Comparative Analysis of Semen Quality and Kinematics in Sexed and Non-Sexed Sperm of Limousin Bulls

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ABSTRACT

The application of spermatozoa sexing technology offers benefits in determining the desired sex in cattle. However, this technology requires particular attention to quality, DNA fragmentation, and kinematics of spermatozoa. This study aimed to evaluate the effect of sexed and non-sexed semen on spermatozoa quality, DNA fragmentation, and kinematics in Limousin bulls. Four Limousin bulls between the ages of four and seven years and weighing between 610 and 814 kg were used in this study. The sexing method followed the bovine serum albumin (BSA) protocol. The upper fraction (5% BSA) was used for X-spermatozoa, and the lower fraction (10% BSA) was used for Y-spermatozoa. The observed variables included the percentage of progressive motility, viability, membrane integrity, acrosome integrity, DNA fragmentation, and spermatozoa kinematics. One-way ANOVA was used to analyse differences in semen quality among bulls. The results showed no significant differences ($p<0.05$) in spermatozoa quality or DNA fragmentation between sexed and non-sexed frozen semen. However, significant differences ($p<0.05$) were found in the kinematic parameters ALH and BCF among non-sexed, sexed-X, and sexed-Y semen. Overall, the BSA sexing method did not reveal notable differences in semen quality and DNA fragmentation between sexed and non-sexed spermatozoa.

Keywords: BSA sedimentation; Limousin bull; sperm sexing; sperm kinematic; semen quality

INTRODUCTION

Indonesia still relies on imports for 40% of its national beef demand (General Secretariat, 2023). This dependence on imports increases the vulnerability of national food security (Kusumaningrum *et al.*, 2024). Therefore, strategic solutions are required, including efforts to enhance reproductive efficiency through the application of reproductive technologies, such as spermatozoa sexing. Spermatozoa sexing enables the selection of the desired sex of offspring, thereby optimising livestock productivity. The process of spermatozoa sexing relies on distinguishing spermatozoa that contain different sex chromosomes (Saputro *et al.*, 2022). There are variations in size, shape, weight, density, motility, charge, and surface biochemical composition between X and Y-chromosome-bearing spermatozoa (Garner *et al.*, 2000).

Limousin cattle are highly desired by farmers in Indonesia. This breed, which originates from France, has been selectively developed to enhance superior traits (Mariadassou *et al.*, 2020). They have several advantages, including adaptability to humid environments in tropical areas such as Indonesia (Nurhidayat *et al.*, 2024), as well as high feed efficiency and carcass yield (Kayar & İnal, 2022)

One of the spermatozoa sexing techniques used in Indonesia is the albumin sedimentation method, which utilises bovine serum albumin (BSA). The effectiveness of this method can vary depending on specific conditions and protocols, but it is relatively straightforward (Pinto-Pinho *et al.*, 2023). In beef cattle, especially Limousin cattle, the quality of semen produced using this method has not been evaluated at the molecular level. Semen quality encompasses not only spermatozoa volume, concentration, and motility but also molecular characteristics, including DNA integrity and spermatozoa kinematics. Research on the impact of spermatozoa sexing on DNA fragmentation and spermatozoa kinematics in Indonesia is still limited. Currently, no research has specifically evaluated this technique in Limousin cattle in Indonesia, making this the first study of its kind. Therefore, this study aimed to assess the effects of spermatozoa sexing using the albumin sedimentation method on semen quality, DNA fragmentation, and spermatozoa kinematics in Limousin cattle.

MATERIALS AND METHODS

Ethical Approval

This research procedure was approved by the ethics committee of the National Research and Innovation Agency (BRIN), Republic of Indonesia, under approval number 070/KE.02/SK/05/2025.

