Research Article

Correlation between Morphometric and Motility Characteristics of Spermatozoa in Semen of Lusitu Boars

Rubaijaniza Abigaba[1,2]*, Pharaoh C Sianangama[1,3], Edwell S Mwaanga[4], Wilson N M Mwenya[1] and Progress H Nyanga[5]

[1]Department of Animal Science, School of Agricultural Sciences, The University of Zambia, P.O. Box 32379, Lusaka, Zambia
[2]Department of Biomolecular & Biolab Sciences, College of Veterinary Animal Resources & Biosecurity, Makerere University, P.O. Box 7062, Kampala, Uganda
[3]Department of Animal Science, School of Agriculture and Natural Resource Management, Palabana University, P.O. Box 50199, Lusaka, Zambia
[4]Department of Biomedical Science, School of Veterinary Medicine, The University of Zambia, P.O. Box 32379, Lusaka, Zambia
[5]Department of Geography and Environmental Studies, School of Natural Sciences, The University of Zambia, P.O. Box 32379, Lusaka, Zambia
*Corresponding author: abigabajzan@gmail.com

ABSTRACT

This study correlated morphometric and motility characteristics of Lusitu boar spermatozoa. A total of 30 ejaculates, with five collected per boar, were used for the study. Data were analyzed using Spearman's rank- and Pearson correlation coefficients. The head length was correlated ($P<0.05$) positively with its derivative shape parameters, namely ellipticity and elongation. The relationship was negative ($P<0.05$) in the correlation of head width with elongation and ellipticity. No head morphometric parameter was associated with midpiece characteristics ($P>0.05$). Head length was positively correlated ($P<0.05$) with the swimming speed parameters, VCL, VAP, VSL, and WOB. In contrast, a negative association between these speed parameters and head width was insignificant ($P>0.05$). Both elongation and ellipticity were associated with BCF ($P<0.05$). Insertion angle was negatively associated with the VCL ($P<0.05$). Spermatozoa hyperactivity was positively associated with head length and perimeter, while its observed significant association ($P<0.05$) with head roughness, midpiece width, and insertion angle was negative. It is concluded that Lusitu boar spermatozoa head and midpiece morphometric characteristics are not correlated; however, some individual traits are associated with the CASA kinematic characteristics. Head length, area, midpiece width, and insertion angle may be additional candidates for improved hyperactivation and spermatozoa fertility prediction in Lusitu boars.

Keywords: Correlation, Lusitu boar, Morphometry, Motility, Spermatozoa

INTRODUCTION

The fertility and reproductive performance of females depend on the quality of a male (boar), thus boar soundness or fertility influences the productivity of the flock (Kondracki et al. 2017). It is critical to optimize the fertilizing capacity of boars to allow for increased productivity and reduce the gap between genetically superior and inferior animals (da Costa Málaga et al. 2022). The fertilizing capacity of boars can be predicted through the evaluation of semen/spermatozoa quantity and quality characteristics, including semen volume, spermatozoa concentration, morphology, and percentage

of spermatozoa with correct motility (Chakurkar et al. 2016; Kondracki et al. 2017). Among these parameters, motility, morphometric, and morphological or DNA integrity tests constitute the crucial parameters used to characterize and understand the reproductive biology of spermatozoa (Valverde et al. 2019).

The study of spermatozoa morphology is considered an essential part of spermatozoa research (Maroto-Morales et al. 2016). Morphological analyses are used to evaluate spermatozoa quality and thereby categorize ejaculates as either approved or rejected (Gaggini et al. 2017). During semen evaluation, spermatozoa abnormalities are routinely considered (Sutkevičienė and Žilinskas 2004).

Nowadays, determination of spermatozoa morphometrics has become an important criterion for classifying morphologically normal spermatozoa or characterizing those with abnormalities in their structure (Kondracki et al. 2023). There are variations in spermatozoa morphometrics which were associated with breed, ejaculate volume, spermatozoa concentration, inter alia (Górski et al. 2021; Kondracki et al. 2023). Understanding these spermatozoa dynamics, more so in indigenous boars, is crucial since some morphometric traits are associated with spermatozoa selection and transportation in the female tract, cryo-resistance, DNA chromatin quantity, inter alia (Maroto-Morales et al. 2016; Gaggini et al. 2017). The relationship between boar spermatozoa morphometry with motility characteristics is one area that remains understudied.

