p-ISSN 2615-787X e-ISSN 2615-790X
Accredited by Directorate General of Higher Education, Research, and Technology, Republic of Indonesia, No. 225/E/KPT/2022

Tropical Animal Science Journal, January 2025, 48(1):1-7
DOI: https://doi.org/10.5398/tasj.2025.48.1.1
Available online at https://journal.ipb.ac.id/index.php/tasj

Variations in Semen Quality and Potential for Frozen Semen Production in Aceh Cattle

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(Received 26-06-2024; Revised 31-10-2024; Accepted 04-11-2024)

ABSTRACT

The quality of fresh and frozen semen as well as the potential to produce frozen semen, vary considerably between individual animals and cattle breeds. This study aimed to analyze the quality of fresh and frozen semen in Aceh cattle and calculate the potential production of frozen semen in Aceh bulls over 1 year. This study used primary data on the quality of fresh and frozen semen from five Aceh cattle and obtained secondary data from artificial insemination centers in Lembang and Singosari in 2022. Semen samples were collected weekly, and the quality of fresh and frozen semen samples were evaluated. The data were analyzed using a one-way analysis of variance at 95% significance level, followed by Tukey's test. The results revealed differences in semen volume and pH. Based on microscopic characteristics, the semen samples from different bulls exhibited no discernible differences in mass movement, sperm motility, viability, morphology, and plasma membrane integrity, except for sperm concentration and acrosomal integrity. Bull 211605 exhibited the highest sperm concentration. Furthermore, the frozen semen samples from Aceh cattle showed no significant differences in viability, plasma membrane integrity, morphology, and acrosomal integrity. The total sperm motility of bull 211710 was higher than that of the other bulls. The highest progressive motility was observed in bulls 211710 and 211605, and the highest intact DNA was detected in bulls 211710 and 211605. The results of this study demonstrated that the quality of fresh and frozen semen in Aceh cattle is distinct, and the potential frozen semen production of Aceh cattle is estimated to range from 3,382 ± 1810 to 11,399 ± 2658 straws/year.

Keywords: Aceh cattle; fresh semen; frozen semen; frozen semen production

INTRODUCTION

Aceh cattle are a local breed in Indonesia that has been officially recognized and approved by the Minister of Agriculture (No. 2907/Kpts/OT.140/6/2011). Aceh cattle were originally distributed in the Aceh Province. They have been bred for generations and serve as a valuable genetic resource for Indonesian livestock. Aceh cattle can adapt readily to various local forage resources, including fresh and dried leaves, grasses, and nuts (Handayani & Safrida, 2023). This adaptability makes Aceh cattle a suitable local candidate for breeding in Indonesia. Aceh cattle are currently bred using natural mating and artificial insemination.

The practice of artificial insemination has the potential to enhance the population size and genetic quality of cattle (Zuidema *et al.*, 2021). The increase in cattle population and enhancement of their genetic quality are contingent upon the availability of high-quality frozen semen. The production of frozen semen from Aceh cattle is currently performed by artificial insemination centers (AICs) in Lembang and Singosari. Frozen semen production is performed according to the Regulations of the Minister of Agriculture of the Republic of Indonesia (No. 10/Permentan/PK.210/3/2016), and the quality requirements for frozen semen have been stipulated by the Indonesian National Standard (SNI) for bovine frozen semen (No. 4869-1:2021) (Badan Standardisasi Nasional, 2021). The utilization of frozen semen from Aceh cattle, produced by the two AICs, is intended to fulfill their insemination requirements within the Aceh province. The current population of Aceh beef cattle is 481,605 (BPS Livestock, 2022), although the precise count remains unknown.