Experimental Design

This study was conducted at the Lembang Artificial Centre (AIC) for frozen semen production and fresh semen evaluation. Subsequent to the thawing process, observations were conducted at the Genomics and Environment Laboratory, National Research and Innovation Agency (BRIN), located in Bogor, Indonesia. A total of 48 samples were analysed, including 12 samples of fresh semen, 12 samples of non-sexed frozen semen, 12 samples of sexed-X frozen semen, and 12 samples of sexed-Y frozen semen. These samples were collected from four Limousin cattle at the Lembang AIC: Commander (BPTU Padang Mangatas ID818134, aged 7 years, 783 kg), Eyrie Park (Australia ID819138, aged 6 years, 814 kg), L P Rossini (Australia ID820179, aged 5 years, 717 kg), and Calvin (BET Cipelang ID821178, aged 4 years, 610 kg). Semen collection was performed twice a week using an artificial vagina according to Iskandar *et al*. (2022). Immediately after collection, semen was evaluated macroscopically and microscopically following the procedures described by Arifiantini (2012). Each ejaculate was divided equally into treatment groups, namely non-sexed semen and sexed semen (sexed-X and sexed-Y).

Macroscopic Evaluation

Semen volume was measured directly using the scale on the collection tube (mL). Semen colour was assessed visually, and acidity (pH) was determined using a pH indicator with a range of 6.4–8.0 (Arifiantini, 2012).

Microscopic Evaluation

Spermatozoa concentration was measured using an SDM 6 photometer (Iskandar *et al*., 2022). Briefly, 3.5 mL of 0.9% NaCl solution was placed in a cuvette, followed by the addition of 35 µL of semen. The cuvette was covered with parafilm, homogenized, and analyzed using the SDM 6 photometer.

Evaluation of individual motility and spermatozoa kinematics using computer-assisted semen analysis (CASA) (Sperm Vision Minitube, Germany). Fresh semen observation was performed according to the Lembang AIC protocol by diluting 25 µL of semen with 725 µL physiological NaCl (1:29) ratio on a glass slide and examining four fields of view at 200× magnification (10 × 20 objective).

Fresh semen viability was assessed by mixing 10 µL of fresh semen and 60 µL of eosin-nigrosine (1:6) (Arifiantini, 2012). The eosin-nigrosine solution was prepared by dissolving 1.67 g of eosin, 10 g of nigrosine, and 2.9 g of sodium citrate in 100 mL of distilled water. Frozen semen viability was evaluated using the same method as fresh semen, using a 1:2 ratio of semen to dye. The preparation was made on an object glass, dried on a warming table at 37 °C, then observed under a microscope at 400× magnification in 10 fields of view with a minimum of 200 cells. Viable spermatozoa were characterised by heads that did not absorb the dye, while non-viable spermatozoa had red-stained heads.

Plasma membrane integrity was assessed using a modified hypoosmotic swelling (HOS) test according to (Singh *et al*., 2019). The test was performed twice, using isosmotic (300 mOsm) (A) and hypoosmotic (150 mOsm) (B) solutions. The isosmotic (300 mOsm) solution was prepared by dissolving 7.35 g trisodium citrate and 13.51 g D-fructose in 1000 mL of double-distilled water (DDW). The hypoosmotic (150 mOsm) solution was obtained by diluting 1 part of the 300 mOsm solution with 1 part of DDW. Briefly, 1000 µL of each solution was mixed with 10 µL of semen in a microtube and incubated at 37 °C for 30 minutes. Subsequently, 10 µL of each suspension was placed on a glass slide and covered with a cover slip. Observations were made under a microscope at 400× magnification, with a minimum of 200 spermatozoa examined per sample. Spermatozoa with damaged membranes exhibited straight tails, while those with intact plasma membranes exhibited curved tails. The difference between the HOS B and HOS A scores represented the integrity of the plasma membrane.

Sexing Sperm Using Albumin Sedimentation

Spermatozoa sexing was performed at Lembang AIC using the 5% and 10% BSA column method (Rasad *et al*., 2020). Briefly, 2 mL of 10% BSA medium (lower fraction) was placed at the base of a test tube, followed by 2 mL of 5% BSA medium (upper fraction). Subsequently, 1 mL of diluted fresh semen was carefully layered on top, yielding three layers: semen, 5% BSA, and 10% BSA. The tube was then subjected to an incubation of 45 minutes. Subsequent to this, the top layer was removed, and the 5% and 10% BSA fractions were collected in separate tubes. Each fraction was centrifuged at 1800 rpm for a duration of 10 minutes. Thereafter, the upper layer of the fraction was discarded, and the spermatozoa pellet was resuspended in 1 mL of Biomed extender. Subsequently, spermatozoa concentration was determined in order to calculate the dilution volume and the number of straws produced.

