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Comparison of Fresh and Cryopreserved Semen Quality of Polled and Horned Bali Bulls

Research Article

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ABSTRACT

A purpose of study is to examine sperm quality of Bali polled and horned bulls. The semen samples collected from 4 Bali polled bulls and 4 horned bulls aged between 5 and 7 years using an artificial vagina, then evaluated macroscopically and microscopically. A macroscopic evaluation revealed no significant differences of semen between polled and horned cattle. However, the microscopic evaluation revealed that sperm concentration, motility, and abnormality of polled bulls were significantly lower than the horned bulls, and there was no apparent difference between viability and membrane integrity. Except for abnormalities and DNA integrity of spermatozoa, all parameters of sperm quality were not significantly different between polled and horned bulls. Sperm abnormalities in polled bulls were significantly higher than in horned ones. However, sperm DNA integrity of the polled bulls was significantly higher than horned bulls. The analysis of CASA revealed that most kinematics were higher in polled bull spermatozoa than in horned ones, except for wobble (WOB) or ration of velocity average path (VAP) and velocity curvilinear (VCL) and amplitude of lateral head displacement (ALH), which were higher in horned bulls than in polled bulls. We therefore concluded that the quality of fresh sperm from horned bulls was better than polled bulls. However, polled bulls semen has greater freezing ability than the horned ones. Furthermore, the polled and horned bulls had the same semen characteristics that enable them for fertilizing ability, and both bulls have good DNA integrity.

KEY WORDS Bali bulls, breed, horned, polled, sperm characteristics.

INTRODUCTION

Indonesia has a comparatively high level of biological diversity, including farm animals. Some native Indonesian cattle, including Bali, Madura, Aceh, and Peranakan Ongole (PO) cattle. Bali cattle (*Bos sondaicus*) is one of the Indonesian native cattle as including quite a large population. Bali cattle are the result of the domestication of wild Banteng (Martojo, 2003; Rahayu, 2014) that were domesticated in Indonesia before 3500 years ago (Garrick and Ruvinsky, 2015). Bali cattle have great economic potential since they have various benefits compared to cattle from other countries. They have a high reproductive rate, minimal calf mortality, good adaptability to new environments, and high carcass production, among other characteristics (Purwantara *et al.* 2012).

However, during its evolution, Bali cattle without horns and known as polled were discovered. Bali polled cattle are those whose horns do not grow naturally (Hasbi *et al*. 2021). Baco *et al*. (2020) reported that Bali polled cattle have advantages in terms of maintenance management, the productivity of the meat, and reducing the risk of injury to livestock caused by horned ones. It can prevent damage to the skin and bruising of the carcass. Therefore, the selection of Bali-polled cattle seems very important, especially in modern livestock management.

Semen quality is one of crucial evaluations in the efficiency of the livestock breeding program. Bali cattle are the most preferred cattle for the small-sized farmer because the individuals of this breed have good fertility, good conception, and a low mortality rate (Purwantara *et al*. 2012). However, unlike Bali horned bull, data regarding the reproductive performance of the Bali polled bull are still very limited. Therefore, this study aimed to examine the characteristics of sperm quality of Bali polled bulls and compare it with the horned ones.

MATERIALS AND METHODS

Ethical approval
The Animal Ethics Commission of Hasanuddin University approved the animal models and experimental design for this study with certificate number 302/UN4.6.4.5.31/PP36/2021.

Study period and location
This study started from April to December 2021, which has a relatively similar climate with temperature of 28-30 ˚C. Semen sample were obtained from all bulls was prepared at the Artificial Insemination Centre (AIC) in Pucak, South Sulawesi, Indonesia. The macroscopic and microscopic examination of semen and sperm cryopreservation was prepared at the *in vitro* Embryo Production Laboratory, Institute for Research and Community Service, Hasanuddin University, Makassar. Immunofluorescences evaluation of sperm liveability was prepared at the Research Centre for Applied Zoology, National Research and Innovation Agency (BRIN).