The quality of fresh and frozen semen exhibits considerable variation between cattle breeds and individual animals (Sukmawati *et al.*, 2014; Indriastuti *et al.*, 2020). Furthermore, a bull's potential to produce frozen semen varies considerably. For instance, Simmental cattle are known to produce 14,699.2 straws/year (Baharun *et al.*,

2020), whereas Pasundan cattle, which are also local to Indonesia, have been documented to produce up to 191.29 straws/ejaculation, which equates to approximately 7,651.6 straws/year (Santoso *et al.,* 2021). Bali cattle, another indigenous Indonesian cattle breed, have been reported to produce 160.58–225.52 straws/ejaculation (Iskandar *et al.,* 2022). It is essential to analyze the potential for frozen semen production in Aceh cattle to determine their population structure within the AIC. The maintenance of an excessive number of Aceh bulls within the AIC is not infeasible, considering the high costs associated with their maintenance and storage of frozen semen samples in liquid nitrogen. The potential for frozen semen production in Aceh bulls can be determined by dividing the total number of motile sperm per ejaculation by the insemination dose (Baharun *et al.,* 2020; Santoso *et al.,* 2021; Iskandar *et al.,* 2022).

The difference in the quality of fresh and frozen semen samples as well as the potential for frozen semen production in Aceh cattle, have not yet been investigated. Therefore, this study aimed to determine the quality of fresh and frozen semen from Aceh cattle and calculate the potential production of frozen semen in Aceh cattle in a single ejaculation and over 1 year.

MATERIALS AND METHODS

Ethical Clearance

The Ethics Committee for the Maintenance and Use of Experimental Animals by the National Research and Innovation Agency (BRIN) reviewed and approved this research protocol (Number: 094/KE.02/SK/05/2023).

Time and Place of Research

The research was performed between April and September 2023 at the AICs in Lembang and Singosari and the Animal Cell Characterization Laboratory in the Genomics Building of BRIN.

Research Sample

Five Aceh bulls, aged 6–8 years, were reared according to the standard operating procedures outlined by the AIC. This study used primary and secondary data on the quality of fresh and frozen semen obtained from both AICs. The secondary data comprised fresh semen data and frozen semen production data of Aceh bulls over 1 year (40 productions) in 2022. The primary data were collected using a five-replicate design.

Collection and Evaluation of Fresh Semen from Aceh Bulls

Semen samples were collected weekly via an artificial vagina. Subsequently, the collected semen samples were subjected to macroscopic and microscopic examinations. The macroscopic evaluation included the assessment of semen volume, color, acidity (pH value), and consistency or viscosity. The microscopic assessment included the analysis of sperm mass movement, concentration, motility, viability, morphology, plasma membrane integrity (PMI), and acrosomal integrity. The collection and evaluation of semen were performed according to the procedures previously described by Arifiantini (2012), Simoes *et al.* (2009), and Prochowska *et al.* (2022).

Frozen semen production was performed at each center using its own established procedures. Singosari AIC utilizes an egg yolk–Tris diluent with 7% glycerol, whereas Lembang AIC employs an egg yolk–skimmed milk diluent with 8% glycerol. The researchers received the frozen semen 24 h after its production.

Macroscopic Evaluation

The semen volume was determined directly by reading the scale (in milliliters) on the semen collection tube. The semen was then examined visually to determine its color. The acidity was measured using a pH indicator (Merck, range 6.4–8.0). The consistency was evaluated by tilting the tube and returning it to its original position. The semen was classified as "dilute or watery" if it returned rapidly to the bottom of the tube, "medium" if it returned slowly, and "thick" if it returned very slowly.

Microscopic Evaluation

Evaluation of sperm mass movement. A total of 10 μL of sperm was deposited onto a glass slide and observed under a microscope at 100× magnification. The scoring categories were as follows: +++ (positive 3) was assigned if a dark cloud was observed or a fast-moving sperm cloud was present.

Evaluation of sperm concentration. The sperm concentration was determined using the methodology outlined in the photometer SDM 6 manual. A 3.5-mL solution of NaCl (0.9%) was added to a 10-mm rectangular 4-mL cuvette, and 35 μL of semen was then added. Subsequently, the cuvette was sealed with a small piece of parafilm or an alternative laboratory film. The sample was thoroughly mixed and gently inverted three times. Subsequently, the "bull concentration" option was selected, and a cuvette containing only the NaCl solution was inserted into the cuvette channel of the photometer for subsequent analysis. Upon selecting the "zero" button, the NaCl control was moved, and the cuvette containing NaCl and semen was inserted into the measurement slot. The "MEASURE" button, as displayed on the device, was selected to initiate the measurement process.