Cryopreservation and Storage of Semen

Fresh and sexed semen diluted with Biomed extender were equilibrated and frozen following the protocol of Lembang AIC. The diluted semen was equilibrated at 4 °C for 4 hours, then packaged using a filling and sealing machine at 4 °C into 0.25 mL straws. The straws were pre-frozen for 7 minutes from 4 °C to -145 °C, followed by freezing in liquid nitrogen at -196 °C. The frozen semen was then stored in a liquid

nitrogen container and subsequently transported to BRIN for post-thawing evaluation.

Post-Thawed Semen Analysis

Semen thawing was performed according to Iskandar *et al*. (2022). Frozen semen straws thawed in water at 37 °C for 30 seconds and maintained at 37 °C during analysis. Post-thawed semen analysis included assessments of motility, kinematics, viability, plasma membrane integrity (PMI), acrosome integrity, and DNA fragmentation index. Motility and kinematics, viability, and PMI were examined using the same methods as for fresh semen, but with different dilution ratios: motility and kinematics were analysed using a 1:1 ratio of semen to NaCl, PMI using a 1:10 ratio of semen and HOST solution, and viability using a 1:1 ratio of semen to dye.

Observation of acrosome integrity was performed using the fluorescein isothiocyanate-peanut agglutinin (FITC-PNA: Sigma-Aldrich, USA) staining method combined with propidium iodide (PI: Sigma-Aldrich, USA), following Maulana *et al*. (2024). Semen samples were placed on a glass slide, air-dried at room temperature, and then fixed with 96% ethanol for 10 minutes. Subsequently, 30 µL of FITC-PNA solution was applied and incubated at 37 °C for 30 minutes, followed by the addition of 5 µL of PI and incubation for 5 minutes. Samples were then rinsed with phosphate-buffered saline (PBS). Acrosome status was observed under a fluorescence microscope (380–420 nm) at 400× magnification, with a minimum of 200 spermatozoa examined per sample. Spermatozoa exhibiting green fluorescence were considered to have intact acrosomes, whereas those showing red fluorescence were classified as having non-intact acrosomes.

DNA fragmentation was assessed using the Halomax® kit (Halotech HT-BT40, Spain) as described by Safa *et al*. (2025). Frozen semen was washed three times with PBS and centrifuged at 1800 rpm to remove the diluent, then diluted to 15–20 million spermatozoa/mL. Agarose was melted at 95–100 °C for 5 minutes and maintained at 37 °C. Subsequently, 25 µL of semen was mixed with 50 µL of agarose and homogenized at 37 °C. Two microlitres of the mixture were placed on a glass slide, covered with a cover slip, and incubated at 2–8 °C for 5 minutes, after which the cover slip was removed. The slide was treated with lysis solution, rinsed with distilled water, and fixed sequentially with 70% and 100% ethanol for 2 minutes each. After drying, 2 µL of a red and green fluorochrome mixture (1:1) was applied to the slide. Samples were observed under a fluorescence microscope at 400× magnification. Spermatozoa with a non-compact halo were considered to have fragmented DNA, while spermatozoa with a compact halo were classified as having normal DNA.

Statistical Analysis

The data were analysed using R Studio software (version 4.4.1). Data analysis was performed using one-way ANOVA with a significance level of 95%, followed by Tukey's test, to determine if there were significant differences. The results are presented as mean ± standard error of the mean (SEM).

RESULTS

Fresh Semen Characteristic of Limousin Bulls

The fresh semen quality of the four bulls presented in Table 1 exhibited favourable characteristics overall. The average of progressive motility was 82.19±2.94%, while total motility reached 87.10±1.94%. The average viability value of the bulls was 90.79±1.36%, plasma membrane integrity was 90.59±1.29%, and spermatozoa concentration reached 1,205.17±55.9 ×10^6/mL. Macroscopic evaluation showed that the average semen volume was 7.21±0.35 mL, with a milky white colour and a pH value of 6.65±0.02.

Total Motility, Viability, and Membrane Integrity of Sperm

Analysis of semen quality between fresh semen and frozen semen (non-sexed, sexed-X, and sexed-Y) showed significant differences (p<0.05) in total motility, viability, and plasma membrane integrity (Table 2). However, no significant differences were observed between sexed (X and Y) and non-sexed frozen semen.

