS-PAGE based on protein MW. The gels were stained with Coomassie brilliant blue, and the molecular mass was determined according to the bands of the MW marker (Karunakaran *et al.,* 2019). The concentrations of the seminal plasma proteins were determined using the Bradford method (Bardford, 1976). According to the Coomassie Protein Kit Use Guide (Bradford). Lastly, the data were analyzed using the Thermo Skanlt RE software Multiskan Go, 3.2. Version.

The proteins were separated using two 12% polyacrylamide gels containing SDS. First, 20 μg of protein was analyzed using 1D-SDS-PAGE with the BM (Spectra Multicolor Broad Range Protein Ladder, Fermentas Life Science) marker, ranging from 5 to 245 kDa. The protein bands were measured and analyzed based on the protein range compared with the walking interval [retention factor; (Rf)]. The results of the Rf analysis and the weight log of the band marker proteins were transformed into linear regression equations. In addition, the proteins were separated at 120 volts for 70 minutes. Later, the color of the photo gel was inverted to identify the protein bands. The intensity of each protein band was determined by ratio analysis using ImageJ (Schneider *et al.,* 2012).

Statistical Analysis

The study used a completely randomized design, two-way ANOVA. The results obtained were tabulated and presented in mean ± standard error for all parameters; a p-value less than 0.05 indicated statistical significance. The Scatter-Plot linearity test (Software SPSS ver. 20) was used to determine the relationship between Bali bulls' protein mass and semen quality.

RESULTS

Bali Cattle Semen Quality

The sperm motility, viability, and abnormalities were significantly different between the bulls ($p<0.05$). The bulls with ID numbers 11437 and 11434 had the highest sperm motility ($p<0.05$), and those with ID numbers 11540 and 11541 had the lowest sperm motility (Table 1). Meanwhile, the best sperm viability was shown by the bull with ID number 11539 and the lowest by the bull with ID number 11541 with a value of 75.25%. Moreover, a bull with ID number 11522 displayed the lowest sperm abnormality, and that with ID number 11540 indicated the highest sperm abnormality. Lastly, the IPM did not differ between the bulls ($p>0.05$) with an average of 94.52% (Table 1).

The frozen semen of Bali cattle produced by the South Sulawesi RAIC is high quality. For example, sperm motility after freezing was 42.50%, similar among the bulls. The bull with ID number 11434 demonstrated the highest sperm viability, and the bulls with ID numbers 11540 and 11541 had the lowest values. The average sperm abnormality of fresh semen, at 6.04±0.17%, increased to 10.01±0.36% after freeze-thawing. Furthermore, the bulls showed the highest IPM of sperm with ID numbers 11521, 11539, 11437, and 11229, and the bull showed the lowest IPM with ID number 11541 (Table 2).

Besides semen quality, it is critical to evaluate the number of semen straws produced by each bull. The results of this study, including semen volume, sperm concentration (per mL), and sperm motility measurements, confirm the secondary data on the productivity of the frozen semen from each bull collected in 2020 and 2021. The three data become a total number of motile sperms in an ejaculate. The total number of motile sperms in the ejaculate divided by the insemination dose for bull is 25×10^6 (BSN, 2017). According to the

Table 1. The quality of the fresh semen samples from Bali bulls at South Sulawesi Regional AI Center

Bull ID	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)	Sperm intact plasma membrane (%)
11521	72.50±0.45^{abc}	81.25±0.39^{cd}	5.05±0.08^{a}	94.00±0.36^{a}
11522	73.75±0.39^{abc}	84.50±0.32^{de}	4.90±0.04^{a}	95.00±0.34^{a}
11434	76.25±0.75^{c}	84.25±0.45^{de}	5.85±0.14^{abc}	95.00±0.31^{a}
11539	75.00±0.64^{bc}	86.50±0.27^{e}	5.77±0.13^{abc}	94.75±0.29^{a}
11437	76.25±0.39^{c}	84.50±0.41^{de}	5.17±0.20^{ab}	96.25±0.23^{a}
11229	71.25±0.39^{ab}	81.75±0.26^{cd}	5.97±0.08^{abc}	95.00±0.31^{a}
11033	72.50±0.45^{abc}	79.50±0.49^{bc}	6.27±0.07^{bc}	93.00±0.31^{a}
11532	71.25±0.39^{ab}	77.25±0.50^{ab}	6.95±0.13^{cd}	93.50±0.47^{a}
11540	70.00±0.00^{a}	78.25±0.52^{abc}	7.82±0.06^{d}	94.75±0.29^{a}
11541	70.00±0.00^{a}	75.25±0.15^{a}	6.67±0.11^{c}	94.00±0.36^{a}
Mean ± SE	72.88±0.53	81.30±0.65	6.04±0.17	94.53±0.32

Note: Means in the same column with different superscripts differ significantly ($p<0.05$).

