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Sperm Morphometry in Lusitu Pigs and the Motility Characteristics Post-incubation in Non-hyperactivating and Hyperactivating Media: A Comparison with the Large White Genotype

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ABSTRACT

This study explored the reproductive status of Lusitu pigs, in comparison with the Large white genotype, in terms of their morphology and motility metrics. At least five ejaculates were collected from each boar, with a total of 60 analyzed for sperm morphology and kinematic parameters. Data were analyzed descriptively using means and standard deviations and inferentially with a Two-Way nested ANOVA. Both Lusitu and Large white genotypes generally had similar sperm head length, width, area, and perimeter ($P>0.05$). However, shape varied, particularly ellipticity and rugosity ($P<0.05$); effect of individual boar on ellipticity and rugosity was also significant ($P<0.05$). In terms of kinematic parameters, especially Wobble, Linearity, and ALH, Lusitu boars were of a lower fertility status than large white considering the observed mean scores ($P<0.05$). Fertility superiority of the Large white boar sperm was more evidenced when incubated with a hyperactivating medium; most kinematic parameters varied significantly between the two breed groups ($P<0.05$). A higher ($P<0.05$) proportion of Large white boar sperm (61.48%) switched to a hyperactive state compared with those of Lusitu (45.20%) boars. All kinematic parameters for Lusitu boar sperm in non-hyperactivating compared with those in hyperactivating medium varied in respect to their mean scores ($P<0.05$). It is concluded that breed had an effect on morphology and kinematic characteristics of boar sperm incubated in the two media. The CASA system can be used to determine the morphology and kinematic characteristics of Lusitu boar semen during fertility evaluation.

Key words: Large white, Lusitu boar, Kinematic parameters, Morphometry, motility, Hyperactivation.

INTRODUCTION

Pigs contribute to the livelihoods of traditional farmers through meat production and for economic gains. Accordingly, there is need to sustain their contribution through increased productivity or production and conserve this genetic resource, particularly indigenous breeds (Abigaba et al. 2022). Adoption of the comprehensive improvement and proper conservation strategies will prevent stagnation of the national flock size (Makhanya 2018; Abigaba et al. 2022). One of the most important prerequisites for pig production improvement and conservation is the characterization of their phenotypic and reproductive attributes, including semen evaluation which is indispensably part of a boar's reproductive attributes (Makhanya 2018; Suárez-Mesa et al. 2021). Additionally, sperm kinematics, morphometric, and morphological tests can be applied to characterize and understand the biology of sperm (Valverde et al. 2019). It is noteworthy that boar soundness influences the performance of sows, hence the production and productivity of the flock.

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Prediction of boar fertility not only carries economic benefits for sow herds but also facilitates the selection of suitable sires for conservation related-activities (Keller and Kerns 2023).

Boar fertility is routinely predicted through semen parameter evaluations, including quality or quantity related-traits or both (Savić et al. 2017). Many studies have characterized different exotic pig breeds by their semen characteristics including computer aided sperm analysis (CASA)-based parameters (Broekhuijse et al. 2012; Tremoen et al. 2018; Savić et al. 2022). However, limited studies have investigated the quality of semen from indigenous breeds of pigs, particularly, using the CASA system. Moreover, a previous study (Chakurkar et al. 2016) recommended the study and incorporation of indigenous pigs into breeding programs which, partly, necessitates characterization of these breeds by their semen quality. The indigenous pigs, including Lusitu and Nsenga genotypes, constitute majority (over 65%) of the national flock in Zambia (MACO 2003). To this end, characterization of these genotypes, at least by their sperm morphology and motility metrics, require urgent consideration. Keller and Kerns (2023) stated that after standard sperm morphology and motility metrics are met, *ca*. 25% of boars have less than 80% conception rates.

Evaluation of sperm morphology helps to determine boar prolificacy (Chakurkar et al. 2016). Additionally, males differ in terms of dimension, shapes, and abnormality levels of their sperm in semen (Saravia et al. 2007; Chakurkar et al. 2016). This, in turn, may contribute to variations in the boars' reproductive performance (Kondracki et al. 2012). For example, Hirai et al. (2001) found that sperm from fertile boars had smaller and shorter heads than in the less fertile ones. Differences in metrics of the boar sperm morphology are attributed to a number of factors, including breed, sperm concentration, age, temperature, staining technique, inter alia (Kondracki et al. 2012; Górski et al. 2021; Szablicka et al. 2022). Kondracki et al. (2012) noted that the size and shape of sperm head have become an important criterion for classifying morphologically normal sperm or characterizing those with abnormalities in their structure. It is not clear how indigenous pigs, particularly a Lusitu genotype that originates and dominates in the hottest part of Zambia, compares with reported metrics for morphology of sperm in other breeds that have been extensively studied.