Spermatozoa motility is the most widely used indicator of boar semen fertility in most boar studs (Tremoen et al. 2018; Savić et al. 2022). It is also the most important trait relied on routinely to predict the fertilizing potential of spermatozoa (Vijayaraghavan 2003; Keller and Kerns 2023). Motility enables ejaculated spermatozoa to traverse the female reproductive tract and reach the fertilization site. It is also essential for penetration of the outer investments of an oocyte including the zona pellucida (Vijayaraghavan 2003). A previous study (Broekhuijse et al. 2012) confirmed that CASA-kinematic parameters showed 9 and 10% of the variations in farrowing rate and total number of piglets born, respectively. Moreover, Keller and Kerns (2023) stated that after standard spermatozoa motility and morphology metrics are met, *ca*. 25% of boars have less than 80% conception rates. Hence there is a need to evaluate these parameters, more so with a CASA system for boar fertility prediction (Mircu et al. 2008). The CASA system is recommended for pre-screening boars prior to artificial insemination (AI) or natural mating (Hwang et al. 2017).

The reliance on multiple tests is crucial for accurate evaluation of semen, since no single spermatozoa parameter may be relied on to predict boar spermatozoa fertility (da Costa Málaga et al. 2022). Some semen characteristics influence other quality parameters; for example, ejaculate volume has an effect on the spermatozoa shape and size (Górski et al. 2021). Hence it is important to appreciate more about the interrelatedness of various semen characteristics. Examining the relationships between morphometric traits, motility patterns, and velocity characteristics may add to the existing knowledge of spermatozoa biology. This is more so with respect to spermatozoa hydrodynamics, fertilizing capacity, and thus proper prediction of spermatozoa fertility, particularly in indigenous pigs intended for breeding (García-Vázquez et al. 2016). There is a dearth of information available on the relationship between spermatozoa shape and size to motility (Gil et al. 2009), especially in the case of the Lusitu pig genotype, hence the need for an urgent investigation. Moreover, there have been suggestions for additional studies with CASA that aim to correlate seminal characteristics with fertility (Gottardi et al. 2020).

This study was, therefore, conducted to 1) determine correlation between the head and midpiece morphometry of spermatozoa obtained from Lusitu boars, 2) correlate the head and midpiece-based morphometric characteristics with motility patterns of spermatozoa in Lusitu boars, and 3) assess the association between head and midpiece-based morphometric parameters with velocity and other motility-derived characteristics of spermatozoa in semen obtained from Lusitu boars.

MATERIALS AND METHODS

Ethical clearance

This study was conducted with approval by Biomedical Research Ethics Committee of the University of Zambia (UNZABREC; approval number 1595-2021).

Experimental animals

This study was conducted at School of Agricultural Sciences, University of Zambia, from August to November, 2023. Six physically healthy and sexually mature indigenous (Lusitu) boars from Gwembe District of Zambia were used in this study. Their average weight was 69.56±2.92kg with a range of 64.00-71.50kg, while the age ranged from 15-24 months with an average of 19.60 months. The boars were housed in individual pens with sufficient ventilation and unrestricted access to drinking water throughout the experiment. Each boar received 1kg of feed (Boar marsh, Novatek Animal Feeds, Lusaka, Zambia) per day. The ambient temperature during the period of study ranged from 19-33°C.

Experimental design

This study employed a correlation study design that investigated the relationship between morphometric and motility characteristics of spermatozoa in Lusitu boars. A total of 30 ejaculates with five per boar were collected and used for the study. Ejaculates used in this study were collected from the boars randomly. Knowledge generation and/or deductions from the study findings were grounded by a positivist paradigm. To obtain data, ejaculates collected from the boars were evaluated for both morphology and spermatozoa 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 boars employed the gloved hand method. Immediately after collection, all samples were transported to the Physiology Laboratory, Department of Biomedical Sciences, School of Veterinary Medicine, The University of Zambia, for analysis. Only acceptable ejaculate samples, following preliminary analysis of semen volume, pH, color, viscosity, gross motility, and concentration, were considered for inclusion. The following morphometric parameters were analyzed: spermatozoa length (μm), width (μm), area (μm^2), perimeter (μm), elongation, ellipticity, rugosity, regularity, acrosome coverage (%), midpiece width (μm), length (μm) and midpiece insertion angle (°). The motility characteristics included: 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 spermatozoa head (ALH; μm), wobble (WOB; %), beat cross frequency (BCF; Hz), hyperactivity (HA; %) and total motility (TM; %).

