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Fresh and Frozen Semen Quality of Horned and Polled Bali Bulls

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Abstract

Semen quality is an important factor in the success of artificial insemination (AI). This study aims to determine the quality of fresh and frozen semen from horned and polled Bali bulls. This research was conducted at the Regional Artificial Insemination Center (RAIC) of South Sulawesi and the In Vitro Embryo Production Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The research materials were the semen of two horned Bali bulls and two polled Bali bulls, each 5-6 years old with 4 repetitions each. Parameters observed in fresh semen include volume, pH, color, concentration, motility, viability, abnormality, and an intact plasma membrane. While the parameters in frozen semen include motility, viability, abnormality, an intact plasma membrane, acrosome integrity, and DNA integrity. Viability and abnormality were tested using eosin-nigrosin, intact plasma membrane was tested using hypoosmotic swelling (HOS), acrosome integrity was tested using formal saline, and DNA integrity was tested using acridine orange (AO). The data obtained were tested with a T-test (Independent sample test).

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The results showed that there was no significant difference (P > 0.05) between fresh and frozen semen of polled Bali bulls and horned Bali bulls on all semen quality parameters. The conclusion is that fresh and frozen semen from polled and horned Bali bulls have the same quality.

Keywords: Horned Bali Bulls; Fresh Semen; Frozen Semen; Semen Quality; Polled.

1. Introduction

The development of Bali cattle is growing with the discovery of hornless Bali cattle (Polled cattle) in the early 1980s in Sidenreng Rappang, South Sulawesi [1]. Although they don't have horns, generally those surveyed have the same characteristics as horned Bali cattle [2]. The trait of not having horns (Polled) is a trait that is inherited through an autosomal dominant pattern [3]. Polled Bali cattle are considered to have superiority and high productivity traits) [1]. However, in reality, there are indications that hornless male Bali cattle have a lower libido than horned ones [4], which has an impact on the difficulty of collecting semen. In order to develop Bali Polled cattle in the community through artificial insemination techniques, it is necessary to know the semen quality characteristics of the two Bali cattle breeds. This is because the quality of semen has an important role in the success of Artificial Insemination, so it is necessary to carry out a thorough and careful examination of the semen of bull bulls. The standard method for evaluating male fertility is predictability of conception by assuring the quality of the semen. Semen quality tests are carried out immediately after being collected or before experiencing dilution which can be done in two ways, namely microscopically including volume, color, consistency and pH of semen, then microscopically including individual motility, survival percentage, concentration and abnormalities [5]. Examination was carried out on fresh and frozen semen. The quality of frozen semen produced must be of good quality according to SNI 4869-1:2017 frozen semen [6] including covering the intact acrosome cap and DNA integrity.

The quality of frozen semen of horned and polled Bali bulls produced is expected to comply with Indonesian National Standard 4869-1:2017 for frozen semen because it will be disseminated to the public and can also increase the success of AI. Therefore, this study was conducted to evaluate the quality of horned and polled Bali bull semen (fresh and frozen), including macroscopic and microscopic quality as well as acrosome integrity and DNA integrity.

2. Materials And Methods

2.1. Materials

The research was conducted from September 2022 to January 2023 at the Regional Artificial Insemination Center (RAIC) of South Sulawesi and the In Vitro Embryo Production Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The research materials were the semen of two horned Bali bulls and two polled Bali bulls, each 5-6 years old.

2.2. Methode

2.2.1. Evaluation of Fresh Semen Quality

Evaluation of the quality of fresh semen of horned and polled Bali bulls conducted at the RAIC of South Sulawesi includes the volume of semen observed directly in the semen collection tube immediately after collection with units of milliliters (ml) [9]. The pH of the semen is observed using a pH meter and then seen; the pH of normal bull semen ranges from 6.4 to 7.8. Semen color in bulls is generally yellowish white or almost milky white. Furthermore, the concentration of spermatozoa is done by mixing 0.035 ml of semen sample with 3.5 ml of 0.9% NaCl solution. The solution was then homogenized for 5-7 seconds, then transferred into a cuvette to measure the concentration using a photometer. Individual movement, or individual motility, was observed under a microscope with 400-fold magnification at a constant temperature of 37 ºC using a cover glass, then determined as the proportion or percentage of spermatozoa that moved progressively [3]. Motility is assessed subjectively by looking at the number of spermatozoa that move straight ahead (progressive). The standard motility assessment is in the range of 0–100%.