Evaluation of sperm motility. Two experienced technicians subjectively evaluated the sperm motility in fresh semen. Overall, 10 μL of fresh semen was combined with 50 μL of saline solution and then homogenized. A total of 4 μL of this solution was applied to the slide and covered with a coverslip. The sperm motility was determined from five fields of view, and the values for progressive motility were expressed as percentages.

Sperm motility in frozen semen was assessed using a computerized sperm analyzer (SpermVision; Minitüb, Tiefenbach, Germany) connected to Carl Zaiss Microimaging GmbH (Göttingen, Germany) and equipped with a 37 °C warming table. The frozen semen was thawed before analysis, and the straws were thawed individually at 37 °C for 30 s. The thawed semen was transferred to a microtube and stored at 37 °C. Subsequently, 10 µL of the thawed semen was diluted with 50 µL phosphate-buffered saline (PBS) and homogenized. A total of 4 µL of this solution was placed on a microscope slide and covered with a coverslip. The total motility and progressive motility values were expressed as percentages.

Evaluation of sperm viability. The sperm viability in fresh semen was determined by mixing 10 µL of semen with 40 µL of eosin–nigrosine solution (1:4). The viability assay was performed similarly for frozen semen and fresh semen, using a 1:2 ratio of semen to dye. Both solutions were homogenized, after which the slides were prepared and dried on a heating table. The slides were then examined under a microscope at 400× magnification. Because dead sperm cells do not absorb any color, sperm viability was determined by observing whether they absorbed color.

Evaluation of sperm morphology. The sperm morphology in frozen semen differs from that observed in fresh semen. Considering that frozen semen comprises diluents, the sperm morphology was evaluated using carbol–fuchsin–eosin staining, also known as William staining (Kavak *et al.*, 2004). A total of 10 µL of the thawed semen was applied onto a slide in a thin layer. Subsequently, the slide was heat-fixed using a Bunsen burner, immersed in absolute alcohol for 3–4 min, and air-dried. Subsequently, the dried slide was rinsed with 2% chloramine solution for approximately 2 min until it appeared clean. The slide was then washed with distilled water and 95% alcohol and finally stained with carbol–fuchsin–eosin dye for approximately 6 min. The slides were then washed under tap water and dried. After staining the slides containing fresh and frozen semen, they were examined under a microscope at 400× magnification. The sperm morphology was evaluated as described by Arifiantini (2012).

Examination of sperm plasma membrane integrity (PMI). The PMI of fresh semen was examined using the hypoosmotic swelling (HOS) test (Prochowska *et al.*, 2022). Briefly, 10 µL of semen was placed in a microtube containing 1000 µL of HOS solution (1:100). The PMI of frozen semen was analyzed similarly, with the exception that the ratio of semen to HOS solution was 1:20 (50 µL of semen in 1000 µL of HOS solution).

The mixture was incubated in a 37 °C water bath for 30 min. To perform PMI examination, a 10-µL sample of the mixture was deposited onto a microscope slide, covered with a coverslip, and observed under a microscope at 400× magnification. The sperm cells were counted randomly from 10 fields of view, with a minimum of 200 cells recorded in each field. A coiled or vesicular tail indicates sperm with an intact plasma membrane, whereas a straight tail indicates sperm without an intact plasma membrane (Arifiantini, 2012).

Evaluation of acrosomal integrity. The acrosomal integrity in fresh and frozen semen was analyzed using the fluorescein isothiocyanate–peanut agglutinin (FITC–PNA) staining method (Rajabi-Toustani *et al.*, 2019). The semen samples were prepared as test samples and aerated at room temperature. The samples were fixed in 96% ethanol for 10 min at room temperature, and the slides were aerated. Overall, 30 µL of PNA lectin solution (100 µg/mL) was added, and the mixture was incubated at 37 °C for 30 min. Subsequently, the mixture was spiked with 5 µL of propidium iodide (PI; 1 µg/µL) (Sigma, St. Louis, MO) and incubated for 5 min. The slides were washed thrice with PBS to remove any unbound reagents and then covered with a coverslip. The acrosome status was analyzed using a fluorescence microscope (Axio Vision Imager z2) at 380–420 nm. A total of 200 sperm cells were observed in each treatment. The results were divided into two categories: sperm with green acrosomes were classified as intact sperm, and sperm with a red color were classified as damaged sperm. The staining procedure was performed in a dark room (Simoes *et al.*, 2009).