Spermatozoa motility

All the spermatozoa motility patterns and velocity parameters were determined using the CASA-Mot module (SCA, Version 6 (Microptic S.L., Barcelona, Spain)). These were determined in semen diluted using phosphate buffer saline medium (PBS; Sigma Aldrich, St. Louis, MO, USA). 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 as previously described (Ntanjana, 2014; Ngcauzele, 2018). The settings used to analyze spermatozoa motility and velocity parameters were adopted from the manufacturer's sperm class analyser® settings for boar/swine species. The microscope used was equipped with a 10x negative-phase contrast objective (AN 0.25). At least two fields, including a total of 500 motile spermatozoa (trajectories), were captured for each sample at ×100 magnification and analyzed accordingly. Before the final analysis, all artifacts incorrectly captured were removed manually.

Spermatozoa morphology

Spermatozoa morphology analysis was done on prepared slides, using an SCA® Morphology-module (SCA® Morphology, Version 6.0, Microptic SL., Barcelona, Spain). The module used automatically detects acrosome, head, and midpiece of spermatozoa. Preparation of slides and staining with a SpermBlue stain-fixative mixture (Microptic SL, Barcelona, Spain) were based on an earlier procedure (van der Horst and Maree 2009). Spermatozoa images were evaluated following capture using a digital camera (Basler ace acA1300-200uc, Ahrensburg, Germany) attached to the microscope (Nikon eclipse E200, Nikon cooperation, Tokyo, Japan). The microscope was set at a 40x objective (bright field optics), with a green filter (Nickon 45mm GF, Tokyo, Japan), that was used to capture images for analysis. A total of 1500 morphologically normal images were analyzed, with 50 normal spermatozoa images being considered per slide.

Data analysis

All the data were analyzed in SPSS IBM® (SPSS IBM 26 Version, USA). The data were checked for normality using Shapiro-Wilk test. The correlational analysis was conducted using Spearman's rank- and Pearson correlation coefficients to establish the strength and direction of association between the different boar spermatozoa variables. In all the tests, significance was taken at P<0.05.

RESULTS

Correlation between the different morphometric characteristics of spermatozoa in Lusitu boars

The correlation coefficient values obtained by comparing bivariate/pair(s) of spermatozoa head and midpiece characteristics are presented in Table 1. Both the head length and width were strongly correlated (P<0.05) with their derivative parameters, including head area, perimeter, ellipticity, and elongation, except for roughness (P>0.05). A significant (P<0.05) negative relationship between head width with ellipticity, elongation, as well as acrosome percentage (covering the spermatozoa head) was observed. Roughness was only associated (P<0.05) with head perimeter, ellipticity, elongation, and acrosome percentage and the relationship was negative. Head area was only associated with head length and width (P<0.05) but not shape parameters (P>0.05). The midpiece characteristics were not associated (P>0.05) with all the studied parameters, except for the negative correlation observed between midpiece area and midpiece width (P<0.05).

Correlation between different spermatozoa velocity and head morphometric characteristics in Lusitu boars

The correlation coefficient values obtained through bivariate comparisons of each spermatozoa head characteristic with individual spermatozoa velocity parameters are presented in Table 2. The spermatozoa head length was positively associated with the VCL (P<0.05), VAP (P<0.05) and VSL (P<0.05). This study did not find a relationship between the spermatozoa head width with any of these spermatozoa velocity parameters (P>0.05). Both head area and perimeter were associated with the VCL (P<0.05), VAP (P<0.05) and VSL (P<0.05) but not BCF (P>0.05). Elongation was positively associated with BCF (P<0.05) but not VCL (P>0.05), VAP (P>0.05), and VSL (P>0.05). Similar observations were made for ellipticity with BCF (P<0.05), VCL (P>0.05), VAP (P>0.05) and VSL (P>0.05). Most of the velocity parameters had a negative relationship tendency with roughness, and regularity, although this relationship was not significant (P>0.05).