The viability of spermatozoa is a parameter observed to determine live and dead spermatozoa. To assess viability, 10 µL of semen was placed on an object glass, 50 µL of eosin-nigrosin dye was added, homogenized and made a smear preparation, and dried on a warming table for 15-20 seconds. The preparations were observed under a microscope using 400-fold magnification. Live spermatozoa do not absorb color, and dead spermatozoa will absorb color. Live and dead spermatozoa were counted in 10 fields of view [10]. The percentage of live spermatozoa was calculated using the formula [11]:

$$Percentage\ of\ Live\ spermatozoa\ = \frac{The\ number\ of\ live\ spermatozoa}{The\ number\ of\ spermatozoa\ counted}\ x100\%$$

Abnormalities of spermatozoa can be classified into three parts, namely abnormalities in the head, center, and tail. Abnormalities in the head such as being too large or small, pointed or blunt, having two heads, acrosome damage, or amorphous. Abnormalities in the center, such as a thick or thin neck, a tail not in the center of the neck, or a crooked neck. While abnormalities in the tail such as a bent tail, short tail, or curled from the tip of the tail [12]. Examination of abnormal spermatozoa using eosin-nigrosin dye. The preparations were observed under a 400-fold magnification microscope at 10 different fields of view, with a minimum of 200 cells. The percentage of abnormal spermatozoa is characterized by abnormalities in spermatozoa cells such as the head, body, or tail of the sperm. The abnormality formula as used by [11]:

$$Percentage\ of\ Abnormality = \frac{The\ number\ of\ abnormal\ spermatozoa}{The\ number\ of\ spermatozoa\ counted}\ x100\%$$

Intact plasma membranes were evaluated using a hypoosmotic swelling (HOS) solution. A total of 10 µL of semen was placed into a microtube containing 1,000 µL of HOS solution (1.351 g fructose and 0.735 g Na-citrate in 100 mL distilled water; osmolarity: 150 mOsm), and homogenized. The solution mixture was kept in a water bath at 37°C. Intact plasma membrane of spermatozoa was performed 30 minutes after incubation by

dripping one drop of the solution mixture on an object glass, covering it with a cover glass, and observing it under a microscope with a 400-fold magnification [10]. Spermatozoa with damaged plasma membranes are characterized by a straight tail, while spermatozoa with intact plasma membranes are characterized by a circular or bulging spermatozoa tail. The number of spermatozoa was observed in a minimum of 200 cells in 10 fields of view [13]. The percentage of spermatozoa that reacted to the HOS solution was calculated using the following formula [11, 14]:

$$Percentage\ of\ Intact\ Plasma\ Membrane = \frac{The\ number\ of\ reacted\ spermatozoa}{The\ number\ of\ spermatozoa\ counted}\ x100\%$$

2.2.2. Evaluation of Frozen Semen Quality

Evaluation of frozen semen quality of horned and polled Bali bulls was conducted at the In Vitro Embryo Production Laboratory, Faculty of Animal Husbandry, Hasanuddin University. Parameters observed included motility, viability, abnormality, and an intact plasma membrane of spermatozoa. Observation procedures were carried out similarly to observations on fresh semen. The success of artificial insemination must be accompanied by good spermatozoa quality, which is seen not only in the progressive motility of spermatozoa but also in the acrosome integrity of spermatozoa [15]. The acrosome cap is a layer covering the nucleus in which there is a collection of enzymes that function to help the nucleus enter the egg cytoplasm during fertilization by damaging the egg wrapping layer through the acrosome reaction [16]. Damage to the acrosome cap of spermatozoa is caused by the process of handling and freezing semen. Ice crystals due to excessive cell dehydration can cause damage to the acrosome cap of spermatozoa [17]. One drop of semen is put into a microtube containing formalin-saline solution (1% formasaline and 0.09% physiological NaCl). Semen that has been thawed is included in the formal saline solution in a ratio of 1:4. It was left for 5 minutes, and one drop was taken, placed on an object glass, and covered with a cover glass [18]. Observations were made under a phase-contrast microscope with 400-fold magnification. Spermatozoa that have an intact acrosome cap are characterized by 1/2 to 2/3 of the anterior part of the head being darker than the posterior part. The examination was performed in 10 different fields of view. Calculation of the percentage of acrosome integrity using the formula:

$$Percentage\ of\ Acrosome\ Integrity = \frac{The\ number\ of\ danaged\ spermatozoa}{The\ number\ of\ spermatozoa\ counted}\ x100\%$$

Damage to the chromatin deoxyribose nucleic acid (DNA) of spermatozoa is an important factor causing infertility [19]. Therefore, it is very important for spermatozoa to have high DNA integrity for successful pregnancy. According to research by [20], high sperm DNA damage will cause low pregnancy rates and an aborted pregnancy.