Another crucial and most important trait relied on to predict the fertilizing potential of sperm is motility (Vijayaraghavan 2003; Keller and Kerns 2023). It is the most widely used indicator of boar semen fertility (Tremoen et al. 2018; Savić et al. 2022). Conventionally, total and progressive motility have been measured subjectively for decades. However, this approach is prone to human error and biasness hence the invention of a CASA system (Masenya et al. 2011; Valverde et al. 2019). The CASA system not only measures conventional motility objectively but also sperm velocity and subpopulations with different motility characteristics simultaneously (Mortimer 2000; Vyt et al. 2008). Broekhuijse et al. (2012) confirmed that CASA parameters explain 9 and 10% of the variations in farrowing rate and total number of piglets born, respectively. Semen parameters described by CASA can serve as predictive values for boar fertility (Mircu et al. 2008).

Recently, hyperactivation, a motility parameter, has increasingly attracted the attention of many researchers because it has been associated with the fertilizing potential of sperm cells (Tremoen et al. 2018). Hyperactivation can also be assessed with a CASA system (Schmidt and Kamp 2004; Tremoen et al. 2018). This notwithstanding, little is known about sperm functionality with regard to this trait as well as its potential application in breeding programs, particularly in the case of indigenous pigs. Tremoen et al. (2018) recommended the consideration of sperm hyperactivation variable when evaluating the fertility potential and approval of ejaculate for use in breeding. There are no studies that have investigated the motility and hyperactivation traits in Lusitu boar sperm. This study was conducted to (1) establish the morphometric characteristics of sperm in semen from Lusitu boars and Large white pig breed, (2) determine the motility traits of sperm in Lusitu and Large white boar semen, and (3) evaluate motility characteristics of Lusitu boar sperm incubated in non-hyperactivating and hyperactivating medium.

MATERIALS AND METHODS

Ethical clearance

The study was approved by Biomedical Research Ethics Committee of the University of Zambia (UNZABREC), under approval number 1595-2021.

Experimental animals

This study was conducted, from August to November, 2023, at School of Veterinary Medicine, University of Zambia. The boars used in this study were physically healthy and sexually mature. The six indigenous (Lusitu) and five Large white boars were sourced from Gwembe and Lusaka Districts of Zambia, respectively. The average weight of Lusitu boars was 69.56±2.92kg with a range of 64.00-71.50kg, while the Large white weighed 162.20±10.14kg with a range of 150.50-175.00kg. The average ages were 19.60 months and 18.00 months, with the range of 15-24 months and 17-20 months, for Lusitu and Large white boars, respectively. The boars were housed in individual pens with sufficient ventilation throughout the experiment. The ambient temperature during the study period ranged from 19-33°C. Large white boars were fed *ca*. 2kg of feed while Lusitu received 1kg of feed per day (Boar marsh, Novatek animal feeds, Lusaka, Zambia). Water was provided *adlib tum* from the water troughs.

Experimental design

This study employed a Two-stage nested design, which incorporated breed as fixed factor and boar nested in breed as a random factor, to generate data on morphology and motility characteristics of sperm in Lusitu pigs for comparison with the Large white contemporaries. The fixed factor had two levels, namely Lusitu and Large white study groups, which was the main factor of interest for the current study. The ejaculates used in this study were collected from boars randomly, thus a consideration of the boar as a random factor. A total of 60 ejaculates with a minimum of five collections per boar were used in the current study. Knowledge generation and/or generalization of the study findings were grounded by a positivist paradigm. To obtain data, ejaculates collected from boars in both groups were evaluated for both

morphological and sperm motion characteristics, using a CASA system (Sperm Class Analyzer (SCA®), version 6, Microptic S.L., Barcelona, Spain).