Table 1: Correlation between the different morphometric characteristics of spermatozoa in Lusitu boars

Variable	HDL	HDW	HDA	HDP	Ellipt	Elong	Rough	MPW	MPA	MPIA	Acros
HDL	1										
HDW	0.300	1									
HAD	0.852*	0.711*	1								
HDP	0.883*	0.478*	0.886*	1							
Ellipt	0.603*	-0.545*	0.071	0.378*	1						
Elong	0.525*	-0.489*	0.149	0.455*	0.956*	1					
Rough	-0.279	0.248	-0.089	-0.477*	-0.480*	-0.517*	1				
MPW	-0.215	0.011	-0.154	-0.351	-0.196	-0.234	0.351	1			
MPA	-0.004	0.058	0.320	0.148	0.003	0.079	0.004	-0.522*	1		
MPIA	-0.004	-0.154	-0.158	-0.217	0.116	0.088	0.238	-0.005	-0.90	1	
Acros	0.209	-0.373*	0.004	0.193	0.499*	0.442*	-0.392*	-0.021	0.210	0.221	1

*=P<0.05; -=Negative correlation; HDL=Head length; HDW=Head width; HAD=Head area; HDP=Head perimeter; Ellipt=Ellipticity; Elong=Elongation; Rough: Roughness; MPW=Midpiece width; MPA=Midpiece area; MPIA=Midpiece insertion angle; Acros=Acrosome.

Table 2: Correlation between spermatozoa velocity and head morphometric characteristics in Lusitu boars

Variable	VCL	VAP	VSL	BCF
HDL	0.425*	0.493*	0.414*	0.325
HDW	0.132	0.153	0.209	-0.050
HAD	0.383*	0.444*	0.420*	0.299
HDP	0.427*	0.492*	0.440*	0.266
Ellipticity	0.221	0.262	0.158	0.396*
Elongation	0.326	0.359	0.275	0.466*
Roughness	0.222	0.231	-0.117	-0.53
Regularity	-0.066	-0.082	-0.050	-0.258
Acrosome	0.039	0.066	-0.002	0.106

*=P<0.05; HDL=Head length; HDW=Head width; HAD=Head area; HDP=Head perimeter; VCL=Curvilinear velocity; VAP=Average path velocity; VSL=Straight-line velocity; BCF=Beat cross frequency.

Correlation between different spermatozoa motility patterns and head morphometric characteristics in Lusitu boars

The results displayed in Table 3 include correlation coefficient values describing relationships between spermatozoa head characteristics and individual motility patterns. There was a positive association between head length with WOB (P<0.05) and spermatozoa hyperactivity (P<0.05), while head width was not associated with any characteristic under spermatozoa motility patterns (P>0.05). Head area was significantly associated with ALH (P<0.05) but weakly related to WOB and spermatozoa hyperactivity with a non-statistical significance (P>0.05). The study found a positive correlation between head perimeter with WOB (P<0.05), ALH (P<0.05), and spermatozoa hyperactivity (P<0.05). There was no significant correlation was observed for the rest of the morphometric characteristics with motility pertain (P>0.05), except for the negative correlation observed between roughness and hyperactivity (P<0.05). The tendency of the relationship between roughness and regularity with most of the motility patterns was generally negative but not significant (P>0.05).

Correlation between midpiece morphometry and motility characteristics of spermatozoa in Lusitu boars

The correlation coefficients and probability results obtained by bivariate comparisons between the midpiece morphometric with motility characteristics of Lusitu boar spermatozoa are presented in Table 4. Midpiece width was negatively correlated with spermatozoa hyperactivity (P<0.05), and no correlation with other motility characteristics was observed (P>0.05). Both spermatozoa hyperactivity and VCL showed a negative relationship with insertion angle (MPIA) (P<0.05), while no association (P>0.05) between MPIA with other motility parameters was found. There was no correlation (P>0.05) between the midpiece area with any of the motility characteristics studied.