South Sulawesi RAIC, bull semen is collected weekly for 40 weeks a year. The average ejaculate production was 225.52 straws per bull (Table 3).

Molecular Weight of Seminal Plasma Protein of Bali Cattle

The seminal plasma proteins of Bali cattle contained protein bands with different MWs (Figure 1). There was no difference in the expression of the number of protein bands among the bulls. In addition, gel electrophoresis results only demonstrated differences in the thickness of protein bands in the bulls with ID numbers 11521, 11522, 11434, 11539, and 11437. The results of linearity analysis for each Bali bull (Figure 2), showed sperm motility (r= 0.281), sperm viability (r= 0.189), sperm abnormalities (r= 0.141), and sperm IPM (r= 0.173). All had positive correlations significant to the confirmed protein band at each MW ($p<0.05$).

DISCUSSION

The Bali bulls in the South Sulawesi RAIC have been well-selected for their semen qualities and frozen semen productions. The selection of superior bull is conducted according to the breeding soundness examination (BSE) technique (Thundathil *et al.,* 2016). The BSE technique includes bull performance, libido, and semen quality testing. The quality of semen contributes to fertility performance by 20%–25% (Diskin *et al.,* 2018). Thus, accurate semen evaluation is critical for assessing the reproductive potential of a superior bull. Moreover, sperm motility is an essential indicator of semen quality (Fraser *et al.,* 2014; Wasilewska *et al.,* 2017; Yoon *et al.,* 2016). In this study, the sperm motility of fresh semen of Bali cattle was >70% (Table 1).

Sperm motility after freeze-thawing is consistent with SNI requirements for cattle number 4869-1: 2017, which is at least 40%. The sperm viability of fresh semen averaged 81.30% and varied between individuals ($p<0.05$) (Table 1). The average viability of the frozen-thawed sperms was 75.60% (Table 2). The decrease in sperm viability from fresh to frozen semen is relatively low, by only 6%–7%, compared to a decrease in sperm motility of almost 30%. These data indicate that the freezing process reduces sperm quality, especially sperm motility.

Meanwhile, this study reported no differences in the IPM of the fresh semen among the bulls; however, after freeze-thawing, the IPM values differed. During freezing, sperm will experience cold and osmotic shocks. Sperm membranes are one of the main damage sites during cryopreservation due to the irreversible modifications of phospholipids during cold shock

Table 2. The quality of the frozen semen samples from Bali bulls at South Sulawesi Regional AI Center

Bull ID	Straw (n)	Sperm motility (%)	Sperm viability (%)	Sperm abnormality (%)	Sperm intact plasma membrane (%)
11521	6	42.50±0.43^{a}	77.67±0.29^{bc}	9.03±0.16^{b}	83.83±0.25^{c}
11522	6	42.50±0.43^{a}	75.50±0.46^{ab}	8.26±0.11^{ab}	80.50±0.48^{ab}
11434	6	42.50±0.43^{a}	78.67±0.64^{d}	11.10±0.16^{c}	81.00±0.26^{ab}
11539	6	42.50±0.43^{a}	76.17±0.18^{abc}	12.45±0.20^{c}	84.17±0.41^{c}
11437	6	42.50±0.43^{a}	74.67±0.43^{ab}	9.00±0.12^{b}	85.17±0.18^{c}
11229	6	43.33±0.40^{a}	75.33±0.27^{ab}	7.70±0.13^{ab}	84.33±0.21^{c}
11033	6	41.67±0.40^{a}	75.50±0.43^{ab}	6.90±0.11^{a}	83.33±0.21^{bc}
11532	6	42.50±0.43^{a}	75.50±0.25^{ab}	12.33±0.32^{c}	80.83±0.55^{ab}
11540	6	42.50±0.43^{a}	73.83±0.27^{a}	12.35±0.19^{c}	80.67±0.38^{ab}
11541	6	42.50±0.43^{a}	73.00±0.36^{a}	11.05±0.12^{c}	79.00±0.28^{a}
Mean ± SE		42.50±0.39	75.60±0.42	10.01±0.36	82.28±0.45

Note: Means in the same column with different superscripts differ significantly ($p<0.05$).