DNA Fragmentation Index and Acrosome Integrity of Sperm

The results of the DNA fragmentation and acrosome integrity analyses (Table 3) showed no significant differences (p>0.05) between sexed and non-sexed frozen semen. However, sexed frozen semen tended to exhibit higher levels of DNA fragmentation compared to non-sexed semen, although the difference was not statistically significant.

Progressive Motility and Sperm Kinematics

Table 4 showed the differences (p<0.05) in progressive motility (PM) parameters, including distance (DAP, DCL, and DSL), velocity (VAP, VCL, and VSL), and movement patterns (STR, LIN, and

Table 1. Fresh non-sexed semen characteristics of Limousin bulls

Variables	Average
Macroscopic quality	
Semen volume (mL)	7.21±0.35
pH	6.65±0.02
Colour	Milky white
Microscopic quality	
Total motility (%)	87.10±1.94
Progressive motility (%)	82.19±2.97
Sperm concentration (10^6/mL)	1,205.17±55.59
Sperm viability (%)	90.79±1.36
Sperm PMI (%)	90.59±1.29

Note: The data are presented as mean ± standard error of the mean (SEM). PMI: plasma membrane integrity.

Table 2. Total motility, viability, and membrane integrity of Limousin bull fresh non-sexed, frozen sexed, and non-sexed semen

Variables (%)	Fresh semen	Frozen semen		
		Non-sexed	Sexed-X	Sexed-Y
Total motility	87.10±1.94[a]	64.19±1.08[b]	60.91±1.71[b]	60.19±2.20[b]
Sperm viability	90.79±1.36[a]	69.93±1.65[b]	66.94±2.07[b]	64.67±2.82[b]
Sperm PMI	90.59±1.29[a]	66.90±1.77[b]	63.89±1.79[b]	63.71±2.60[b]

Note: The data are presented as mean ± standard error of the mean (SEM). Different superscripts within the same row indicate significant differences (p<0.05). PMI: plasma membrane integrity.

Table 3. Sperm DNA fragmentation index and acrosome integrity of Limousin bull frozen semen

Variables (%)	Non-sexed	Sexed-X	Sexed-Y
DNA fragmentation index	3.54±0.22	4.03±0.38	4.36±0.31
Acrosome integrity	96.74±0.37	96.36±0.23	96.72±0.39

Note: The data are presented as mean ± standard error of the mean (SEM).

Table 4. Progressive motility and sperm kinematics of Limousin bull fresh non-sexed and frozen semen

Variables	Fresh semen	Frozen semen		
		Non-sexed	Sexed-X	Sexed-Y
PM (%)	87.10±1.94[a]	52.33±2.04[b]	52.21±1.94[b]	51.87±1.98[b]
DAP (μm)	14.01±1.04[a]	30.24±0.74[b]	31.55±0.66[b]	29.87±0.74[b]
DCL (μm)	29.17±2.52[a]	44.92±1.40[b]	46.93±1.05[b]	44.78±1.27[b]
DSL (μm)	11.49±0.78[a]	20.78±0.62[b]	22.4±0.61[b]	21.76±0.71[b]
VAP (μm/s)	80.46±3.53[a]	70.0±2.02[b]	73.38±1.35[b]	67.86±1.35[b]
VCL (μm/s)	163.52±8.69[a]	103.86±3.5[b]	108.89±2.27[b]	101.40±2.87[b]
VSL (μm/s)	69.52±2.43[a]	47.97±1.33[b]	52.23±1.23[b]	49.41±1.63[b]
STR (%)	86.75±1.24[a]	69.25±1.27[b]	71.17±0.84[b]	72.83±1.99[b]
LIN (%)	43.17±1.09[a]	47.00±0.95[ab]	46.70±0.42[ab]	47.75±1.09[b]
WOB (%)	49.50±0.67[a]	67.58±0.50[b]	67.00±0.47[b]	67.00±0.54[b]
ALH (μm)	1.61±0.10[a]	5.33±0.15[b]	5.27±0.09[b]	4.51±0.06[c]
BCF (Hz)	10.51±0.44[a]	26.5±0.73[b]	27.22±0.33[b]	29.22±0.44[c]

Note: The data are presented as mean ± standard error of the mean (SEM). Different superscripts within the same row indicate significant differences (p<0.05). PM: progressive motility, DAP: distance average path, DCL: distance curved line, DSL: distance straight line, VAP: average path velocity, VCL: curvilinear velocity, VSL: straight line velocity, STR: straightness, LIN: linearity, WOB: wobble, ALH: amplitude of lateral head displacement, BCF: beat cross frequency.