DISCUSSION

Spermatozoa morphometry has recently become an important criterion for classifying morphologically normal spermatozoa or for characterizing those with abnormalities in their structure (Kondracki et al. 2023). This study compared the within-head morphometric characteristics as

Table 3: Correlation between spermatozoa motility patterns and head morphometric characteristics in Lusitu boars

Variable	WOB	ALH	PM	RPM	LIN	STR	HA
HDL	0.363*	0.281	0.052	0.110	0.234	0.023	0.454*
HDW	-0.008	0.056	-0.136	-0.004	0.038	0.099	0.209
HAD	0.329	0.361*	-0.003	0.114	0.190	0.033	0.342
HDP	0.408*	0.388*	-0.048	0.115	0.197	-0.042	0.465*
Ellipticity	0.343	0.212	0.161	0.114	0.189	-0.55	0.075
Elongation	0.337	0.210	0.206	0.146	0.192	-0.22	0.161
Roughness	-0.335	-0.147	0.109	0.031	-0.134	0.153	-0.389*
Regularity	-0.188	-0.031	-0.185	-0.117	-0.211	-0.245	0.090
Acrosome	0.104	0.107	0.142	0.039	0.100	0.075	0.114

*=P<0.05; -=Negative correlation; LIN=Linearity; STR=Straightness; WOB=Wobble; ALH=Average lateral displacement of spermatozoa head; PM=Progressive movement; RPM=Rapid progressive movement; HA=Hyperactivity; HDL=Head length; HDW=Head width.

Table 4: Correlation between spermatozoa motility and midpiece morphometric characteristics in Lusitu boars

Var.	VCL	VAP	VSL	LIN	STR	WOB	ALH	BCF	PM	RPM	HA
MPW	-0.150	-0.079	0.002	0.041	0.285	-0.104	-0.153	0.196	0.140	0.152	-0.406*
MPA	0.224	0.227	0.167	0.126	0.036	0.198	0.156	0.119	0.015	0.098	0.060
MPIA	-0.371*	-0.327	-0.279	-0.167	0.010	-0.279	-0.339	-0.089	0.052	-0.125	-0.404*

*=P<0.05; VCL=Curvilinear velocity, VAP=Average path velocity; VSL=Straight-line velocity; LIN=Linearity; STR=Straightness; WOB=Wobble; ALH=Average lateral displacement of spermatozoa head; BCF=Beat cross frequency; PM=Progressive movement; RPM=Rapid progressive movement; HA=Hyperactivity; MPW=Midpiece width; MPA=Midpiece area; MPIA=Midpiece insertion angle; Var.=Variable

well as between spermatozoa heads with midpiece parameters. As expected, it was found that many of the head morphometric characteristics of Lusitu boar spermatozoa were correlated. The head length was positively associated with many of the head-derived morphometric traits, such as spermatozoa head area, perimeter, elongation, and ellipticity. The current findings support those of an earlier study (Gómez Montoto et al. 2011), which reported that head length directly determines these derived spermatozoa head parameters. Our findings confirmed that Lusitu boar spermatozoa with more elliptical and elongated heads are associated with a less rough plasma membrane. This, in turn, suggests a more reduced resistance to spermatozoa during locomotion in the female tract since head shape has an effect on spermatozoa hydrodynamics (Malo et al. 2006; Gil et al. 2009).

The head width relationship with shape parameters, elongation, and ellipticity was generally negative. It is plausible that Lusitu boar spermatozoa with lower head width and longer head length scores are considerably more competitive in swimming speed. This notion is based on an earlier study (Araya-Zúñiga et al. 2024) which stated that spermatozoa with elongated heads swim faster with less resistance. Spermatozoa swimming speed and proportion of those with normal morphology determine male fertility (Gomendio et al. 2007). While this appears to suggest a preference for boars bearing more spermatozoa with elongated heads, it was previously found that spermatozoa with higher elongation values are associated with higher risks of abnormality in semen quality, including cytoplasmic droplets, abnormal acrosin activity, and chromatin instability (Gaggini et al. 2017; Chen et al. 2019).