Table 3. The productivity of the frozen semen samples of Bali bulls at South Sulawesi Regional AI Center

Bull ID	Semen volume (mL)	Sperm motility (%)	Sperm concentration ($\times10^{6}$/mL)	Total motile sperm/ ejaculate	Total straw/ ejaculate
11521	7.32±0.36	72.50±0.45	1262.25±52.95	6698.76	267.95
11522	7.50±0.17	73.75±0.39	1298.50±37.55	7182.32	287.29
11434	6.75±0.22	76.25±0.75	1139.75±35.89	5866.15	234.64
11539	6.40±0.31	75.00±0.64	1521.00±66.62	7300.80	292.03
11437	5.85±0.20	76.25±0.39	900.00±39.30	4014.56	160.58
11229	7.20±0.23	71.25±0.39	936.75±27.32	4805.52	192.22
11033	6.57±0.33	72.50±0.45	1345.00±73.25	6406.57	256.26
11532	5.32±0.09	71.25±0.39	1282.25±49.04	4860.36	194.41
11540	6.10±0.19	70.00±0.00	1216.50±51.95	5194.45	207.77
11541	6.32±0.36	70.00±0.00	945.25±33.91	4181.78	167.27
Mean ± SE	6.53±0.25	72.88±0.53	1184.72±52.74	5638.81±191.24	225.52±7.64

Note: Means in the same column with different superscripts differ significantly ($p<0.05$).

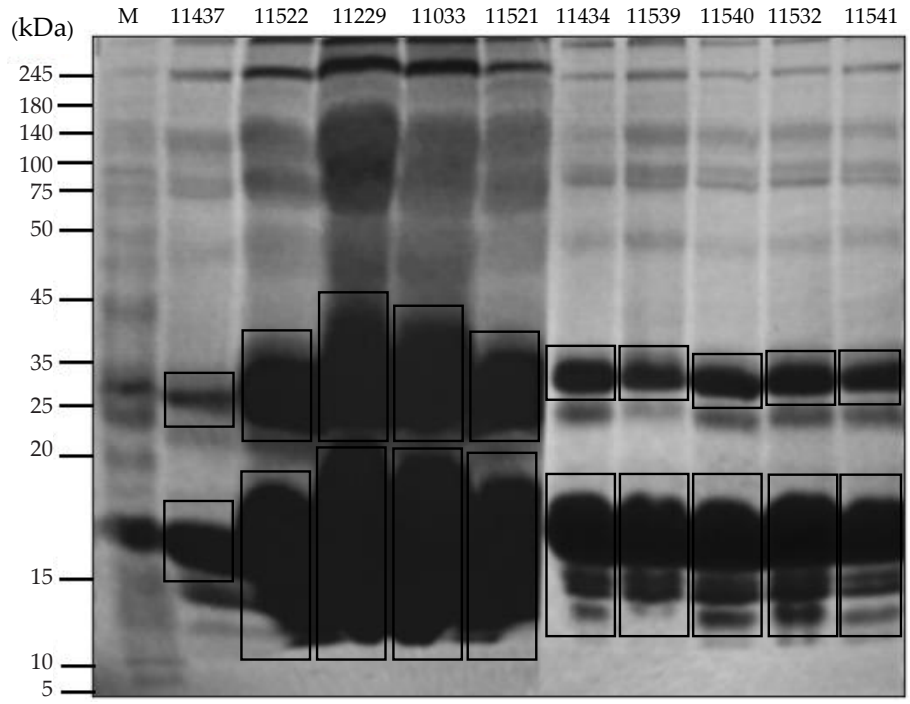

Figure 1. The protein profile of the seminal plasma of Bali cattle at the South Sulawesi Regional AI Center