Frozen semen was taken and made into a smeared preparation on an object glass, then dried using a Bunsen burner. After that, it was fixed in Carnoy (200 ml) for 2 hours and then rinsed with distilled water. After that, soak in acridine orange (AO) overnight, then rinse with distilled water and dry. Clean the bottom of the glass object and cover it using cover glass. The preparations were observed under a fluorescent microscope at 400-fold magnification. The heads of spermatozoa with good chromatin integrity will be green, while those with

reduced chromatin integrity will be red, yellow, or orange [11]. The examination was conducted on 200 spermatozoa for each sample. The percentage of spermatozoa DNA integrity was calculated using the formula:

$$Percentage\ of DNA\ Integrity = \frac{Total\ percentage\ of\ reacting\ spermatozoa}{The\ number\ of\ spermatozoa\ counted}\ x100\%$$

2.3. Data Analysis

The data obtained will be analyzed using a comparative test, namely the T-test (T-test Independent sample), to compare semen samples of horned and polled Bali bulls.

3. Result and discussions

3.1. Fresh Semen Quality Of Horned And Polled Bali Bulls

Fresh semen of horned and polled Bali bulls that have been collected are immediately observed for semen quality. Semen quality tests conducted include macroscopic and microscopic quality. Macroscopic quality testing includes volume, color, and pH of semen, while microscopic quality testing includes concentration, motility, viability, abnormality, and an intact plasma membrane. The results of the fresh semen quality evaluation of horned and polled Bali bulls can be seen in Table 1.

Table 1. Quality Evaluation of Fresh Semen of Horned and Polled Bali Bulls

Parameters	Bali Bulls		Statistic
	Horned	Polled	
Macroscopic			
Color	Cream	Cream	
pH	6.4±0.00	6.4±0.00	Ns
Volume (ml)	5.9±1.21	5.07±1.74	Ns
Microscopic			
Concentration (x10^6/ml)	1.035±0.30	0.921±0.05	Ns
Motility (%)	70±0.00	67.5±2.88	Ns
Viability (%)	91.51±0.58	91.40±0.67	Ns
Abnormality (%)	5.41±0.40	5.50±0.65	Ns
Intact Membrane Plasma (%)	90.89±0.45	90.73±0.84	Ns

3.1.1. Color of Semen

The color of the semen of horned and polled Bali bulls obtained in this study is cream-colored. This indicates that the semen color obtained in this study was categorized as normal. This is in accordance with the opinions of [21] and Feradis [22] that normal bull semen was milky white or whitish cream and cloudy. [3] also reported that, in general, bull semen was yellowish white or almost milky in color, the cloudier the color, usually the more spermatozoa per milliliter.

3.1.2. pH of Semen

pH is the degree of acidity in the semen, which indicates whether the semen has an acidic or alkaline pH. Table 1 shows that the pH of fresh semen from horned and polled Bali bulls was 6.4±0.0. The pH value is the normal

pH of the semen. This is in accordance with the opinions of [3] and [9] that the pH of bull semen is relatively normal, ranging from 6.4 to 7.8. Furthermore, [23] reported that in general, the pH value of Bali bull ranges from 6.2–6.8.

The pH value of bull semen tends to be acidic or too low, or it can be caused by high levels of lactic acid, which is the result of metabolism. In addition, a pH of semen that is too acidic can also be caused by damage to the plasma membrane or acrosome [24]. A pH value that was too high or tended to be alkaline was caused by excessive fluid production by the accessory glands. In addition, the pH of the semen that was too alkaline could also be caused by the large number of dead spermatozoa [25].

3.1.3. Volume of Semen

Semen volume can be seen on the scale of the tube used to hold the cement. The volume of semen varies for each shelter.Table 1 showed that the average fresh semen volume of a horned Bali bull was 5.9±1.21 ml and that of a polled Bali bull was 5.07±1.74 ml. There was no significant difference ($P > 0.05$) in the fresh semen volume of the two Bali bulls. The volume of fresh semen produced by the two Bali bulls is still included in the normal condition. In accordance with the opinion of [9], the volume of bull semen varies with each collection, which is between 1–15 ml or 5-8 ml. [26] reported that normal bull semen characteristics have a volume of 5-8 ml. Even the semen volume in Bali bulls showed higher results than those reported by [27], which was 5.52 ± 0.91 ml but lower than the report of [11], which was 6.27 ± 1.79 ml.