Semen collection and evaluation

The collection of ejaculates from both breed groups employed the gloved hand method (King and Macpherson 1973). All samples were collected into a semen collection flask that was pre-warmed at 37℃, and transported to the Department of Biomedical Science, Physiology Laboratory, for analysis within 20mins. Preliminary analysis of semen volume, pH, color, viscosity, gross motility, and concentration were done to aid the selection of ejaculates that are fit for further analysis. Progressive motility (PM; %), non-progressive motility (NPM; %), rapid progressive motility (RPM; %), straightness (STR; %), linearity (LIN; %), curvilinear velocity (VCL; (µm/s), average path velocity (VAP; µm/s), straight line velocity (VSL; µm/s), average lateral displacement of the sperm head (ALH; µm), wobble (WOB; %), beat cross frequency (BCF; Hz), hyperactivity (HA; %) and total motility (TM; %) were used to constitute sperm motility patterns and velocity. The dimensions of sperm were determined using length (µm), width (µm), area (µm^2), perimeter (µm), acrosome coverage (%), midpiece width (µm), length (µm) and midpiece insertion angle, while elongation, ellipticity, rugosity, and regularity were used to evaluate the boar sperm shape.

Sperm motility

Sperm motility patterns and velocity parameters were determined using the CASA-Mot module (SCA, version 6 (Microptic S.L., Barcelona, Spain)). These were determined for sperm incubated in a non-hyperactivating (phosphate buffer saline (PBS; Sigma Aldrich, St. Louis, MO, USA)) and hyperactivating (5mM procaine hydrochloride in PBS; procaine) medium. The flash technique was used to load a pre-heated (37˚C) chamber (20µm deep) of the Leja-4 chamber slide (Leja Products B.V., Nieuw Vennep, The Netherlands) using a positive displacement pipette (Ngcauzele 2018; Ntanjana 2014). The settings used to analyze sperm motility and velocity parameters were adopted from manufacturer, sperm class analyser® settings for boar/swine species. A color digital camera (Basler acA1300-200uc, Ahrensburg, Germany) mounted onto a Nikon Eclipse E200 microscope (Nikon eclipse E200, Nikon cooperation, Tokyo, Japan) was used to capture the sperm trajectories. The microscope was equipped with a 10x negative-phase contrast objective (AN 0.25) and a heated stage (37˚C). At least two fields with a minimum of 500 motile sperms (trajectories) in total, were captured for each sample (×100) and analyzed accordingly. The number of objects incorrectly identified as sperm was removed manually before final analysis.

Sperm morphology

The computer-aided sperm morphology analysis (CASMA), which automatically detects the acrosome, head, and midpiece of sperm, was used to obtain their metrics. The analysis was done on sperm following appropriate smear preparation and staining with a SpermBlue stain-fixative mixture (Microptic SL, Barcelona, Spain) as earlier described (van der Horst and Maree 2009). Evaluation of sperm images was done using a 40x objective (bright field optics), with a green filter (Nickon 45mm GF, Tokyo, Japan) on the microscope (Nikon eclipse E200, Nikon cooperation, Tokyo, Japan). A digital camera (Basler ace acA1300-200uc, Ahrensburg, Germany) attached to the microscope was used to capture images for analysis, under a morphology module (CASA-Morph) of the SCA®, version 6.0 (Microptic SL., Barcelona, Spain). A total of 3000 morphologically normal images were analyzed, of which the number considered per slide was 50 normal sperm images.

Data analysis

All data were analyzed in SPSS IBM® (SPSS IBM 26 version, USA). The data were checked for normality using Kolmogorov-Smirnov test, while homogeneity of variance was tested using Lavene's test. Non-normally distributed data were subjected to the log or square root transformations prior to analysis. The data were descriptively analyzed using means and standard deviations (SD), while analysis of variance (ANOVA) was the inferential statistic employed. The F-test in ANOVA (Two-Way nested ANOVA) was used to test the effect of breed and boar on various sperm morphometric and kinematic variables. The effect of medium and boar factors on sperm kinematic variables was tested using a Two-Way ANOVA. In the Generalized Linear Model (GLM, univariate) used, breed and medium were treated as fixed factors and boar as a random factor. In all the tests, significance was taken at $P<0.05$. For transformed data, results (means and SD) are reported as untransformed to ease comparisons with findings from other studies.