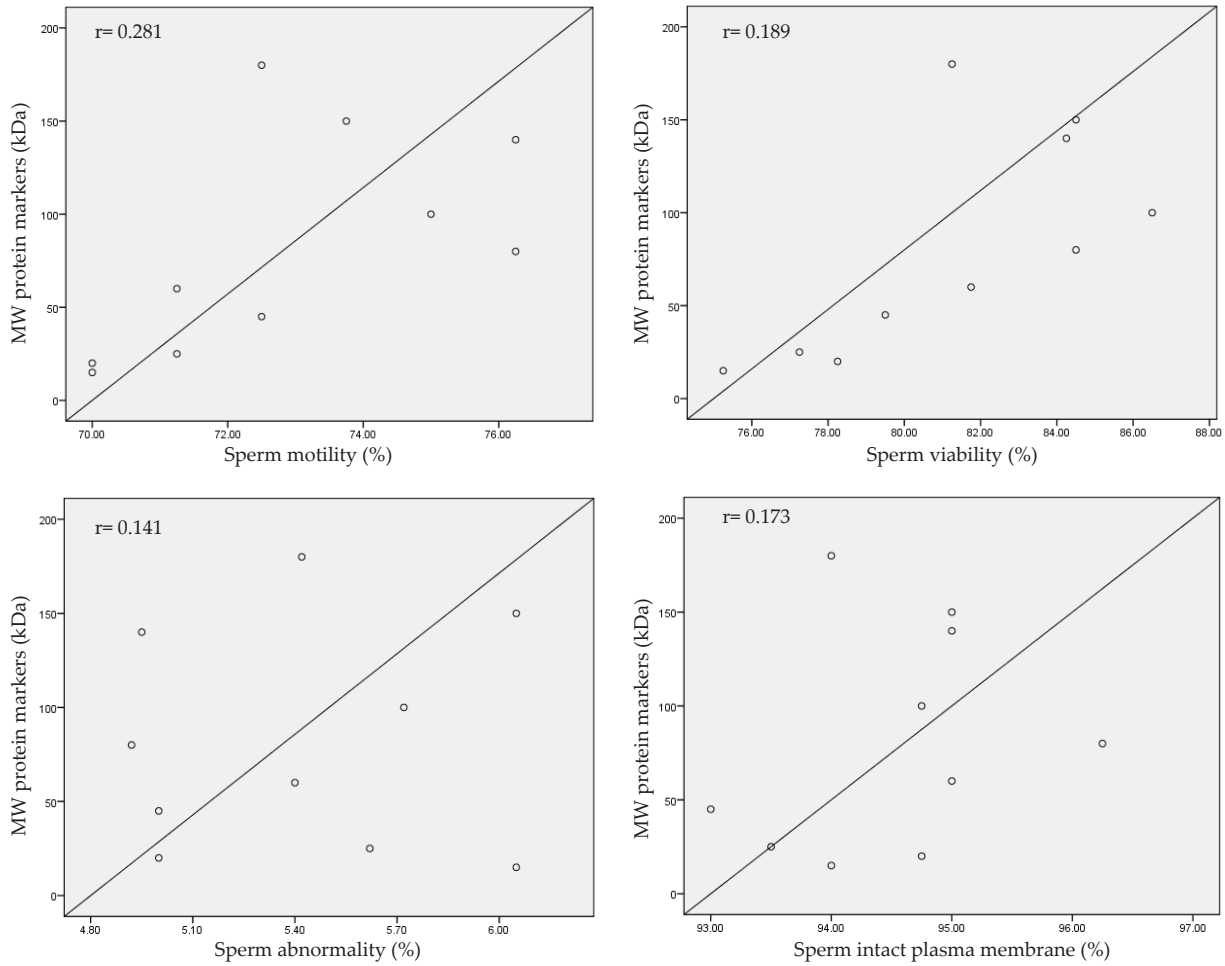

Figure 2. Correlation between sperm motility (a), viability (b), abnormality, and intact plasma membrane with molecular protein weight of Bali cattle at the South Sulawesi Regional AI Center

(Sieme *et al.*, 2015). In addition, the cryo-survival of sperms differs among individuals (Indriastuti *et al.*, 2020). Thus, the HOS test was used in this investigation to predict the functional integrity of the sperm plasma membrane in the sperm tail area. IPM evaluations were based on the principle of sperm swelling when exposed to hypotonic media. During the HOS test, water will flow through the plasma membrane into a cell, causing the cell to re-establish equilibrium between the extracellular and intracellular fluid compartments. The increase in cell volume expands the membrane area, causing the flagellum to coil (Sunter *et al.*, 2015). A sperm will display a coiled tail if the plasma membrane remains intact.

All parameters of semen quality were significantly related to 15-180 kDa (Figure 2). Protein bands with different MWs are indicators of fertility levels in males. According to Baharun *et al.* (2021), sperm motility, normal sperm morphology, and sperm concentration in Simmental bulls were correlated with proteins with MWs between 71 and 100 kDa. In addition, the production of frozen semen is influenced by sperm motility and concentration. The production of frozen semen differs among bulls. For example, frozen semen production ranged from 167 to 292 straws in this study. Meanwhile, the bull with ID number 11539 demonstrated the highest frozen semen production.

On the other hand, the seminal plasma proteins of Bali bulls exhibited different protein bands with different MWs (Figure 1). There was no difference in the expression of the number of protein bands among the bulls. In addition, gel electrophoresis results only indicated differences in the thickness of the protein band in the bulls with ID numbers 11521, 11522, 11434, 11539, and 11437. The proteins in a seminal plasma play an essential role in regulating sperm quality (Fu *et al.*, 2019) and capacitation (Lu *et al.*, 2011). Proteins are also associated with the binding of sperms to egg cells, fertilization, and initiation of embryonic development (Rodriguez-Villamil *et al.*, 2016).