3.1.4. Concentration of Spermatozoa

The spermatozoa concentration of horned Bali bulls was $1.035 \pm 0.30 \times 10^6$ cells/ml, and that of polled Bali bulls was $0.921 \pm 0.05 \times 10^6$ cells/ml. There was no significant difference ($P>0.05$) between the concentration of spermatozoa in both Bali bulls. The spermatozoa concentration of polled Bali bulls was lower than that of horned Bali cattle. [28] revealed that the concentration, motility, and abnormality of polled Bali bulls' semen were significantly lower than those of horned Bali bulls. The concentration obtained was even lower, ranging from $0.49 \pm 0.15 \times 10^6$ cells/ml in horned Bali bulls to $0.31 \pm 0.12 \times 10^6$ cells/ml in polled Bali bulls.

The higher the concentration of spermatozoa, the thicker the consistency of the semen. Meanwhile, if the concentration is low, the consistency of the semen will also be thinner [29]. According to [30], one of the factors affecting fresh-semen concentration is the quality of feed given to each animal.

3.1.5. Motilty of Spermatozoa

Motility is the ability of spermatozoa to fertilize the ovum [31]. Spermatozoa require progressive movement while in the female reproductive tract in order to reach the place of fertilization with the ovum [32]. The results (Table 1) showed that the average percentage of spermatozoa motility in horned and polled Bali bulls was 70±0.00% and 67.5±2.88%, respectively. There was no significant difference ($P > 0.05$) between the sperm motility of horned and polled Bali bulls. It implies that the semen of horned and polled Bali bulls has similar quality. The percentage of motility of spermatozoa in horned Bali bulls and polled Bali bulls meets the standard and is suitable for further processing. In accordance with [33], the requirement for bovine spermatozoa to be

alive and moving forward (motile) after dilution should be 55%. Furthermore, research by [28], that the motility of polled Bali bulls' spermatozoa after dilution, equilibration, and post-thawing is lower than that of horned Bali bulls.

3.1.6. Viability of Spermatozoa

Viability, or the percentage of live spermatozoa in fresh semen of polled horned Bali bulls obtained in this study, which is 91.51 ± 0.58% and 91.40 ± 0.67 showed no significant difference (P > 0.05). It means that fresh semen from horned and polled Bali bulls is suitable to be processed into frozen semen. The observation of sperm viability in horned and polled Bali bulls can be seen in Figure 2.

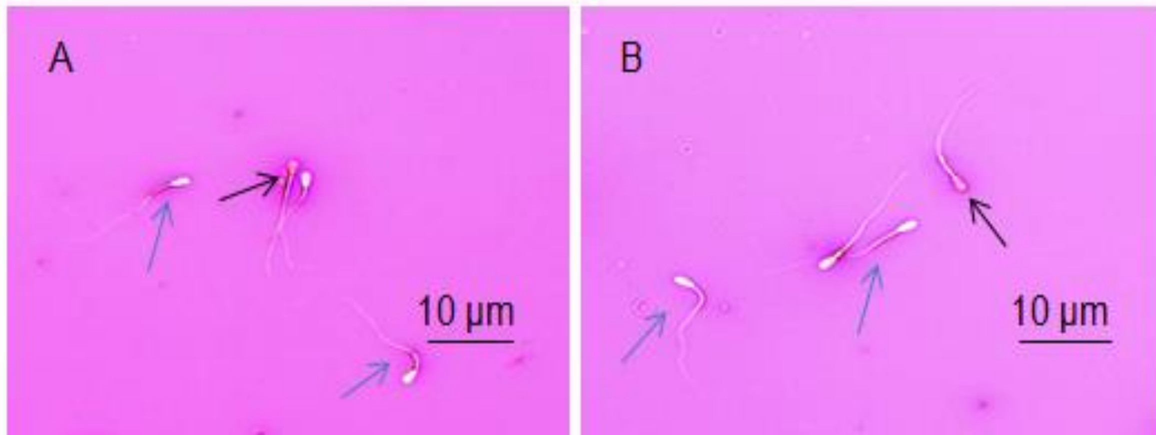

Figure 2: Observation of Spermatozoa Viability (A: Horned Bali Bull Spermatozoa; B: Polled Bali Bull Spermatozoa). Blue arrows: live spermatozoa; black arrows: dead spermatozoa.

The results of the study were in accordance with the statement [34] that fresh semen to be processed must contain at least 70% live spermatozoa. The results of research by [35] reported that the sperm characteristics of Bali bulls assessed using CASA obtained an average fresh semen viability of 74.7%. [14] added that the viability of fresh Bali bulls' semen averaged 96.91 ± 1.59%.