RESULTS

Breed means for sperm morphometric attributes in Lusitu and Large white boars reared in Zambia

The means for sperm morphometric traits in Lusitu and Large white boars and their corresponding p-values are presented in Table 1. Both Lusitu and Large white boar sperm had similar mean head length ($P>0.05$). The difference in breed mean values for sperm head width, head area, and perimeter were not significant ($P>0.05$). Lusitu boar sperm had a significantly lower mean ellipticity score than that of the Large white ($P<0.05$), while the mean elongation was, in contrast, higher ($P<0.05$). The mean scores of midpiece width, area, and insertion angle for Lusitu boar sperm and that of the Large white sperm did not differ significantly ($P>0.05$). The percentage proportion of acrosome covering the sperm head in Lusitu pigs was similar ($P>0.05$) to that of the Large white group. The effect of the boar factor was significant for elongation, ellipticity, and acrosome variables ($P<0.05$), while no significant effect of individual boar was observed for the rest of the variables ($P>0.05$).

The breed means for sperm motility characteristics evaluated by the CASA system for sperm incubated in phosphate buffer saline medium

The mean scores for different sperm motility traits, including those under motility patterns, velocity, and their derivatives, in Lusitu and Large white boars are presented in Table 2. This study did not find a difference in mean total motility scores between the two breed groups ($P>0.05$).

Table 1: Mean scores for sperm morphometric attributes in Lusitu and Large white pigs reared in Zambia

	Lusitu pigs	Large white pigs	Breed	Breed (Boar)
Variable	Mean±SD	Mean±SD	P-value	P-value
Head length (µm)	9.85±0.22	9.97±0.25	0.150	0.086
Head width (µm)	4.67±0.09	4.62±0.07	0.176	0.001
Head area (µm^2)	41.77±1.25	41.67±1.07	0.762	0.411
Head perimeter (µm)	21.02±0.36	21.23±0.36	0.112	0.090
Elipticity	2.11±0.05	2.16±0.03	0.046	0.001
Elongation	0.36±0.01	0.37±0.01	0.067	0.001
Roughness	1.19±0.02	1.16±0.02	0.006	0.054
Regularity	0.87±0.01	0.87±0.01	0.503	0.002
Midpiece width (µm)	1.00±0.03	1.00±0.02	0.836	0.489
Midpiece area (µm^2)	8.97±0.63	9.37±0.47	0.498	0.156
MIA	2.34±0.46	2.35±0.42	0.905	0.664
Acrosome (%)	57.01±0.35	57.18±0.32	0.216	0.022

P<0.05=Significant effect; P>0.05=Non-significant effect; Breed (Boar)=Boar nested in Breed; %=Percentage; µm=micrometer; µm^2=square micrometer; MIA=Midpiece Insertion Angle.

Table 2: Breed means for motility characteristics of sperm incubated in non-hyperactivating (phosphate buffer saline (PBS)) medium

	Lusitu pigs	Large white pigs	Breed	Breed (Boar)
Variable	Mean±SD	Mean±SD	P-value	P-value
Motility pattern				
PM (%)	78.20±6.72	79.21±6.80	0.603	0.331
NPM (%)	14.01±5.57	14.48±5.74	0.773	0.324
RPM (%)	66.11±11.96	69.16±10.60	0.227	0.781
STR (%)	79.10±5.39	75.63±4.74	0.036	0.323
LIN (%)	54.78±6.27	49.29±5.49	0.010	0.281
Sperm velocity				
VCL (µm/s)	76.62±20.19	79.56±13.14	0.060	0.994
VAP (µm/s)	50.82±15.28	50.08±10.21	0.913	0.947
VSL (µm/s)	41.18±11.24	39.46±8.47	0.336	0.932
Other motility traits				
ALH	1.65±0.34	1.81±0.29	0.002	0.997
WOB (%)	66.61±4.69	62.20±5.42	0.014	0.267
BCF (Hz)	15.05±1.99	14.08±1.91	0.175	0.070
HA (%)	1.91±1.50	2.45±1.54	0.179	0.324
TM (%)	92.28±2.62	93.89±2.19	0.097	0.029

P<0.05=Significant effect; P>0.05=Non-significant effect; Breed(Boar)=Boar nested in Breed; %=Percentage; µm=micrometer; µm/s=micrometer per second; Hz=Hertz; PM=Progressive Motility; NPM=Non-progressive Motility; RPM=Rapid Progressive Motility; STR=Straightness; LIN=Linearity; VCL=Curvilinear Velocity; VAP=Average Path Velocity; VSL=Straight Line Velocity; ALH=Average Lateral Displacement of the sperm head; WOB=Wobble; BCF=Beat Cross Frequency; HA=Hyperactivity; TM=Total Motility.