The cryo-tolerance of sperms during freezing is affected by seminal plasma components (Yeste, 2016; Recuero *et al.*, 2019). The role of the proteins in the seminal plasma is manifested in different molecular processes. For example, seminal plasma proteins are absorbed into the sperm surface, affecting cell functions and properties (Purdy, 2006). Here, semen quality analysis uncovered individual variations among the Bali bulls; however, all bulls have the same protein expression. This study found that bands with MWs below 50 kDa had a thickness band level, indicating more protein in those bands.

The thickness of a plasma protein band indicates protein concentration, suggesting an increase in the extracellular proteins from the accessory glands or epididymis that bind more proteins to the phospholipids of the sperm membrane. Protein bands with greater thickness and color intensity are expressed as influential bands (Subagyo, 2015). This study also demonstrated a significant correlation between sperm motility, viability, abnormalities, and sperm IPM with protein band levels (Figure 2).

The confirmed relationship between semen quality and protein MW indicated that all bulls had a reasonable fertility rate. The proteins with MWs of 15 to 180 kDa were found in all bulls. The bulls with ID numbers 11521, 11522, 11434, and 11539 showed higher intensities of the protein bands with MWs of 10 to 45 kDa than the bulls with ID numbers 11229, 11033, 11532, 11540, and 11541 (Figure 1). Moura *et al.* (2010) found several proteins in Holstein bulls with MWs between 71 and 100 kDa indicated as Arylsulfatase A, a protein derived from the cauda epididymis.

Another protein associated with semen quality is a protein with MWs between 10 and 45 kDa. For example, according to Druart *et al.* (2013), a protein with MW of 10 to 25 kDa in bulls may be associated with males with high fertility. In addition, proteins with MW of 10 to 25 kDa are associated with the presence of bovine seminal plasma (BSP) protein. Chacur (2012) also suggested that BSP contained BSP-A1/-A2, BSP-A3, and 30 kDa BSP, collectively referred to as BSP proteins. BSP protein is about 60% found in all *Bos taurus* bulls, with almost the same amount found in *Bos indicus* (Rego *et al.*, 2014).

In this study, a 45-kDa protein related to semen quality was also found in the Bali cattle. Similarly, Karunakaran *et al.* (2019) reported that a 48-kDa protein was positively correlated with sperm motility and IPM. In addition, a 15-kDa protein was found in all the bulls in this study. Moreover, a protein with a BM of 17-18 kDa was reported called A-kinase anchor protein 3 (AKAP3) (Frayne & Hall, 2002). AKAP3, synthesized by both spermatids and sperms, is localized in the flagellum and involved in sperm motility (Hillman *et al.*, 2013). In addition, the 14-kDa proteins contain in the seminal plasma fluids (Rodriguez-Villamil *et al.*, 2016).

Previously, Sarsaifi *et al.* (2015) generated a 2D SDS-PAGE reference map of Bali cattle seminal plasma proteins that provided some new information on the seminal plasma proteins of *Bos taurus* compared with *Bos indicus*. However, some of the essential proteins in *Bos taurus* seminal plasma were not detected in Bali cattle. Moreover, the seminal plasma of Bali cattle semen contains high albumin, clusterin, seminal ribonuclease, and cationic trypsin, which increases the penetration capacity of oocytes (Sarsaifi *et al.*, 2015).

The high albumin concentration in the seminal plasma of Bali cattle semen likely contributes to the thickness of the protein bands in this study. However, further research using more samples is needed to determine the differences in the MWs of seminal plasma proteins between fertile and unfertile Bali cattle. The existing correlation between some seminal plasma proteins in this study could be an additional reference for selecting superior bulls. However, further analysis, such as protein profiling based on MWs using liquid chromatography-mass spectrometry (LC-MS/MS), is needed to confirm a protein in the seminal plasma as a biomarker for selecting superior Bali bulls.

CONCLUSION

In Bali bulls, there is a positive correlation between the molecular weights of the protein markers and their

levels. Protein band thickness of Bali cattle seminal plasma could be used as a candidate biomarker for the selection of superior Bali bull. Protein profiling based on MWs using LC-MS/MS is needed to confirm specific proteins in seminal plasma as markers of superior Bali cattle.

CONFLICT OF INTEREST

The authors declared that there was no conflict of interest in the publication of this paper.

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