Animals
The semen samples were collected from 8 Bali bulls 5-7 years old with mean body weight 385-478 kg selected from AIC in Pucak, South Sulawesi. The bulls were maintained according to the standard ethical protocol of animal care by AI Centre. They were housed individually in pens equipped with a supply of food and water throughout the experiment. Each bull was fed 10% fresh forage and 1% concentrates. Components of ration and the daily amount provided to each animal must mention in the current study based on their daily body weight. They were fed twice a day at 08.00 a.m. and 16.00 p.m. The experiment divided into two equal groups: 4 Bali polled bulls and 4 Bali horned bulls.

Semen collection
Semen samples were collected using an artificial vagina in mid-morning (06.00-10.00 a.m.) after an extended period of the routine collection twice a week, with 32 ejaculated samples. The semen quality was then evaluated using Olympus microscope Model CX23. The microscopic assessment was conducted by examining the mass motility and the morphology of the sperm using the technique from Baracaldo *et al*. (2007). Sperm motility was assessed using an Olympus CX23 microscope. Ejaculates used for further analysis contained sperm motility (70%) and normal sperms (80%).

Cryopreservation
Semen was diluted with AndroMed® extender to provide a concentration of 25 million spermatozoa in a 0.25 mL polyvinyl straw. Packed semen in 0.25 mL straw was then equilibrated at 4 ˚C for 2 hours, then the straws were placed horizontally on a rack and frozen in a vapor 5 cm above liquid nitrogen for 10 minutes, then dipped into liquid nitrogen tank at -196 ˚C (Said *et al*. 2015).

Sperm concentration
Photometer SDM 6 (Minitube, Germany) was used to measure sperm concentration. Aliquots of 35 µL of fresh semen were diluted in a cuvette with 3.5 mL NaCl 2% for displaying the sperm concentration.

Sperm viability, abnormality, and membrane integrity
Sperm viability (%) and abnormality (%) were assessed according to the modified method of Arif *et al*. (2020). Twenty microliters of semen with 40 µL of Eosin-Nigrosin solution (1:4 ratio) were homogenized, smeared, and dried above a heated plate. Sperm cells that did not take up the Eosin-Nigrosin stain were considered alive, whereas those that did were considered dead. Sperm membrane integrity (%) was determined by calculating the percentage of sperm having an intact plasma membrane by hypo-osmotic swelling (HOS) test, performed by incubating 100 µL of semen sample in 1 mL of 150 mOsm/L hypo-osmotic solutions for 60 min at 37 ˚C. Sperm cells with intact plasma membranes were indicated by curled or swollen tails, while straight tails indicated defective or dead sperm (Paddrik *et al.* 2012). The stained slides were evaluated using a light microscope at 400× magnification and at least 200 sperm cells counted. The viability, abnormality, and membrane integrity values are expressed as a percentage (%).

Recovery rate

Recovery rate is the number of spermatozoa from frozen semen that have recovered after thawing with a minimum recovery rate of 50% (BSN, 2017). The values of recovery rate were calculated from the following equation:

Recovery rate: (post thawing motility / fresh semen motility) × 100

Computer-assisted semen analysis (CASA)

Sperm kinematics (progressive motility (PMot %), VCL (velocity curved line, μm/s), velocity straight line (VSL μm/s), velocity average path (VAP μm/s, amplitude of lateral head displacement (ALH μm), beat cross frequency (BCF Hz) and the ratios straightness (STR VSL/VAP), linearity (LIN VSL/VCL), and path wobble (WOB VAP/VCL) were assessed objectively by using a CASA system (Oliveira *et al.* 2013).

The microscope (Axioo-Scope A1, Carl Zeiss, Germany) was set to one phase contrast 200× magnification and connected to the Spermvision™ 3.7.8 (Minitube, Germany). An aliquot (5 μL) of semen was deposited on a warmed microscope slide at 38 ˚C and covered with a coverslip (18×18 mm). Sperm images in 8 fields were digitized for analysis of the kinematic patterns using the SpermVision software. The mean values were calculated for each of the parameters based on approximately 1000 spermatozoa (Maulana and Said, 2019).