The mean progressive motility percentage for Lusitu boar sperm was similar (P>0.05) to that of the Large white. A non-significant difference (P>0.05) was found for mean rapid progressive motility between the two breed groups. The study found mean differences in STR (P<0.05) and LIN (P<0.05) proportions of sperm between Lusitu and Large white groups. The mean VCL, VAP, and VSL of sperm did not differ between Lusitu and Large white pigs (P>0.05). Statistical differences were observed for the case of ALH (P<0.05) and WOB (P<0.05) but not BCF (P>0.05) scores between the two breed groups. The proportions of hyperactive sperm were not different between the two groups (P>0.05). The observed boar effect on all the measured sperm traits was not significant (P>0.05) apart from total motility (P<0.05).

The breed means for motility characteristics evaluated by the CASA system for sperm incubated in procaine medium

The mean scores for the different motility traits of Lusitu and Large white boar sperm exposed to procaine medium are presented in Table 3. The mean total motility of sperm in Lusitu and the Large white group did not differ significantly (P>0.05). The mean scores for each of the sperm traits under motility patterns, including progressive motility, non-progressive motility and rapid progressive motility differed significantly between Lusitu and Large white boars (P<0.05). Similar observations (P<0.05) were made for STR and LIN variables. In the case of sperm velocity traits, statistical differences were observed between Lusitu and Large white boar sperm for VCL (P<0.05) and VAP (P<0.05) but not for VSL (P>0.05). Analysis results for other kinematic variables, ALH and BCF, revealed significantly different mean scores (P<0.05) between the two breed groups, except for the case of WOB (P>0.05). A significantly higher (P<0.05) proportion of hyperactivated sperm was observed in Large white (61.48%) compared to its Lusitu counterpart (45.20%). The observed effect of individual boar on mean scores for each of the sperm trait was not significant (P>0.05).

Table 3: Breed means for motility characteristics of sperm incubated in hyperactivating (procaine) medium

	Lusitu pigs	Large white pigs	Breed	Breed (Boar)
Variable	Mean±SD	Mean±SD	P-value	P-value
Motility pattern				
PM (%)	47.35±12.44	34.82±12.56	0.001	0.800
NPM (%)	46.67±13.71	58.72±10.56	0.001	0.945
RPM (%)	44.58±12.97	33.41±12.41	0.002	0.777
STR (%)	46.77±7.87	38.35±6.84	0.001	0.655
LIN (%)	22.49±4.41	19.27±3.63	0.005	0.731
Sperm velocity				
VCL (µm/s)	160.23±35.05	191.07±36.73	0.003	0.783
VAP (µm/s)	78.06±20.22	93.75±19.61	0.006	0.720
VSL (µm/s)	33.98±8.11	35.46±9.96	0.565	0.363
Other motility traits				
ALH (µm)	3.84±0.89	4.84±0.81	0.001	0.931
WOB (%)	47.96±4.55	48.11±2.71	0.847	0.802
BCF (Hz)	13.60±2.99	9.37±2.77	0.002	0.080
HA (%)	45.20±11.50	61.48±11.46	0.001	0.945
TM (%)	93.16±4.03	93.55±4.86	0.305	0.738

P<0.05=Significant effect; P>0.05=Non-significant effect; Breed(Boar)=Boar nested in Breed; %=Percentage; µm=micrometer; µm/s=micrometer per second; Hz=Hertz; PM=Progressive Motility; NPM=Non-progressive Motility; RPM=Rapid Progressive Motility; STR=Straightness; LIN=Linearity; VCL=Curvilinear Velocity; VAP=Average Path Velocity; VSL=Straight Line Velocity; ALH=Average Lateral Displacement of the sperm head; WOB=Wobble; BCF=Beat Cross Frequency; HA=Hyperactivity; TM=Total Motility.