Evaluation of Sperm DNA Integrity in Frozen–Thawed Semen

Sperm DNA integrity was assessed exclusively in frozen–thawed semen samples. The semen samples were diluted with PBS to a concentration of 10-15 × 10^6 sperm/mL. Agarose was melted on a heating table at 95 °C–100 °C and cooled to 37 °C. From each sample, 25 µL of semen was added to a microtube containing 50 µL of liquid agarose and mixed gently. The cell suspension was then deposited into a well of a specific glass slide, which was then covered with a coverslip. The slide was subsequently stored at 4 °C for 5 min to allow the agarose to solidify. The slide was removed from the refrigerator, and the coverslip was removed by carefully sliding it off.

The lysis solution was added to the wells in a controlled manner, ensuring complete immersion, and the mixture was incubated for 5 min. The slide was tilted to remove residual lysis solution and positioned horizontally, ensuring that the mixtures in all wells were at the same height. The slide was then subjected to a 5-min wash and a 2-min dehydration process using a disposable pipette and 70% ethanol. The slide was further stained with 100% ethanol for 2 min. Before visualization and analysis under a fluorescence microscope, the fragmented sperm samples and slides were stained with a highly sensitive fluorochrome, such as fluoGreen or fluoRed. A final volume of 2 µL proved sufficient to stain each fragmented well. The number of sperm cells with fragmented or unfragmented DNA was calculated from 10 fields of view.

Potential for Frozen Semen Production

The potential for frozen semen production per ejaculation was calculated by multiplying the semen volume by the sperm concentration and motility and then dividing by the insemination dose. The annual potential for frozen semen production was calculated by multiplying the frozen semen production per ejaculation by the number of frozen semen collections and productions.

Statistical Analysis

The data were analyzed using Minitab statistical software (Minitab 18). A one-way analysis of variance at a 95% significance level was performed, followed by Tukey's test. The data were presented as the mean ± standard deviation.

RESULTS

Fresh Semen Quality

Fresh semen samples from five bulls exhibited variation in several quality indicators. Macroscopic evaluation revealed that two bulls, identified by codes 211501 and 211608, had a higher semen volume (6.1 and 6.2 mL, respectively) than the other bulls ($p<0.05$), which had semen volumes ranging from 3.7 to 4.2 mL. The pH of the semen exhibited considerable variation among the bulls but remained within the normal range. The color and consistency of the semen were also within the normal parameters (Table 1).

Based on the microscopic characteristics of Aceh bulls, semen samples exhibited no differences in sperm mass movement, motility, viability, morphology, or sperm PMI. However, compared with the other bulls, a significant difference was observed in the sperm concentration of bull 211605 (1,541 ± 79.9 × 10^6 sperm/mL), which exhibited the highest concentration. In fresh semen samples from Aceh cattle, the lowest recorded sperm concentration was 1,077 × 10^6 sperm/mL. Bull 211501 exhibited the highest acrosomal integrity at 93.80 ± 0.55. Nevertheless, all bulls exhibited high acrosomal integrity, thus demonstrating their suitability for cryopreservation and utilization in artificial insemination (Figure 1).

Frozen Semen Quality

The frozen semen samples diluted with two different diluents did not exhibit notable variation in quality. All variables showed similar values in semen samples diluted with egg yolk–skimmed milk diluent. However, bull 211710 exhibited a higher progressive motility than the other bulls (Table 2). In contrast, the semen samples diluted with Tris–egg yolk diluent showed no differences in any variables (Table 3).