3.1.7. Abnormality of Spermatozoa

Abnormality of spermatozoa of horned and polled Bali bulls showed no significant difference (P > 0.05). The abnormality of the spermatozoa of horned Bali bulls obtained was 5.41±0.40% and that of polled Bali bulls was 5.50±0.65%. The results of this study indicate that fresh semen is suitable for further processing. [26] reported that spermatozoa abnormalities should not exceed 15% in bulls. The observation results of abnormalities in horned and polled Bali bull spermatozoa can be seen in Figure 3.

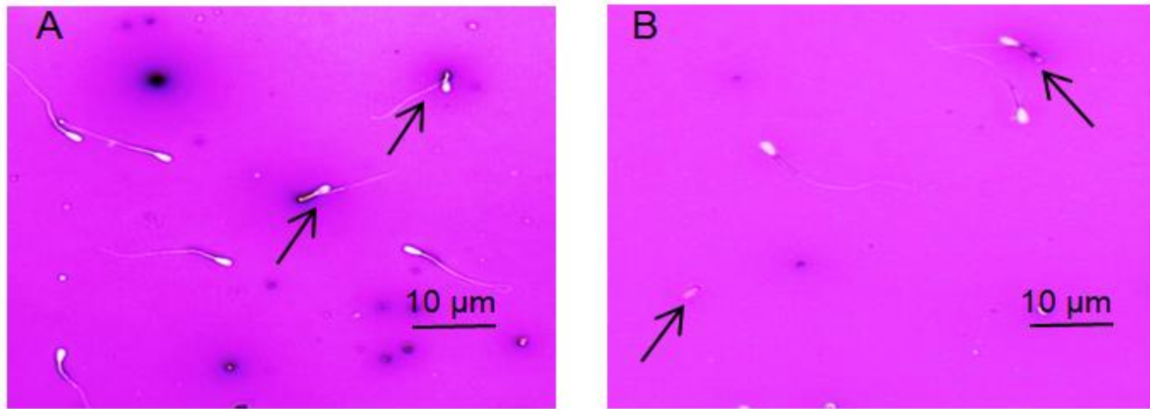

Figure 3: Observation of Spermatozoa Abnormality (A: Horned Bali Bull Spermatozoa; B: Polled Bali Bull Spermatozoa).

Abnormalities of spermatozoa can occur during the process of spermatozoa formation in the seminiferous tubules (a primary abnormality) or when spermatozoa pass through the male reproductive organs (a secondary abnormality). Abnormality can also occur due to improper [36]. This result has a lower abnormality value than the research of [37], with an average abnormality value of 10.34% in Aceh bulls from the normal range of 12-23%.

3.1.8. Intact Plasma Membrane of Spermatozoa

The intact plasma membrane test is one of the vital parameters to be observed because it is related to the permeability of the plasma membrane, which plays a role in protecting spermatozoa [12]. The percentage of intact plasma membrane of spermatozoa in this study was 90.89±0.45% for horned Bali bulls and 90.73±0.84% for polled Bali bulls, respectively. The two Bali bulls showed no significant difference (P>0.05) in the parameter of intact plasma membrane. However, when viewed from the average intact plasma membrane value obtained, horned Bali bulls have a relatively higher intact plasma membrane value than polled Bali bulls. The resulting intact plasma membrane values are already included in the viable category because they are more than 60%. [36] states that fresh semen that has a percentage of intact plasma membrane less than 60% can be categorized as infertile semen. The results of this study are higher than previous studies by [37] that the MPU value of fresh semen for horned Bali bulls is 72.43 ± 7.32 and for Bali polled bulls is 76.58 ± 6.53.The observation of plasma membrane of spermatozoa in horned and polled Bali bulls can be seen in Figure 4.

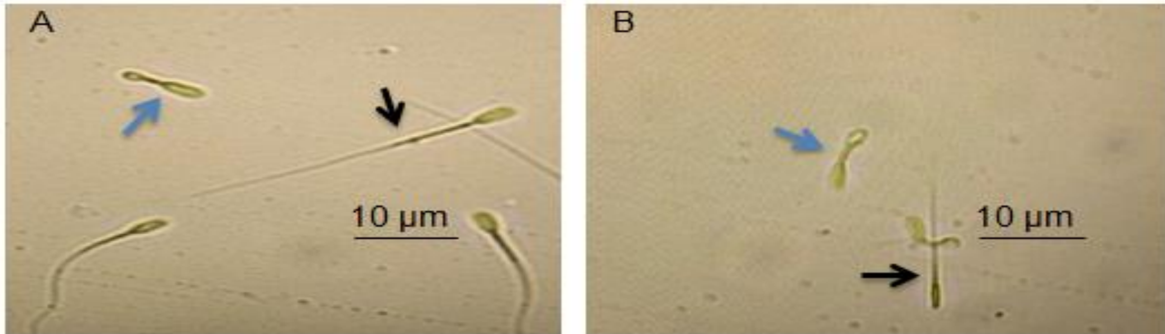

Figure 4: Observation of the Intact Plasma Membrane of Spermatozoa (A: Horned Bali Bull Spermatozoa; B: Polled Bali Bull Spermatozoa). Blue Arrows: Intact plasma membrane; Black Arrow: Incomplete plasma membrane.