Sperm chromatin structure assay

DNA integrity of frozen-thawed spermatozoa was evaluated by using the acridine orange staining technique. Samples of spermatozoa were smeared on glass slides, air-dried, fixed for 2 h in acetic alcohol (1-part glacial acetic acid plus 3 parts 100% methanol), and air-dried again. After fixation, sperm samples were stained with acridine orange solution (at 1000x dilution with GL-PBS) overnight (Said *et al.* 2003).

After staining, each slide was washed with distilled water and sealed with synthetic resin to against drying. Slides were examined with a fluorescence microscope (Axiophot Zeiss; 490/530 nm excitation/barrier filter). Two hundred cells were analyzed in each treatment slide. Sperm with normal DNA content presents a green fluorescence, whereas sperm with abnormal DNA content emits fluorescence in a spectrum varying from yellow to red. The percentage of sperm with intact chromatin was calculated by dividing the number of green-stained sperm by the total number of sperm and multiplying the result by 200 (Said *et al.* 1999).

Acrosomal status

Acrosomal status of semen samples was evaluated by using lectin peanut agglutinin (FITC-PNA, Sigma St. Luis MO) fluorescence stain combine with propidium iodide. Samples were smeared on glass slides. Fixed for 10 min in 96% ethanol at room temperature. They were air-dried and then drop 30 μL (100 μg/mL) PNA dropped over and incubated for 30 min at 37 ˚C. Two hundred cells per slides were examined with fluorescence microscope (Axiovision Zeiss; 380/420 nm excitation/barrier filter). Spermatozoa with intact acrosome present a green fluorescence in acrosome, whereas those with non-intact acrosome present red (Rajabi-Toustani *et al.* 2019).

Protamine status

Frozen semen was thawed in a 37 ˚C water bath for 30 seconds. The semen sample was removed from the straw and placed in a microtube. During the experiment, the semen was kept in a 37 ˚C water bath. The Chromomycin A3 (CMA3) technique was used to test for protamine deficiency (Simoes *et al.* 2009). The samples were washed twice in phosphate buffered saline (PBS) by centrifugation and fixed in Carnoy's solution for 8 minutes and centrifuged, then spread on slides rinsed with APES and air-dried. All procedures were carried out at 4 ˚C until the CMA3 treatment was performed. Each slide was stained in 100 μL of CMA3 solution (Sigma Chemical Co., St. Louis, USA) (0.25 mg/mL) in McIlvain buffer, pH 7.0, containing 10 mM MgCl for 30 min. The slides were then rinsed in McIlvain buffer, air-dried, and covered with antifade solution (Fluoprep, BioMerieux, France). The preparations were observed with a fluorescent microscope with a wavelength of 460-470 nm. Spermatozoa with protamine deficiency will appear bright yellow in colour, while those with good protamine will appear dark yellow/dull.

Statistical analysis

The data were analyzed by using Minitab Statistical Analysis programme pversion 18.1 (Minitab for Windows, Minitab, Inc. USA). Data normality was tested by using Shapiro and Wilk test, then homogeneity was tested by using the Levene test. The data were normally distributed and homogeneously varied, so the test continued by using paired T-test.

RESULTS AND DISCUSSION

Fresh semen quality

Data on the quality of fresh semen for polled and horned Bali bull are shown in Table 1.

Table 1 Macroscopic and microscopic evaluation of fresh semen of Bali polled and Bali horned bulls

Parameters	Bali polled	Bali horned	Significance
Volume (Mean±SD)	6.02±2.22	5.29±1.79	NS[1]
pH (Mean±SD)	6.4±0.00	6.4±0.00	NS
Color	Creamy	Creamy	-
Smell	Distinctive	Distinctive	-
Consistency	Tick milky	Tick milky	-
Concentration (×10^9)	0.31±0.12	0.49±0.15	P < 0.01
Motility (%)	73.33±2.50	77.78±2.64	P < 0.01
Viability (%)	92.73±1.83	91.92±1.51	NS
Abnormality (%)	4.01±0.94	5.52±0.92	P < 0.01
Membrane integrity (%)	76.58±6.53	72.43±7.32	NS

NS: non significant.