Frozen Semen Production Potential

Table 4 shows the potential for frozen semen production in Aceh cattle. The study found significant

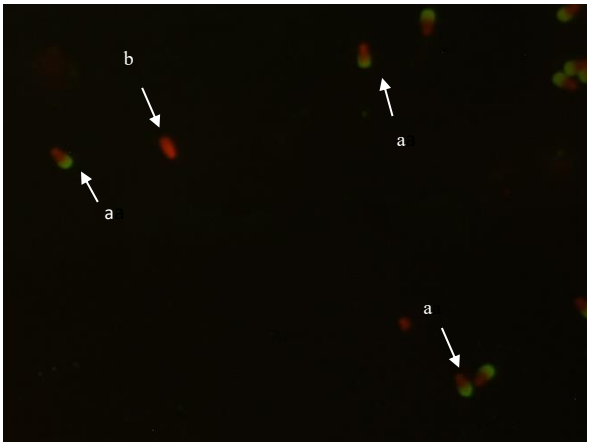

Figure 1. The integrity of the sperm acrosome in Aceh bulls. The sperm cells were stained with fluorescein isothiocyanate–peanut agglutinin (FITC–PNA). The presence of an intact acrosome is indicated by green fluorescence (a), whereas damaged acrosomes exhibit red fluorescence (b). The samples were examined under a fluorescence microscope at 400× magnification.

Table 1. Fresh semen quality of Aceh cattle based on data from five bulls

Variables	Bull codes				
	211710	211609	211608	211501	211605
Macroscopic (semen)					
Semen volume (mL)	3.8 ± 0.75^{b}	3.7 ± 0.84^{b}	6.2 ± 1.25^{a}	6.1 ± 0.67^{a}	4.12 ± 1.93^{ab}
pH	6.6 ± 0.10^{a}	6.5 ± 0.05^{ab}	6.7 ± 0.04^{a}	6.4 ± 0.17^{c}	6.4 ± 0.00^{bc}
Color	Milky white	Milky white	Milky white	Milky white	Milky white
Consistency	Average	Average	Average	Thick	Thick
Microscopic (sperm)					
Mass movement	2 ± 0.00	2 ± 0.00	2 ± 0.00	2 ± 0.55	2 ± 0.45
Progressive motility (%)	71.88 ± 2.58	73.78 ± 2.12	72.47 ± 3.58	74.36 ± 6.8	76.30 ± 3.79
Concentration (10^6/mL)	1,122 ± 117.3^{b}	1,276 ± 129.9^{ab}	1,077 ± 173.9^{b}	1,229 ± 379^{ab}	1,541 ± 79.9^{a}
Viability (%)	74.00 ± 3.62	74.21 ± 2.27	76.44 ± 3.21	73.48 ± 2.5	75.10 ± 5.6
Normal morphology (%)	83.28 ± 5.59	88.04 ± 4.65	83.53 ± 4.64	83.75 ± 2.56	82.21 ± 2.63
Plasma membrane integrity (%)	79.15 ± 4.57	79.77 ± 4.59	78.59 ± 3.30	74.82 ± 3.81	77.37 ± 3.81
Acrosomal integrity (%)	93.58 ± 0.24^{ab}	92.80 ± 0.25^{b}	93.00 ± 0.14^{b}	93.80 ± 0.55^{a}	93.10 ± 0.67^{ab}

Note: The data are presented as the mean ± standard deviation (SD) of 25 ejaculations. Means in the same row with different superscripts differ significantly ($p<0.05$).

differences ($p<0.05$) in the potential for frozen semen production in Aceh cattle between individuals. Bulls 211501 and 211605 were higher than bulls 211609 and 211608, while bull 211710 had the lowest production potential.

The production of frozen semen per ejaculation ranged from 97.05 to 268.9 straws, with an annual yield of 3,382 ± 1,810 to 11,399 ± 2,658 straws. Semen volume, concentration, and motility are all factors that influence each individual's potential for frozen semen production. Table 4 shows that these factors differ between individuals, resulting in different production potentials.

DISCUSSION

The findings of this study indicate that there were variations in the semen volume and pH between individual animals. Nevertheless, the semen volume and pH generally remained within the normal range. The semen volume of indigenous and local Indonesian cattle is generally lower than that of exotic cattle (Wahyudi *et al.*, 2022). This can be attributed to the relatively small stature of indigenous and local cattle. An evident correlation between body size and the size of the testes and accessory glands has been documented (Coulter & Foote, 1977; Rodrigues *et al.*, 2018).