The percentage of intact plasma membrane in fresh semen of horned and polled Bali bulls, which is not significantly different, was also reported by[39], where the percentage of fresh semen in polled Bali bulls was 76.90% while that in horned Bali bulls was 72.42%. The integrity of the plasma membrane determines the life and death of spermatozoa; therefore, the percentage of intact plasma membrane should not differ much from the value of live spermatozoa. [40] added that a damaged plasma membrane can cause spermatozoa to die.

3.2. Frozen Semen Quality Of Horned And Polled Bali Bulls

Frozen semen that has been made at RAIC of South Sulawesi is immediately observed for quality at the In Vitro Embryo Production Laboratory, Faculty of Animal Husbandry, Hasanuddin University. The results of the study of frozen semen quality in horned and polled Bali bulls can be seen in Table 2.

Table 2: Quality Evaluation of Frozen Semen of Horned and Polled Bali Bulls.

Parameters	**Bali Bulls**	
	Horned	**Polled**
Motility (%)	63.75±1.44	58.12±2.39
Viability (%)	90.35±0.38	89.87±0.25
Abnormality (%)	7.14±1.13	8.15±0.37
Intact Membrane Plasma (%)	89.61±0.42	89.33±0.39
Acrosome Integrity (%)	90.06±0.47	89.79±0.39
DNA Integrity (%)	90.16±0.51	89.76±0.67

3.2.1. Motilty of Spermatozoa

Table 2 shows the observation results of spermatozoa motility in frozen semen of horned and polled Bali bulls. The study results showed no significant difference ($P > 0.05$) in both Bali bulls. The percentage of sperm

motility in horned Bali bulls was 63.75 ± 1.44% and that in polled Bali bulls was 58.12 ± 2.39%. The percentage of frozen semen motility obtained in this study meets the requirements set by Indonesian National Standard 4869.1-2017 for frozen semen, which is frozen semen motility of at least 40%. The average value of the percentage of motility of Bali polled bulls post-thawing was lower than that of horned Bali cows. This means that polled Bali bull spermatozoa have a lower resistance to freezing compared to horned Bali bull spermatozoa. A study by [28] showed that the motility of spermatozoa of polled Bali bulls has a lower resistance after freezing compared to horned Bali bulls, with the percentage of motility of polled Bali bulls being 45.41±0.83 and that of horned Bali bulls being 49.58±0.83.

The decrease in the percentage of sperm motility after freezing is caused by significant changes in temperature. These conditions can reduce the quality of spermatozoa, resulting in a decrease in the percentage of motility. [41] explain that the decrease in spermatozoa motility after freezing and thawing can occur in the range of 24-64%. [42] also added that the freezing process can reduce motility by 30–60% and cause changes in spermatozoa morphology, mitochondrial damage, and acrosome damage.

3.2.2. Viability of Spermatozoa

The results of the study of the spermatozoa viability of horned and polled Bali bulls were 90.35±0.38% and polled 89.87±0.25%, respectively. The viability of spermatozoa from both Bali bulls showed no significant difference (P>0.05). The results of this study are higher than the report of [28], where the viability of spermatozoa from horned Bali bulls was 72.55±2.00% and that of polled Bali bulls was 56.95 ± 3.74%. Another report was also reported by [43] that the viability of spermatozoa in polled Bali bulls was 56.05±4.58% and in horned Bali bulls was 54.58±6.53%. The viability of spermatozoa in polled Bali bulls tends to be lower than the viability of spermatozoa in horned Bali bulls. This indicated that spermatozoa in polled Bali bulls are more susceptible to death when frozen than spermatozoa in horned Bali bulls, a phenomenon thought to be caused by the effect of cold shock. According to [10], cold shock can cause spermatozoa to be damaged, especially in the plasma membrane. The plasma membrane is a protector of spermatozoa that is directly affected by environmental factors. If the spermatozoa membrane is damaged in the head, it will be detected in the observation of viability, so that many spermatozoa will be colored when stained.