A macroscopic evaluation (volume, pH, color, and consistency) revealed no significant differences between polled and horned Bali cattle (Table 1). However, the sperm concentration (0.31±0.12 mL), motility (73.33±2.50%), and abnormality (4.01±0.94%) of Bali polled were significantly (P<0.01) lower than those of horned bull (0.49±0.15 mL), (77.78±2.64%), and (5.52±0.92%), respectively, but there was no apparent difference between viability and membrane integrity (Table 2).

Frozen-thawed semen quality
The quality of frozen-thawed semen of Bali polled and horned bulls are presented in Table 2. Except for abnormalities and DNA integrity of spermatozoa, all parameters of sperm quality (motility, viability, membrane integrity, acrosome integrity, protamine status, and mitochondrial membrane potential) were not significantly different between polled and horned bulls. Sperm abnormalities in polled Bali bull (8.85±2.87%) were significantly (P<0.01) higher than those of horned bulls (6.21±1.33%). However, sperm DNA integrity of polled Bali bull (99.35±0.51%) was significantly (P<0.05) higher than those of horned ones (98.12±1.75%).

Except for LIN, the sperm kinematics were significantly different for the two types of semen (Table 2). Most kinematics were higher in polled Bali bull spermatozoa than those in horned bulls, except for WOB and ALH, which were higher in horned bulls than those in polled ones.

In this study, the ejaculate volumes of both Bali polled and horned bull in this study was almost the same as reported before, which is between 6.32 mL (Indriastuti et al. 2020) and 6.44 mL (Nabilla et al. 2018). The pH was consistently the same (6.4±0.00) in both types of bulls. According to Garner and Hafez (2016), normal bull semen had a pH range of 6.4-7.8. The pH level of semen has implications for physiological circumstances as well. The characteristic of pH in the seminal plasma can be changed by bacteria if there is a contamination (Okazaki et al. 2010), and

the bacteria could also affect the function of sperm (Bussalleu et al. 2011). As a result, an acidic environment caused by a pathological condition or by producing the lactic acid from fructolysis can decrease sperm function (Contri et al. 2013).

According to the findings of this study, the quality of fresh semen from horned Bali bull was better than those of polled bull in terms of sperm concentration, motility, and abnormalities. Sperm concentration is frequently associated with semen volume, however, in this study, semen volume tends to be higher in polled bulls while sperm concentration is lower (Table 1). This finding is consistent with prior findings that semen volume is negatively correlated with sperm concentration (Karoui et al. 2011; Mapel et al. 2022). In addition, sperm concentration is not related to fertility (Mapel et al. 2022). The concentration of sperm in bull semen is influenced by the testicle size and the frequency of semen collection. The scrotal circumference had a positive correlation with semen volume, sperm concentration, and motility in the Bali bulls (Saputra et al. 2017). The volume, creamy color, consistency, and concentration of sperm are inter-correlated parameters because the color of semen is influenced by the concentration of sperm and manifested in sperm consistency. The color and consistency of Bali polled bull semen was the same at that of horned bulls, consistency of Bali polled bull semen was the same as that of Bali horned bull semen, and these findings are consistent with those reported in Pasundan (Santoso et al. 2021) and Sumba Ongole cattle (Maulana and Said, 2019).

The fresh semen motility of polled Bali cattle was significantly lower than that of horned ones in this study, however, sperm abnormalities were significantly higher in horned Bali cattle. This finding contradicts with that of Gilani and Gilani (1998), who found that a rise in the number of normal sperm morphology causes an increase in motility. Furthermore, Karabulut and Tekin (2013) showed that increasing sperm motility was significantly related to normal sperm morphology.