The observed head area, despite the absence of a relationship with elongation and ellipticity, was associated positively with the VCL, VAP, VSL, and ALH. This suggests a positive association between the head area of spermatozoa and swimming speed. Moreover, spermatozoa head size is associated with chromatin structure and hence related to fertility (Barquero et al. 2021). Based on findings by Gomendio et al. (2007), the current findings suggest that Lusitu boar spermatozoa with larger head areas swim faster and, therefore, are expected to reach the fertilization site faster. However, successful fertilization depends on spermatozoa reaching the oviduct and maintaining their fertilizing ability while the ova are viable (Firman and Simmons 2010). Thus, it is detrimental if all their ATP supplies is expended with a resultant loss of the fertilizing ability of spermatozoa prior to ova maturation. An earlier study found that smaller and shorter spermatozoa were associated with boars that were more fertile (Hirai et al. 2001). According to Helfenstein et al. (2010), spermatozoa with smaller heads relative to their flagellum showed a higher swimming velocity; also, the shorter spermatozoa were found to live longer. It is likely that spermatozoa with a higher swimming speed have an advantage only when they find the ovum at the appropriate maturation stage. Nevertheless, this study cannot allow for a more robust conclusion in terms of cause-effect relationship between head size and fertility due to the scope and design used.

Although both head and midpiece are part and parcel of the spermatozoa bauplan, there was no inter-relatedness observed in the current study. Malo et al. (2006) did not find a relationship between the head length and midpiece parameters, which supports part of the current study findings. However, the current findings contradict those of Gil et al. (2009), who reported a positive association between midpiece width and head width (P<0.001), head area (P<0.001), and head perimeter (P<0.001). These authors also reported a negative relationship between midpiece width with elongation (P<0.001) and ellipticity (P<0.001). The disparity between our findings and those of Gil et al. (2009) was probably attributed to differences in the boars' lines, evaluation technique, and/or settings used (Gaggini et al. 2017). It is also plausible that the head parameters of Lusitu boar spermatozoa may be instead related to the flagellar length as a whole. This is because of the reported effect of spermatozoa head size relative to flagellum on the swimming speed of house sparrow spermatozoa (Helfenstein et al. 2010). Notably, it is the flagellar beating that generates the force that drives the spermatozoa forward (Malo et al. 2006).

Both motility and morphology status of spermatozoa are associated with fertility of the boars (García-Vázquez et al. 2016). Malo et al. (2006) reasoned that the actual swimming speed achieved by spermatozoa is a combination of several factors: the size of the component whose movement generates the force in relation to the size of the components that have to be driven forward and the degree of resistance offered by the head when spermatozoa swim forward. The current study also found that head length was positively correlated with the VCL, VAP, and VSL, which disagreed with Gil et al. (2009), who reported a non-significant relationship with head length. However, a positive association between head length and VAP was reported in another study (Malo et al. 2006). A study on bull spermatozoa confirmed that VCL, VAP, and VSL were significantly correlated with non-return rates in cows (Nagy et al. 2015). These authors found that VAP was more correlated with fertility than VCL or VSL, which supports the higher correlation value (r=0.493) for the head length-VAP association than the case with VCL or VSL. The observed correlation between head length and velocity characteristics is suggestive of the potential for using the former as a biomarker for fertility prediction in Lusitu boars.

The observed non-significant correlation between head width with any of the Lusitu boar spermatozoa motility patterns and velocity characteristics was in agreement with the findings of Gil et al. (2009), who did not find a relationship (P>0.05) between head width with the VCL, VAP, VSL, LIN, STR, WOB, ALH, and BCF. Also, the observed trend for negative correlation tendency between head width and some of these kinematic parameters was in agreement with the previous study (Gil et al. 2009). Hence it is not surprising that an earlier study on Arabian horses reported an association between spermatozoa with smaller head width, area, and perimeter with stallions having higher fertilizing capacity (Waheed et al. 2015). Notably, head width and length determine the size and shape of spermatozoa, which, in turn, influence the spermatozoa motility and/or function. The current study has shown that Lusitu spermatozoa head width is negatively correlated with ellipticity and elongation, which have a considerable impact on spermatozoa hydrodynamics (Malo et al. 2006).