The mean motility characteristics for Lusitu boar sperm evaluated by the CASA system post-incubation in phosphate buffer saline and procaine medium

The results of motility parameter evaluations by CASA for the Lusitu boar sperm exposed to non-hyperactivating (phosphate buffer saline (PBS)) and hyperactivating (Procaine hydrochloride) medium are presented in Table 4. The difference in mean total motility

Table 4: Mean scores for motility characteristics of Lusitu boar sperm incubated in non-hyperactivating (PBS) and hyperactivating (procaine hydrochloride) medium

	Sperm in PBS	Sperm in Procaine	Medium	Boar	Medium*Boar
Variable	Mean±SD	Mean±SD	P-value	P-value	P-value
Motility pattern					
PM (%)	77.97±6.34	47.35±12.44	0.001	0.682	0.432
NPM (%)	14.01±5.57	46.67±13.71	0.001	0.898	0.513
RPM (%)	66.11±11.96	44.65±12.97	0.001	0.682	0.654
STR (%)	78.93±5.60	46.77±7.87	0.001	0.783	0.477
LIN (%)	54.86±6.38	22.49±4.41	0.001	0.680	0.395
Sperm velocity					
VCL (µm/s)	77.29±19.53	160.23±35.05	0.001	0.138	0.966
VAP (µm/s)	50.82±15.28	78.06±20.22	0.001	0.058	0.994
VSL (µm/s)	41.18±11.24	33.98±8.11	0.005	0.146	0.897
Other motility traits					
ALH (µm)	1.82±0.48	3.84±0.89	0.001	0.536	0.487
WOB (%)	67.18±4.99	47.96±4.55	0.001	0.288	0.829
BCF (Hz)	15.05±1.99	13.59±2.99	0.033	0.095	0.698
HA (%)	1.97±1.43	45.20±11.50	0.007	0.830	0.409
TM (%)	92.28±2.62	93.16±4.03	0.360	0.397	0.422

P<0.05=Significant effect; P>0.05=Non-significant effect; Medium*Boar=Medium-Boar interaction; Hz=Hertz; PM=Progressive Motility; NPM=Non-progressive Motility; RPM=Rapid Progressive Motility; STR=Straightness; LIN=Linearity; VCL=Curvilinear Velocity; VAP=Average Path Velocity; VSL=Straight Line Velocity; ALH=Average Lateral Displacement of the sperm head; WOB=Wobble; BCF=Beat Cross Frequency; HA=Hyperactivity; TM=Total Motility.

of sperm in PBS and that of procaine was not significant (P>0.05). In terms of motility patterns, higher mean scores of sperm progressive motility (P<0.05), rapid progressive motility (P<0.05), STR (P<0.05), and LIN (P<0.05) were observed for sperm incubated in PBS than the case of those subjected in procaine medium, except for NPM which was significantly lower (P<0.05). In the case of velocity traits, the mean VCL and VAP values were significantly higher for sperm in procaine than those in PBS medium (P<0.05) except for the VSL which was significantly lower (P<0.05). The mean ALH and hyperactivity scores were higher (P<0.05) for sperm in procaine than the case of PBS medium, while those of WOB and BCF were significantly lower (P<0.05) in procaine medium. There was no significant effect (P>0.05) of individual boar on the mean scores for each of the studied parameters. This study revealed no significant interaction (P>0.05) between the medium used with individual boar factors, at least for each of the boar sperm parameter studied.

DISCUSSION

Sperm motility and morphology status are associated with the fertility of boars (García-Vázquez et al. 2016). The morphology status, in terms of dimensions and shape, is crucial for sperm locomotion within the genital tract and reach fertilization site to fertilize ova (García-Vázquez et al. 2016). Sperm dimensions influence the post-copulation selection of sperm (Kondracki et al. 2012), which could be due to association with motility. The results of sperm dimensions in this study were generally inconsistent with the earlier findings by Kondracki et al. (2012) who found differences between Duroc and Petrian boar sperm dimensions. Górski et al. (2021) also demonstrated differences between Large white and Landrace boars with regard to various sperm dimensions. On the other hand, Saravia et al. (2007) found differences for particular dimensions between some breeds as well as similarity between other breeds, where the latter case agrees with some findings in the current study. It is plausible that sperm from some pig breeds have similar dimensions, but some others do not. Our observations add to the importance of exploring the sperm dimensions for each breed of pigs. It is noteworthy that a sperm head is the genetic information carrier during fertilization process, hence the potential effect of varied dimensions and shape in this process (Kondracki et al. 2012).