WOB), between fresh and frozen semen. However, no significant differences were observed among the sexed frozen semen groups. Significant differences (p<0.05) were found in spermatozoa head movement parameters (ALH and BCF) between fresh and frozen semen, and between sexed-Y semen and both sexed-X and non-sexed semen.

DISCUSSION

The progressive and total motility of fresh semen averaged more than 80%, and the viability values for all bulls also exceeded the standard threshold of 80%, indicating that semen was suitable for further processing (Safa *et al*., 2025). The plasma membrane integrity exhibited greater values than motility, as reported by Baharun *et al*. (2023), suggesting that the spermatozoa are capable of optimal functionality. Macroscopic evaluation showed that the average semen volume, colour, and pH values were within the normal range, as reported by Royan *et al*. (2021).

The quality of frozen sexed and non-sexed semen, such as total motility, viability (Figure 1A), and plasma membrane integrity (Figure B), decreased after

freezing due to physical damage, cold shock, mitochondrial dysfunction, and oxidative stress (Hai *et al*., 2024). Spermatozoa undergo dehydration during freezing, which may reduce the intracellular ice formation (Hine *et al*., 2019). However, dehydration can alter the structure and function of the plasma membrane, leading to the formation of micropores and a loss of semipermeability (Sun *et al*., 2020). The quality of frozen sexed and non-sexed semen did not show significant differences, in contrast to the findings of Nurgina *et al*. (2024), who reported significant differences in semen quality between sexed and non-sexed semen. These differences may be due to the conventional albumin sedimentation method (Yata, 2022), indicating that it remains highly dependent on the protocol and procedures.

Sexed and non-sexed frozen showed no significant differences in DNA fragmentation (Figure 2A) and acrosome integrity (Figure 2B, Table 3), because the BSA sedimentation method allows high-quality spermatozoa to pass through the gradient, while low-quality spermatozoa remain in the upper layer (Mundana *et al*., 2023). Al-Kass *et al*. (2025) reported that centrifugation at 1800 rpm for 10 minutes did not reduce spermatozoa quality. This finding is consistent with that

Figure 1. (A) Spermatozoa viability of Limousin bulls; arrow (a) indicates viable spermatozoa, while arrow (b) indicates non-viable spermatozoa. (B) Spermatozoa plasma membrane integrity of Limousin bulls; arrow (a) indicates spermatozoa with intact plasma membranes, whereas arrow (b) indicates spermatozoa with damaged plasma membranes.
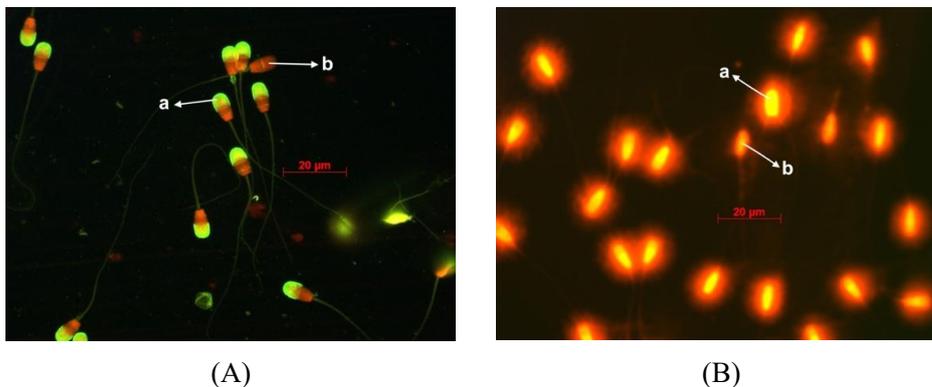