Table 2 The quality of frozen-thawed semen of Bali polled and Bali horned bulls

Parameters	Bali bulls				Significance
	Polled	SD	Horned	SD	
Motility (%)	52.79	6.53	47.98	6.98	NS[2]
Viability (%)	56.05	4.58	54.58	6.53	NS
Abnormality (%)	8.85	2.87	6.21	1.33	$P < 0.01$
Membrane integrity (%)	54.79	3.73	53.08	7.23	NS
DNA integrity (%)	99.35	0.51	98.12	1.75	$P < 0.05$
Acrosomal integrity (%)	92.98	5.18	89.49	5.16	NS
Protamine status (%)	97.92	1.88	96.43	2.02	NS
Recovery rate (%)	71.72	9.83	61.62	10.46	NS (0.071)
Pmot (%)	46.28	7.06	39.60	6.82	$P < 0.05$
DAP (µm/s)	31.03	3.97	27.74	3.03	$P < 0.01$
DCL (µm/s)	45.79	7.43	43.78	15.39	$P < 0.05$
DSL (µm/s)	22.32	3.19	18.64	2.30	$P < 0.01$
VAP (µm/s)	72.43	8.71	67.34	7.70	$P < 0.05$
VCL (µm/s)	106.52	16.55	95.09	12.47	$P < 0.05$
VSL (µm/s)	52.11	6.36	45.58	5.43	$P < 0.01$
STR (%)	71.50	4.34	67.25	3.59	$P < 0.05$
LIN (%)	48.75	4.03	47.41	3.20	NS
WOB (%)	68.00	3.38	70.41	2.10	$P < 0.05$
ALH (µm)	4.78	0.91	5.46	0.82	$P < 0.05$
BCF (Hz)	25.33	2.96	22.11	2.69	$P < 0.01$

DAP: distance average path; DCL: distance curve linear; DSL: distance straight line; VAP: velocity average path; VCL: velocity curvilinear; VSL: velocity straight line; STR: straightness; LIN: linearity; WOB: wobble; ALH: amplitude of lateral head and BCF: beat cross frequency.
NS: non significant.

This is presumed to be due to the fact that horned bulls have much larger sperm concentrations, which may enhance oxidative stress, resulting in increased sperm abnormalities. According to a study on fresh bull semen, high sperm concentration may cause an increase in oxidative stress (Murphy *et al.* 2013), and it was proposed that ROS damages sperm DNA and would lead a number of morphological defects (Aitken *et al.* 1993).

Sperm motility was positively correlated with bull fertility (Gredler *et al.* 2007; Gebreyesus *et al.* 2021). Analysis of non-return rates to artificial insemination (AI), and *in vitro* fertilization (IVF) studies, the notion of compensable and uncompensable abnormalities has been developed (Barth, 2007). As the sperm head contains the genetic material and key effectors of fertilization, most abnormalities of the head are associated with a marked impairment of fertility (Soderquist *et al.* 1991). Abnormal condensation of chromatin (Johnson, 1997) and abnormal nuclear shape (Ostermeier *et al.* 2001) are closely associated with reduced fertility. The misshapen pyriform head abnormality impairs both fertilization rate and subsequent embryonic development (Thundathil *et al.* 1999), with failure of cleavage being the primary outcome.

In general, the frozen semen quality parameters for polled and horned Bali bulls were not significantly different, except for sperm abnormalities and DNA integrity. Given the fact that fresh semen motility was significantly higher in horned bulls compared to polled ones, as indicating that Bali polled bull semen was more tolerant for the freezing process. The capacity of sperm to survive during the freezing process is an important aspect for bull selection. Even though sperm abnormalities were dramatically increased in frozen polled Bali bull sperm. Sperm motility is a major aspect of frozen sperm quality. The sperm motility of frozen-thawed semen of polled in this study was $52.79 \pm 6.53\%$ and horned $47.98 \pm 6.98\%$. Sorensen (1979) reported that motility decreased about 40% during the freezing process. In addition, the viability of sperm will be reduced due to hyperosmotic diluent and temperature change (Len *et al.* 2019; Indriastuti *et al.* 2020). In the present study, polled bull sperm was able to recover better after the freezing process ($71.72 \pm 9.83\%$) as compared to those of horned bulls ($61.62 \pm 10.46\%$) (Table 2). This may indicate that polled bull semen has better freezing ability than those of horned bulls. The results of the recovery rate (RR) obtained in Bali cattle in this study were still higher