The mean head length in this study was on a higher side compared with the 9.41µm and 9.35µm, for Duroc and Large white boar sperm, respectively (Banaszewska et al. 2011; Kondracki et al. 2012). However, our mean scores for sperm head width, in both boar genotypes, were similar to a 4.67µm score that was reported previously for the 28-month old Large white boars (Banaszewska et al. 2011). On the other hand, it was generally smaller than the one reported by Górski et al. (2021) for Large white (4.76µm) and Landrace (4.78µm). The current mean scores for head area, regardless of the breed, were similar to the one (41.94µm) reported for the 28-month old Large white boars (Banaszewska et al. 2011), but higher than that of the Landrace by *ca*. 1µm (Górski et al. 2021). The current mean perimeter values were *ca.* 2µm lower than the earlier reported figures (Banaszewska and Andraszek 2021). The above subtle but important variations between our study and the previous ones may be attributed to differences in breeds studied, age, sperm concentration, incubation temperature, storage time, and staining technique (Saravia et al. 2007; Czubaszek et al. 2019; Szablicka et al. 2022). It is opined that standardization of morphology protocol(s) may be needed for consistent and reliable results.

This study revealed breed differences with regard to sperm shapes which agrees with an earlier study that found variations in elipticity and rugosity of sperm head between different pig breeds (Saravia et al. 2007). The same study did not find differences in mean regularity and elongation scores between different breeds which is supported by the findings of the current study where no

differences in mean values were found between the study breeds. Ellipticity indicates the degree to which sperm heads are oval or conical (Banaszewska and Andraszek 2021). Its mean score in this study was generally similar to an earlier reported value (2.0) for Duroc and Risco boars (Saravia et al. 2007). However, sperm rugosity, which specifies the regularity or amorphous shape of the sperm head, was found with a mean value higher than the previous findings for Duroc, Large white, and Landrace (6.0-7.0) (Saravia et al. 2007; Banaszewska and Andraszek 2021). Similarly, elongation, which represents roundedness of the sperm head, was on the higher side than the mean score (0.32) reported previously by Szablicka et al. (2022). Sperm regularity, which indicates the symmetry of sperm head and degree to which it is pyriform, had a mean score similar to the one reported previously (0.88) for Petrian (Barquero et al. 2021) and Landrace boars (Szablicka et al. 2022). Our study reveals a generally shorter and more rugose Lusitu boar sperm head which may have a negative effect on motility/velocity during transportation in the genital tract compared with those of the Large white. This is in view of the reported effect of head shape upon sperm hydrodynamics (García-Vázquez et al. 2016). For example, sperm with elongated head swim faster (Malo et al. 2006), which, in turn, may positively impact on post-copulation selection, as well as their transportation to the fertilization site and fertilizing ability.

The close relationship between sperm morphology and motility (García-Vázquez et al. 2016), accordingly, necessitates exploration of the motility characteristics of indigenous boar sperm. With regard to the medium, both Lusitu and Large white sperm did not switch to hyperactivation state since phosphate buffer saline is known to be non-hyperactivating to sperm (Ngcauzele et al. 2020). Most of the observed motility characteristics for Large white boar sperm were on the higher side compared to those of Lusitu, except for straightness, linearity, straight line velocity, and wobble. A similar trend was also observed between the indigenous (Kolbroek) and Large white boar sperm in South Africa (Masenya et al. 2011). It is likely that the observed status of sperm dimension and shapes contributed partly to the varied motility scores, this is in view of earlier studies that confirmed a relationship between sperm morphology and motility metrics (García-Vázquez et al. 2016). Furthermore, many motility traits in this study had mean scores similar to those reported in earlier studies that incubated boar sperm in Tyrode's complete medium and Tyrode's basal medium (García Herreros et al. 2005) as well as Androstar Plus extender (Tremoen et al. 2018).

The current findings on most sperm motility patterns and velocity characteristics, particularly for Lusitu boar sperm, generally favor Lusitu pigs for breeding. This is in view of their similarity with those reported for sperm from Kolbroek, an indigenous breed reared in South Africa, that was found to possess fertility scores acceptable for breeding (Makhanya 2018). García-Vázquez et al. (2016) confirmed motility as one of the factors that determines initial sperm selection in the genital tract, with the highly motile sperm being favored most. Moreover, it is a crucial trait that enables sperm to wiggle through the tract system to reach the fertilization site and fertilize ova. Motility is also required for sperm to traverse cumulus oophorus and zona pellucida to access the ovum, more so when sperm are hyperactivated (Vijayaraghavan 2003; Schmidt and Kamp 2004). Broekhuijse et al. (2012) reported that CASA-kinematic parameters explain 9 and 10% of the variations in farrowing rate and total number of piglets born, respectively, hence their correlation with fertility.