Figure 2. (A) Spermatozoa acrosome integrity of Limousin bulls; arrow (a) indicates spermatozoa with intact acrosomes, while arrow (b) indicates spermatozoa with damaged acrosomes. (B) Spermatozoa DNA integrity of Limousin bulls; arrow (a) indicates spermatozoa with non-fragmented DNA, whereas arrow (b) indicates spermatozoa with fragmented DNA.

of Masrizal et al. (2024), who reported that spermatozoa sexing using the BSA method did not lead to increased DNA fragmentation. Meanwhile, the flow cytometry method reported by Silva et al. (2016) revealed genetic material damage resulting from DNA staining and laser exposure. However, the centrifugation process can increase the production of reactive oxygen species (ROS), which can trigger DNA fragmentation due to damage to the plasma membrane (Dasrul et al., 2012). Spermatozoa DNA damage can interfere with pronuclear fusion during fertilisation, causing embryo death (Priyanto et al., 2015).

Acrosome integrity demonstrated stability between sexed and non-sexed semen, due to its higher lipid and protein composition compared to the plasma membrane. Lipids in the acrosome can increase its resistance to lipid peroxidation, which often damages the plasma membrane of spermatozoa (Nofa et al., 2017). Additionally, the structural integrity of the acrosome is maintained during cryopreservation by integral proteins, including protease enzymes (Ferrer et al., 2012). Furthermore, acrosome resistance is significant to ensure that the acrosome reaction occurs in a timely and effective manner during fertilisation (Bernecic et al., 2021).

Progressive motility and spermatozoa kinematics showed significant differences between fresh semen

and frozen semen, likely due to mitochondrial damage incurred during the freezing process (Pezon et al., 2020). Mitochondria in spermatozoa produce adenosine triphosphate (ATP), which is the primary source of energy for spermatozoa (Pezo et al., 2021). Mitochondria are responsible for the generation of adenosine triphosphate (ATP), which serves as the primary energy source for spermatozoa (Nofa et al., 2017). Damage to spermatozoa mitochondria can reduce motility, so that good spermatozoa can adapt to the remaining extreme conditions. Progressive motility did not show significant differences between sexed and non-sexed frozen semen. Safa et al. (2025) also reported no significant difference in progressive motility between sexed and non-sexed frozen semen.

The differences in distance parameters (DCL, DAP, and DSL) between fresh and frozen semen may be due to changes in spermatozoa membrane fluidity and cytoplasm consistency during freezing (Grötter et al., 2019), which can affect spermatozoa flagella motility. On the other hand, the effect of spermatozoa sexing did not show significant differences in distance parameters. Fertilisation success indicators can be determined by DCL, DAP, and DSL values (Utami et al., 2025). The overall spermatozoa trajectory is indicated by the DCL value (Víquez et al., 2020). The average distance travelled by spermatozoa along the trajectory

is indicated by the DAP value (Sinha *et al.*, 2021). The DSL value indicates the minimum distance between the starting and ending points of movement (Foutouhi *et al.*, 2023). The ability of spermatozoa to penetrate the oocyte layer with sufficient speed and movement is crucial for successful penetration (Tumova *et al.*, 2021).

The velocity parameters (VSL, VCL, and VAP) differ significantly between fresh and frozen semen, which can be attributed to mitochondrial damage during the freezing process, affecting flagellar activity and overall spermatozoa motility (Utami *et al.*, 2025). In contrast, no significant differences were observed among non-sexed, sexed X, and sexed-Y frozen semen. These results are in line with the study by Safa *et al.* (2025), which reported no significant differences in frozen semen spermatozoa velocity parameters after the sexing process. Spermatozoa 275 of fresh and frozen semen showed values above the minimum VSL threshold of 25 μm/s (Maulana *et al.*, 2021). The VCL values obtained in this study were greater than 90 μm/s, which is categorised as fast (Křížková *et al.*, 2017). The parameters VCL, VSL, and VAP have been proven to be effective predictors of fertility (Maulana *et al.*, 2019). Specifically, VCL and VSL are directly related to fertilisation capacity, while VAP has been shown to have a strong correlation with pregnancy success (Nagy *et al.*, 2015).