than the report by Sukmawati *et al*. (2014) in Limousine (59.70±3.23%), Simmental (58.46±1.06%), and Friesien Holstein (57.53±1.74%). The quality of semen produced by each breed and individual varies, influencing the quality of frozen semen produced at last.

Spermatozoa motility is the most important characteristic associated with fertilization ability, and it is one of the quality controls of insemination dose before and after thawing (Puglisi *et al*. 2012). There were significant differences in the sperm motility of polled and horned Bali cattle on parameters of Pmot (%), DCL (μm/s), VAP (μm/s), VCL (μm/s), VSL (μm/s), STR (%), WOB (%) and ALH (μm) ($P<0.05$) as well as parameters of DAP (μm/s), DSL (μm/s), and BCF (Hz) ($P<0.01$). The values of VCL, VAP, and VSL of spermatozoa to be able to penetrate into the ovum is VCL >70 μm/s, VAP and VSL >45 μm/s (Inanc *et al*. 2018). Spermatozoa with BCF value >20 Hz and ALH value >2.5 μm to <6.5 μm, indicate that sperm movement seems optimal and it has good fertility value (Belala *et al*. 2019). In the present study, we found that according to the kinematic sperm data obtained (Table 2), polled and horned bulls have the same.

DNA damage in spermatozoa has an effect on embryogenesis failure and is one of the factors that contribute to low fertility (Simon *et al*. 2011; Dogan *et al*. 2015; Khezri *et al*. 2019). Acridine orange (AO) staining was used in this study to test for DNA damage (Figure 1-1). In this study, the percentage of spermatozoa DNA damage was significantly different between polled (99.35±0.51%) and horned (98.12±1.75%) bulls ($P<0.05$) (Table 3).

Bochenek *et al*. (2001) reported a decrease in the fertility of males with DNA damage of more than 10%. In another study, it was also stated that DNA damage in semen being less than 15% was still in normal conditions, while 15% to 25% reduced fertility, and DNA damage above 25% was classified in infertile males (D'Occhio *et al*. 2013).

Based on the data on spermatozoa DNA integrity in polled and horned Bali bulls, this study found that, while polled bulls had better DNA integrity than those of horned ones, however, both had good DNA integrity.

For examination of protamine deficiency, fluorescent CMA3 staining was used (Figures 1-2 and Table 2), in which the results showed no significant difference between polled (97.92±1.88%) and horned (96.43±2.02%) Bali bulls ($P<0.05$). These data also show that the protamine deficiency of both breeds of bulls is less than 5%. The CMA3 is a guanine-cytosine-specific fluorochrome that competes with protamine binding sites on DNA, causing chromomycin to bind to DNA and fluorescence stain spermatozoa when protamine levels are low (Bianchi *et al*. 1996). The association of protamine deficiency testing with CMA3 and spermatozoa DNA damage have been widely reported and used (Bianchi *et al*. 1996; Zandemami *et al*. 2012; Carreira *et al*. 2017). The nucleus of sperm cells dominated by protamine. Protamine plays a role in the final compaction of chromatin, as observed in mature sperm in the cauda epididymis until ejaculated (Bao and Bedford, 2016). Protamine as the main protein is needed for the stability of the very dense DNA wrapping structure of sperm (Hutchison *et al*. 2017). Disruption of protamine cause a variety of changes, including DNA instability, which results in high DNA damage (Kumaresan *et al*. 2020) and morphological abnormalities of sperm heads (Oehninger and Kruger, 2021).