The circumference of the scrotum in bulls is correlated with the volume of semen produced. In Brahman and native crossbred cattle, a scrotal circumference of 31 cm is associated with a semen volume of 2.5 mL, whereas a scrotal circumference of 36–37 cm is associated with a semen volume of >5 mL (Rashid *et al.*, 2015). In contrast, Balinese bulls in the Baturiti AIC, with a body weight of 700–800 kg, had a semen volume of 4.56–8.50 mL (average 6.32 ± 0.07 mL) (Indriastuti *et al.*, 2020). Another study reported that Bali cattle in the South Sulawesi AIC, with an average body weight of 600 kg, had a semen volume of 5.32–7.50 mL (Iskandar *et al.*, 2022). The sperm concentration of Aceh bulls in this study was considered satisfactory, considering their small body size and scrotal circumference of only 30 cm. The semen concentration of Balinese cattle with a scrotal circumference of >32 cm has been reported to range from 1,164.81 ± 9.10 × 10^6 (Indriastuti *et al.*, 2020) to 1,184.72 ± 52.74 × 10^6 sperm/mL (Iskandar *et al.*, 2022). Furthermore, the semen concentration of Pasundan cattle was 1,355.85 ± 6.06 × 10^6 sperm/mL. The sperm concentration depends on several factors, including the scrotum size and spermatogenic activity. In addition, the collection method and frequency of semen collection can influence the sperm concentration (Arifiantini, 2012). The effect of individual factors on the quality of fresh semen in Balinese cattle was previously investigated by Indriastuti *et al*. (2020).

Two different types of diluents were used in the two AICs; the Lembang AIC uses skimmed milk–egg yolk diluent, whereas the Singosari AIC uses Tris–egg yolk diluent. As shown in Tables 2 and 3, the quality of frozen semen produced showed promising results, indicating their suitability for insemination. After freeze–thawing, the variables differed between frozen semen diluted in skimmed milk–egg yolk and frozen semen diluted in Tris–egg yolk. The semen samples from Aceh bulls stored in the Tris–egg yolk diluent

Table 2. Quality of frozen–thawed semen of Aceh cattle from three bulls in skimmed milk–egg yolk diluent

Variables (sperm)	Bull codes		
	211710	211609	211608
Total motility (%)	63.31 ± 4.37^{a}	58.78 ± 1.5^{ab}	60.16 ± 1.9^{ab}
Progressive motility (%)	53.98 ± 5.15^{a}	45.50 ± 3.27^{b}	47.16 ± 5.93^{ab}
Viability (%)	66.22 ± 1.75	65.85 ± 2.90	65.47 ± 0.77
Normal morphology (%)	66.84 ± 1.96	68.09 ± 1.32	66.19 ± 2.06
Plasma membrane integrity (%)	66.31 ± 1.97	64.72 ± 2.28	67.39 ± 1.20
Acrosomal integrity (%)	90.42 ± 0.54	90.90 ± 0.60	89.5 ± 1.41
DNA integrity (%)	95.05 ± 1.59^{a}	94.58 ± 2.02^{ab}	92.17 ± 0.86^{b}

Note: The data are presented as the mean ± standard deviation (SD) of 15 ejaculations. Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 3. Quality of frozen–thawed semen of Aceh cattle from two bulls in Tris–egg yolk diluent

Variables (sperm)	Bull codes	
	211501	211605
Total motility (%)	57.17 ± 4.10	59.65 ± 3.11
Progressive motility (%)	52.58 ± 3.02	55.27 ± 3.78
Viability (%)	64.72 ± 2.61	66.58 ± 2.61
Normal morphology (%)	67.45 ± 1.12	65.69 ± 2.98
Plasma membrane integrity (%)	67.92 ± 2.00	67.52 ± 1.97
Acrosomal integrity (%)	90.38 ± 0.79	90.88 ± 0.58
DNA integrity (%)	94.86 ± 1.63	96.00 ± 1.07

Note: The data are presented as the mean ± standard deviation (SD) of 10 ejaculations. Means in the same row with different superscripts differ significantly ($p<0.05$).