The findings of this study agree with those of Gil et al. (2009) with regard to the absence of correlation between midpiece parameters, including its width, area, and insertion angle, with those of the spermatozoa kinematics. The only exception was that this study found a negative correlation between insertion angle and VCL (P<0.05), whereas Gil et al. (2009) found a negative association with the VAP (P<0.05). A weak negative correlation was also observed with VAP in our study, although not significant, probably due to the difference in boar line and/or CASA settings used. Since VCL is an indicator of the swimming speed of sperm (Gil et al. 2009), it is plausible that Lusitu boar fertilizing capacity could be predicted based on the size of the insertion angle. The optimal insertion angle or range that can be used to predict boars with better fertility has not been established, considering the scope of this study. A previous study (Firman and Simmons 2010) reported that midpiece length is associated with the swimming speed of spermatozoa, which may be an additional predictor of boar fertility although not evaluated considering the scope of this study.

The positive correlation suggests that spermatozoa with a higher head length may easily shift to a hyperactive state. Also, the proportion of hyperactive spermatozoa is potentially higher in Lusitu boars, whose spermatozoa possess less-rugose plasma membranes, smaller midpiece widths, and insertion angles. There is currently little information available that can be used to compare with these findings. Nevertheless, previous studies reported that spermatozoa hyperactivation, a biomarker of male fertility, was associated with the VCL, ALH, LIN, and WOB, with a hyperactive spermatozoon possessing >97µm/s, >3.5µm, >32%, and <71%, respectively (Schmidt and Kamp 2004; Mircu et al. 2008; Ntanjana 2014). Notably, hyperactivity association with midpiece width and insertion angle was negative. It is opined that head length, midpiece width, and insertion angle are candidate parameters which could supplement motility characteristics for fertility prediction on the basis of their relationship with spermatozoa hyperactivation.

Conclusion

Correlation of morphometric and motility characteristics of spermatozoa may be relied on to improve the predictability of boar fertility. This study has found that spermatozoa head length and area are correlated with parameters of spermatozoa swimming speed, including VCL, VAP, VSL, and WOB, while their relationship with head width was not significant. There was no relationship between spermatozoa head characteristics with those of the midpiece. The midipiece parameters were not associated with motility characteristics except for the observed negative insertion angle-VCL correlation. Spermatozoa hyperactivity was positively correlated with head length, VCL, VAP, and ALH but negatively associated with roughness, midpiece width, and insertion angle. The head length, area, and insertion angle could be candidate biomarkers for Lusitu boar fertility prediction based on their relationship with kinematics and spermatozoa hyperactivity. Future studies utilizing causal-effect models or designs, more so with a bigger sample size, are suggested to confirm the current findings and establish parameter reference values or ranges for fertility prediction.

REFERENCES

Araya-Zúñiga IA, Sevilla F, Molina-Montero R, Roldan ERS, Barrientos-Morales M, Silvestre MA and Valverde A, 2024. Kinematic and morphometric assessment of fresh semen, before, during and after mating period in Brahman bulls. Animals 14: 132. http://dx.doi.org/10.3390/ani14010132

Barquero V, Roldan ERS, Soler C, Yániz JL, Camacho M and Valverde A, 2021. Predictive capacity of boar sperm morphometry and morphometric sub-populations on reproductive success after artificial insemination. Animals 2021(11): 920. https://doi.org/10.3390/ani11040920

Broekhuijse MLWJ, Šoštarić E, Feitsma H and Gadella BM, 2012. Application of computer-assisted semen analysis to explain variations in pig fertility. Journal of Animal Science 90: 779–789. http://dx.doi.org/10.2527/jas.2011-4311

Chakurkar BE, Naik SS, Barbuddhe BS, Karunakaran M, Naik KP and Singh PN, 2016. Seminal attributes and sperm morphology of Agonda Goan pigs. Journal of Applied Animal Research 44(1): 130-134. https://doi.org/10.1080/09712119.2015.1021807