3.2.3. Abnormality of Spermatozoa

The results of spermatozoa abnormalities in horned and polled Bali bulls were 7.14±1.13% and 8.15±0.37%, respectively. There was no significant difference (P > 0.05) in the abnormality parameter for both Bali bulls. Table 2 showed that, on average, post-thawing spermatozoa abnormality in polled Bali bulls was higher than that in horned Bali bulls. The research of [44] was even higher, with the value of sperm abnormality in horned Bali bulls at 15.90±5.70% and polled Bali bulls at 19.18 ± 6.39%. Furthermore, Hasbi and colleagues (2023) also reported that the abnormality of Bali polled bulls' spermatozoa was higher than that of horned Bali bulls at 8.85±2.87% and 6.21±1.33%.

Abnormal spermatozoa, either primary or secondary, cannot fertilize the egg. In accordance with the standards of the Regulation of the Minister of Agriculture of the Republic of Indonesia No. 10/Permentan/PK.210/3/2016,

frozen semen produced and circulated must have an abnormality of less than 20%. If the abnormality of the spermatozoa passes 30 to 35%, it indicates infertility.

3.2.4. Intact Plasma Membrane of Spermatozoa

The results of the study of intact plasma membranes of spermatozoa in horned Bali bulls were 89.61 ± 0.42% and polled Bali bulls were 89.33 ± 0.39%. Intact plasma membrane parameters showed no significant difference ($P > 0.05$) in both Bali bulls. The percentage of intact plasma membranes post-thawing in this study decreased from fresh semen. The decrease in the percentage of intact plasma membrane is because, during the freezing process, spermatozoa experience many extreme temperature drops. [45] states that a significant decrease in temperature is a major factor in spermatozoa experiencing cold shock, which can damage spermatozoa, including plasma membranes. The results of this study are higher than [39], where the frozen semen of polled Bali bulls was 36.65% and the frozen semen of horned Bali bulls was 35.70%. Furthermore, [43] reported that the intact plasma membrane of horned Bali bull spermatozoa was 53.08±7.23% and polled Bali bull spermatozoa was 54.79±3.73%. The plasma membrane in spermatozoa plays a role in protecting organelles in the cytoplasm and as a medium for electrolyte transport, which is important for spermatozoa metabolism [36, 40] added that a damaged plasma membrane can cause spermatozoa to die.

3.2.5. Acrosome Integrity of Spermatozoa

The results of the study of acrosome integrity of spermatozoa in horned and polled Bali bulls were 90.06±0.47 and 89.79±0.39%, respectively. The parameter of spermatozoa acrosome integrity showed no significant difference ($P>0.05$) in both frozen semen of Bali bull. The acrosome integrity of horned Bali bull spermatozoa is relatively higher when viewed from the average value compared to polled Bali bull spermatozoa. The observation of acrosome integrity of spermatozoa in horned and polled Bali cattle can be seen in Figure 5.

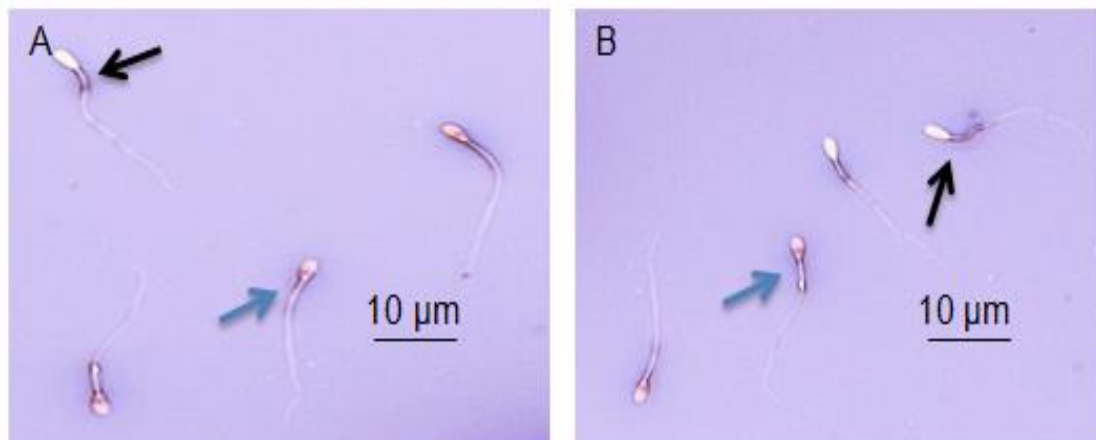

Figure 5: Observation of Spermatozoa acrosome integrity (A: Horned Bali Bull Spermatozoa; B: Polled Bali Bull Spermatozoa). Blue Arrows: acrosome integrity; Black Arrow: a damaged acrosome.