Table 4. Potential production of frozen semen from Aceh cattle

Variables	Bull codes				
	211710	211609	211608	211501	211605
Semen volume (mL)	3.44 ± 1.00^{b}	3.83 ± 1.08^{b}	5.22 ± 1.33^{a}	5.46 ± 1.08^{a}	5.32 ± 1.28^{a}
Sperm progressive motility (%)	70.12 ± 0.79^{c}	70.79 ± 1.81^{c}	70.25 ± 1.04^{c}	72.84 ± 4.97^{b}	75.67 ± 4.38^{a}
Sperm concentration (×10^6/mL)	1,182 ± 132.6^{bc}	1,309 ± 212.8^{bc}	1,112 ± 133.6^{c}	1,385 ± 310.7^{ab}	1,559 ± 119.0^{a}
Total straw/collection	97.05 ± 45.25^{c}	134.72 ± 48.06^{b}	152.59 ± 53.01^{b}	268.9 ± 70.40^{a}	285.0 ± 66.4^{a}
Total straw/ year	3,382 ± 1810^{c}	5,389 ± 1922^{b}	6,104 ± 2021^{b}	10,758 ± 2817^{a}	11,399 ± 2658^{a}

Note: The data are presented as the mean ± standard deviation (SD) of 65 ejaculations, comprising 40 ejaculations from secondary data from the year 2022 and 25 ejaculations from primary data. Means in the same row with different superscripts differ significantly ($p<0.05$).

showed no difference in any variables (Table 3). The semen samples from Aceh bulls stored in skimmed milk–egg yolk diluent differed only in progressive motility. This may be because of the limited number of samples. As mentioned previously, the need for frozen semen from Aceh cattle is limited to the Aceh province; therefore, the AIC does not maintain a large number of these bulls.

This finding differs from that of the other studies on Balinese or Pasundan cattle. In Balinese cattle native to Indonesia, the sperm motility after thawing was 69.37% ± 0.41% (Indriastuti *et al.*, 2020), and that in Pasundan cattle, a local breed of cattle, ranged between 32.93% and 46.52% (Santoso *et al.*, 2021). The difference in sperm motility observed between Aceh, Bali, and Pasundan cattle supports the notion that breed influences sperm motility, as previously documented by Sukmawati *et al*. (2014). The sperm motility observed after thawing in this study was satisfactory and met the SNI quality requirements for frozen bovine semen in Indonesia, which stipulates a minimum motility of 40%.

Compared with fresh semen, the motility, viability, sperm morphology, and PMI values of frozen semen decreased by approximately 20%–25%. This can be explained by the fact that dilution, equalization, freezing, and thawing inevitably cause damage to the sperm. This damage can be attributed to the addition of glycerol-containing diluents, which are hyperosmotic (Santoso *et al.*, 2021). The processes of lowering the semen temperature to 5 °C during equilibration, freezing in liquid nitrogen, and thawing at 37 °C have been shown to cause damage to the sperm membrane (Said *et al.*, 2015).

In contrast, compared with fresh semen, the acrosomal integrity of frozen semen decreased merely by 2%–3%. This reduction in acrosomal integrity is minimal because the plasma membrane of the sperm as well as the outer and inner acrosome membranes, protect the acrosome. The sperm DNA is located within the innermost part of the sperm head and is protected by the outer and inner membranes of the acrosome and the plasma membrane. It is, therefore, a relatively protected area. The DNA integrity of frozen semen was relatively high, ranging from 92.17 ± 0.86% to 96.00 ± 1.07% (Figure 2). Other local cattle breeds, such as Pasundan cattle, exhibited lower DNA integrity, ranging from 86.83% to 89.81% (Santoso *et al.*, 2021). However, Balinese cattle demonstrated a higher DNA integrity, reaching 97.00% (Indriastuti *et al.*, 2020).

In this study, frozen semen production of Aceh cattle was classified into three categories. The high production category included bulls 211501 and 211605, which yielded 268.9 ± 70.40 and 285.0 ± 66.4 straws/collection, respectively; the medium production category comprised bulls 211608 and 211609, yielding 134.72 ± 48.06 and 152.59 ± 53.01 straws/collection, respectively; and the low production category comprised bull 211710, yielding 97.05 ± 45.25 straws/collection.