Significant differences in STR WOB values between fresh and frozen semen may be due to cold stress. Cold stress on spermatozoa is known to affect the plasma membrane, increasing ion permeability, which primarily causes the influx of calcium ions (Ca^{2+}) into the spermatozoa cytosol (Treulen *et al.*, 2018). which in turn affects spermatozoa motility patterns. In this study, semen sexed-Y had higher LIN values compared to semen non-sexed, indicating that semen containing a higher proportion of Y-chromosome-bearing spermatozoa can move more linearly. Daloglu *et al.* (2018) reported that the linearity (LIN) of Y-chromosome-bearing spermatozoa consistently showed higher values in trajectory. The identification of hyperactive spermatozoa that move quickly and strongly, but not progressively and linearly, can be explained by STR and LIN values (Hansen *et al.*, 2006). Spermatozoa with STR values greater than 50% and LIN higher than 35% are categorised as spermatozoa that move linearly (O'Meara *et al.*, 2022). Sexed and non-sexed semen in this study had STR values above 50% and LIN exceeding 35%, indicating that the sexing using the albumin sedimentation method can maintain linear movement. The WOB values in this study were higher than those reported by Utami *et al.* (2025). The actual oscillatory movement along the trajectory can be demonstrated by the WOB value (Valverde *et al.*, 2019). The balance between stability and flexibility of spermatozoa movement, as indicated by a high WOB value, is the key to successful fertilisation (Utami *et al.*, 2025).

Fresh and frozen semen exhibited different ALH and BCF values, indicating alterations in spermatozoa head motility patterns resulting from the freezing and thawing process. Factors such as increased intracellular calcium ions, changes in membrane flexibility, and cold stress during freezing can affect these motility patterns (Afriani *et al.*, 2024). In addition, sexed-Y frozen semen showed different ALH and BCF values compared to non-sexed and sexed-X semen, which may be related to the spermatozoa sexing procedure. During this process, spermatozoa undergo physical and chemical manipulation that affects spermatozoa head movement patterns (Sringarm *et al.*, 2022). The results of this study indicate that sexed-Y semen exhibits higher BCF and lower ALH values than the other groups, consistent with the findings of Afriani *et al.* (2024). This variation is likely associated with the genetic content of the spermatozoa, as X-chromosome-bearing spermatozoa contain more DNA. In contrast, Y-chromosome-bearing spermatozoa have higher levels of miRNA and tsRNA (Zhou *et al.*, 2020). These differences may explain the relatively higher motility efficiency observed in Y-chromosome-bearing spermatozoa.

The ALH values of frozen sexed and non-sexed semen were in the range of 4–6 μm. The ideal ALH values for increasing fertility potential range from 2.5 to 6.5 μm (Belala *et al.*, 2019). indicating that the spermatozoa in this study can support successful fertilisation. The observed BCF values were higher than those reported by Prastiya *et al.* (2023). Higher BCF reflects more stable and regular motility patterns (Ratnawati *et al.*, 2020) as well as greater energy expenditure by spermatozoa (Vigolo *et al.*, 2022). Moreover, Y-chromosome-bearing spermatozoa exhibited higher BCF values compared to X-chromosome-bearing spermatozoa and non-sexed semen, indicating a more stable and regular motility pattern. It should be noted, however, that this study has certain limitations, as only frozen sexed and non-sexed semen were evaluated, without including semen before freezing. Nevertheless, these findings provide valuable insight into the effects of spermatozoa sexing on semen quality and spermatozoa kinematics in Limousin cattle for artificial insemination.

CONCLUSION

Sexing spermatozoa using the BSA method does not affect spermatozoa quality in Limousin bulls. The kinematic profile, characterised by lower ALH and higher BCF values in sexed-Y, indicates that Y-chromosome-bearing spermatozoa have a more stable and regular movement pattern, which may provide better fertilisation capacity compared to X-chromosome-bearing spermatozoa and non-sexed spermatozoa. These findings imply that spermatozoa sexing using the BSA method can be used in artificial insemination without compromising spermatozoa quality and may support improved fertilisation efficiency and sex control in cattle breeding.

CONFLICT OF INTEREST

J. Jakaria serves as editor of the Tropical Animal Science Journal but has no role in the decision to publish this article. The authors also declare that there is no conflict of interest in this study.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used Grammarly to improve the writing structure and grammar of this article. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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