This study showed higher results than [10] in terms of the acrosome integrity of frozen semen of bulls, where the acrosome integrity obtained was 71.91%, but lower results than the findings of [43] for horned and polled

Bali bulls, which were 89.49±5.16% and 92.98±5.18% respectively. [46] recommends that at least 65% of the acrosome integrity be present in frozen semen, indicating that the acrosome integrity in Bali bulls is in the good category.

The observation of acrosome integrity is considered important to do because it is useful to determine the ability of spermatozoa in the fertilization process. [16] explained that the acrosome cap is a layer covering the nucleus, and there is a collection of enzymes in it that have a function to help the nucleus enter the cytoplasm of the ovum during fertilization through the acrosome reaction.

The spermatozoa head is divided into two parts: the anterior acrosome, wrapped by the acrosome cap, and the posterior acrosome. In the acrosome cap, there are acrosin, hyaluronidase, and other hydrolytic enzymes that are involved in the fertilization process [47]. For that, the acrosome cap must remain intact to protects the release of enzymes and genetic material before it is released in the female reproductive organs [49].

3.2.6. DNA Integrity of Spermatozoa

The results of DNA integrity of spermatozoa in horned and polled Bali bulls were 90.16±0.51% and 89.76±0.67%, respectively. Both frozen semen in Bali cattle showed no significant difference (P>0.05) in DNA integrity parameters. This means that the DNA integrity of spermatozoa in horned and polled Bali cattle is in the good category.

Recent research conducted by [43] found that the DNA integrity of spermatozoa in polled Bali bulls reached 99.35 ± 0.51, while in horned Bali bulls it reached 98.12 ± 1.75. According to [10], the average percentage of spermatozoa DNA integrity in several types of cattle is 92.06 ± 2.4%. [49] also reported that DNA damage reaching 37.11% can affect miscarriage in Brahman and Balinese cattle, but sperm DNA damage of 10.66% did not affect miscarriage in these cattle. The observation results of DNA integrity in spermatozoa of horned and polled Bali bulls can be seen in Figure 6.

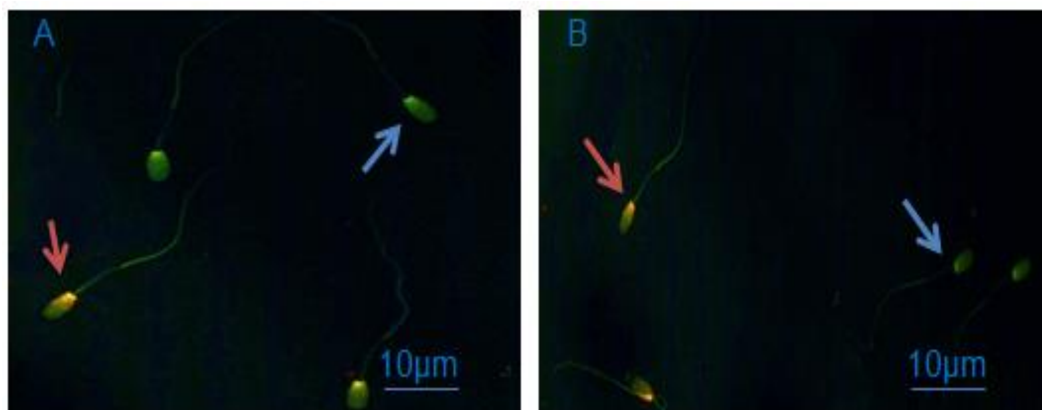

Figure 6: Observation of DNA Integrity of Spermatozoa (A: Horned Bali Bull Spermatozoa; B: Polled Bali Bull Spermatozoa). Blue arrow: intact spermatozoa DNA; red arrow: damaged spermatozoa DNA.

Spermatozoa with high DNA damage are one of the causes of miscarriage in cattle. Therefore, high spermatozoa DNA integrity is very important to achieve a successful pregnancy. Research by [20] showed that high spermatozoa DNA damage can lead to low pregnancy rates and high miscarriage rates. The standard of spermatozoa DNA damage that is not recommended for fertilization in pigs is 6%, in cattle it is 10–20%, in horses it is 28%, and in humans it is 25–30%. [48] also added that spermatozoa DNA damage in cattle of 10–20% is not recommended for use because it can inhibit fertilization.

4. Conclusion

Based on the results of the research conducted, it can be concluded that the quality of fresh and frozen semen from horned and polled Bali bulls does not show a significant difference or have the same quality.

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