In this study, acrosome integrity (Figure 1-3), revealed no significant difference between polled (92.98±5.18%) and horned (89.49±5.16%) Bali bulls ($P<0.05$). This result is higher than that of Priyanto *et al*. (2015), who reported an acrosome integrity percentage ranging from 71.92%. According to the India Agri Ministry (2014), the percentage of acrosomal integrity in frozen semen was at least 65%, so the percentage of acrosomal integrity was still within the normal range herein.
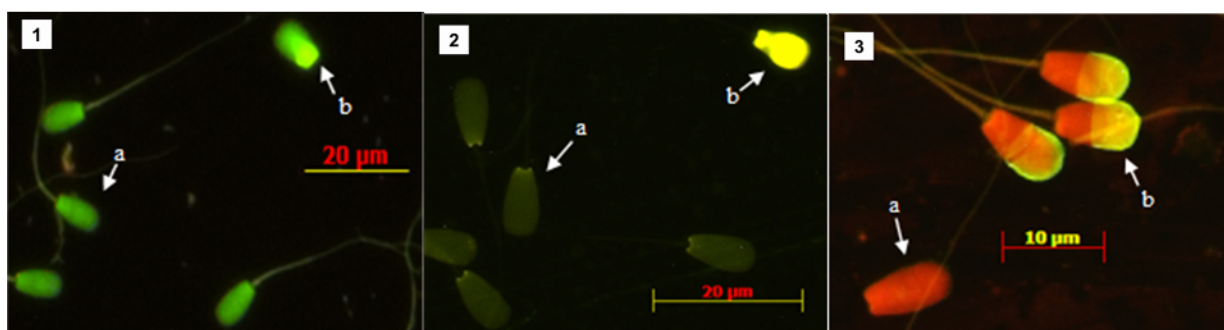

Figure 1 Magnification 60x, microscope fluoresence, Imager Z7, Carl Zeiss, Germany. (1). DNA integrity (Magnification 40x, microscope fluoresence, Imager Z7, Carl Zeiss, Germany), 1a. Normal DNA (green fluoresence), 1b. Fragmented DNA (yellow-green to red fluoresence); (2). Protamine status, 2a. Normal/complete protamine (dark/dull green fluoresence), 2b. Protamine deficiency (yellow fluoresence); (3). Acrosomal status, 3a. Non-intact acrosome (red fluoresence no acrosome), 3b. Intact acrosome (green fluoresence in the acrosome)

The higher the proportion of spermatozoa with intact acrosomes, the higher the chances of spermatozoa being able to penetrate the zona pellucida and fuse with the oocyte plasma membrane (Celeghini *et al.* 2010).

The presence of an acrosome, which can activate the acrosomal response and cause oocyte fusion, determines the success of fertilization. One of the most important processes in mammalian fertilization is the acrosomal reaction (AR), which is normally initiated when the sperm penetrates the ootid zona pellucida. The AR takes place when the plasma membrane and the underlying outer acrosomal membrane fuse, allowing the acrosomal contents to be released (Costa *et al.* 2010). Acrosomes are essential in the fertilization process; when sperm cells bind to the zona pellucida, the AR is stimulated, resulting in the release and activation of acrosomal enzymes, allowing sperm to pass through the zona pellucida (Miranda *et al.* 2009). To be able to fertilise oocytes, sperm cell must be acrosome-intact; if the AR occurs before the sperm reach the fertilisation stage, the sperm will lose its ability to fertilise the oocyte.

CONCLUSION

Based on current findings, it has been concluded that the quality of fresh semen from horned Bali bulls was better than polled Bali bulls. However, polled bull sperm has better freezing ability than those of horned ones. Furthermore, Bali polled and horned bulls have the same fertility ability, and while polled Bali bulls had better DNA integrity than those of horned bulls, both breeds of bulls had good DNA integrity.

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