The frozen semen productivity per collection exhibits considerable variation, with the frozen semen production in Pasundan cattle ranging from 144.18 to 191.29 straws/collection (Santoso *et al.*, 2021) and that in Bali cattle ranging from 246.94 to 270 straws/collection (Iskandar *et al.*, 2022). The mean frozen semen production at the Singosari AIC for Ongole grade and Simmental cattle was 334 ± 9.76 and 374 ± 10.4 straws/collection, respectively (Isnaini *et al.*, 2019).

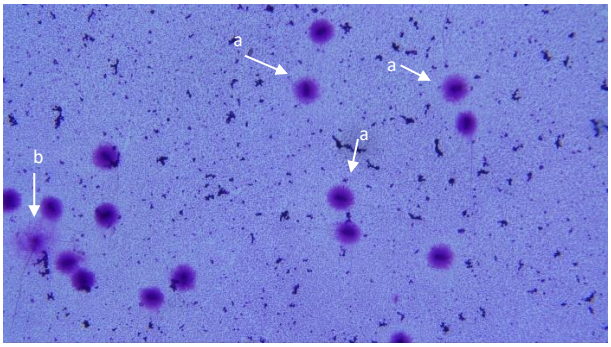

Figure 2. DNA fragmentation in Aceh bull sperm. Sperm cells were processed using the Sperm–Bos–Halomax kit and stained with propidium iodide. Sperm with a slight halo (a) exhibit a normal status of DNA fragmentation, and those with a big halo (b) contain a high proportion of fragmented DNA. The samples were examined under a fluorescence microscope at 400× magnification.

The annual frozen semen production potential of the local cattle state in the Roadmap for Indonesia's Superior Bull 2018–2022 document is 7,500 straws/year. Two bulls, 211501 and 211605, are referenced in this context and can produce more than 7,500 straws annually. Bulls 211710, 211608, and 211609 produced 3,382 ± 1,810 to 6,104 ± 2,121 straws/year. Moreover, the results of this study demonstrated that bulls 211501 and 211605 produced 10,758 ± 2817 and 11,399 ± 2658 straws/year, respectively. The increased production potential of bulls 211501 and 211605 can be attributed to their high sperm motility and concentration.

The results of this study do not accurately reflect the actual productivity of Aceh bulls. From a physiological standpoint, bull semen can be collected two to three times per day; however, the production of frozen semen occurs on a biweekly basis. If collection is performed for 40 weeks, frozen semen will be produced 80 times per year. Therefore, all bulls can double their frozen semen production. Bull 211710 was the least productive, and even though the semen was harvested 80 times, the resulting straws produced were less than 7,500 per year.

The rationale for calculating frozen semen production for 40 weeks rather than 52 weeks per year is to accommodate the variable health status of the bulls and the occurrence of public holidays. It is reasonable to conclude that the AIC's policy is to produce frozen semen from Aceh cattle for artificial insemination in the Aceh Province once weekly for 40 weeks per year. The limitation of this study is the small number of cattle available. The collection and production of semen from Aceh cattle were not performed according to the physiological potential of the cattle. Further research is warranted to determine the potential of local cattle

in Indonesia. This will enable AICs to rear animals according to the frozen semen requirements and the frozen semen production potential of each breed.

CONCLUSION

There was considerable variability in the quality of fresh semen obtained from Aceh cattle. There was no difference in the quality of frozen semen, except for the higher progressive motility in semen diluted with skimmed milk–egg yolk. The Aceh cattle exhibited the highest potential for frozen semen production, with an average yield of 11,399 ± 2,658 straws/year. Moreover, the lowest potential was observed in one individual (211710), with an average yield of 3,382 ± 1,810 straws/year.

CONFLICT OF INTEREST

We declare that there is no conflict of interest with regard to any financial, personal, or other relationships with individuals or organizations related to the material discussed in this manuscript.

ACKNOWLEDGEMENT

The authors express their gratitude and greatest appreciation to the technicians and staff of Singosari and Lembang Artificial Insemination Centers, National Research, and Innovation Agency (BRIN) for facilitating the research.

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