Recently, another important motility parameter known as sperm hyperactivation has increasingly attracted the attention of researchers because of its association with the fertilizing potential of sperm (Schmidt and Kamp 2004; Tremoen et al. 2018). It is characterized by vigorous and non-linear movement caused by an increased amplitude of flagellar beats, whiplash movement of the sperm (Schmidt and Kamp 2004). It is opined that sperm undergo hyperactivation to prevent entrapment in the folds and crypts of oviductal epithelium, improve chances of oocyte contact, maintain microenvironment, assist in penetration of cumulous oophorus as well as zona pellucida by means of a high force generated during hyperactivation (Mortimer 2000). Consequently, it is recommended that sperm hyperactivation routinely be evaluated as a biomarker for male fertility. Additionally, the use of procaine as one of the time-saving and cheap media during evaluation of this sperm function test was previously recommended (Mircu et al. 2008; Ntanjana 2014). Furthermore, Schmidt and Kamp (2004) established threshold values considered for boar sperm to be judged as hyperactive, these being VCL >97µm/s, ALH >3.5µm, LIN >32%, and WOB <71%.

There are many different compounds known to induce hyperactivation, including Ca^{2+} ionophore A23187, progesterone, and CatSper activators like 4-aminopyridine and procaine (Schmidt and Kamp 2004; Sharif et al. 2022). When Ca^{2+} ionophore A23187 was used in a previous study (Schmidt and Kamp 2004), approximately 60% of the incubated sperm switched from non-hyperactive to hyperactive motility. In this study, that employed procaine medium, *ca.* 61.48% and 45.20% proportions of sperm in Large white and Lusitu boars, respectively, were found to be in a hyperactive state. In both breeds, the mean values for VCL and ALH increased significantly while those of linearity and wobble decreased, this is typical of sperm hyperactivation (Mircu et al. 2008). In view of the observed proportions of hyperactive sperm for the two pig genotypes and opined physiological roles of sperm hyperactivation (Mortimer 2000), Large white boar sperm may have higher chances of reaching the fertilization site and or fertilizing ova compared with those of Lusitu boars.

It is noteworthy that a 45% proportion of sperm switching to hyperactive motility was a good score for fertility potential of Lusitu boar sperm. Ngcauzele et al. 2020) confirmed acceptable sperm fertility on the basis of mammalian sperm proportion of more than 20% switching to hyperactivation state, which Lusitu boar sperm satisfies considering the current 45% proportion in a hyperactive state. Moreover, comparisons of most kinematic parameters revealed significant mean differences between Lusitu boar sperm incubated in non-hyperactivating and hyperactivating medium.

Conclusion

Sperm morphology and motility metrics are associated with boar fertility. This study has revealed that Lusitu and

Large white boars produced sperm with generally similar dimensions but varied in shapes. Their kinematic characteristics generally varied, of which Large white boar sperm scores demonstrated a generally superior quality compared to those of Lusitu boar, more so when there are incubated in a hyperactivating medium. This notwithstanding, the observed sperm parameters for Lusitu boars were promising and indicative of acceptable quality for breeding. This study recommends the following: (1) further studies be conducted to establish the actual proportion of sperm needed to switch to a hyperactive state for ejaculate or boar acceptability as fertile for breeding purposes, (2) standardization of the protocol(s) for kinematic analysis in terms of time, medium, dilution rate, temperature, and perhaps CASA model must be considered for reliability of the results on boar sperm fertility, and (3) *in vivo* studies be conducted to supplement or confirm the current *in vitro* study findings.

Authors' contributions

RA conceived and designed the project, collected and analyzed data, and wrote the manuscript draft; PCS designed and supervised study and reviewed the manuscript; ESM designed and supervised study and reviewed the manuscript; PN supervised study; and WNMM supervised study. All authors read the final draft and approved manuscript submission for publication.

Conflict of interest

All authors declare that there is no conflict of interest in the work presented in this manuscript.

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