Chen F, Gao L, Zhou H, Guo L, Chen Q, Gan Y, Sun X, Li Q and Wang K, 2019. The association between sperm head elongation and semen quality. Andrology 7: 840–845. http://dx.doi.org/10.1111/andr.12619

da Costa Málaga F, Siqueira HA, Rauber LP, Marques MG, Peripolli V, Schwegler E, Oliveira Júnior JM, Moreira F, Lopes MS and Bianchi I, 2022. In vitro and in vivo parameters for identification of landrace pigs with low reproductive performance. Semina: Ciências Agrárias Londrina 43(2): 573-584. http://dx.doi.org/10.5433/1679-0359.2022v43n2p573

Firman RC and Simmons LW, 2010. Sperm midpiece length predicts sperm swimming velocity in house mice. Biology Letters 6: 513–516. https://doi.org/10.1098/rsbl.2009.1027

Gaggini TS, Rocha LO, Souza ET, de Rezende FM, Antunes RC and Beletti ME, 2017. Head morphometry and chromatin instability in normal boar spermatozoa and in spermatozoa with cytoplasmic droplets. Animal Reproduction 14(1): 1253-1258. http://dx.doi.org/10.21451/1984-3143-AR921

García-Vázquez AF, Gadea J, Matás C and Holt WV, 2016. Importance of sperm morphology during sperm transport and fertilization in mammals. Asian Journal of Andrology 18: 844–850. https://doi.org/10.4103/1008-682X.186880

Gil MC, García-Herreros M, Barón FJ, Aparicio IM, Santos AJ and García-Marín LJ, 2009. Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. Theriogenology 71: 254–263. http://dx.doi.org/10.1016/j.theriogenology.2008.07.007

Gomendio M, Malo AF, Garde J and Roldan ERS, 2007. Sperm traits and male fertility in natural populations. Reproduction 134: 19–29. http://dx.doi.org/10.1530/REP-07-0143

Gómez Montoto L, Sánchez MV, Tourmente M, Martín-Coello J, Luque-Larena JJ, Gomendio M and Roldan ERS, 2011. Sperm competition differentially affects swimming velocity and size of spermatozoa from closely related muroid rodents: head first. Reproduction 142: 819–830. https://doi.org/10.1530/REP-11-0232

Górski K, Kondracki S, Iwanina M, Kordan W and Fraser L, 2021. Effects of breed and ejaculate volume on sperm morphology and semen parameters of boars. Animal Science Journal 92(1): e13629. https://doi.org/10.1111/asj.13629

Gottardi EM, Marques TC, Leão KM, de Araújo Neto FR and Fernandes LG, 2020. Correlation between the sperm characteristics evaluated by a computerized system (CASA) and the reproductive performance of sows. Research, Society and Development 9(6): e09963389. http://dx.doi.org/10.33448/rsd-v9i6.3389

Helfenstein F, Podevin M and Richner H, 2010. Sperm morphology, swimming velocity, and longevity in the house sparrow Passer domesticus. Behavioral Ecology and Sociobiology 64: 557–565. http://dx.doi.org/10.1007/s00265-009-0871-x

Hirai M, Boersma A, Hoeflich A, Wolf E, Föll J, Aumüller R and Braun J, 2001. Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): Relation to fertility and seminal plasma growth factors. Journal of Andrology 22(1): 104-110. https://doi.org/10.1002/j.1939-4640.2001.tb02159.x

Hwang I-S, Lee S-C, Kim SW, Kwon D-J, Park M-R, Yang H, Oh KB, Ock S-A, Woo J-S, Im G-S and Hwang S, 2017. Analysis of semen parameters in α1,3-Galactosyltransferase$^{-/-}$ Boars. Journal of Embryology Transfer 32(2): 53-58. https://doi.org/10.12750/JET.2017.32.2.53

Keller A and Kerns K, 2023. Sperm capacitation as a predictor of boar fertility. Molecular Reproduction and Development 2023: 1-7. https://doi.org/10.1002/mrd.23690

Kondracki S, Górski K, Iwanina M, Kordan W and Lecewicz M, 2023. Association between sperm morphology and sperm count of boar semen. Polish Journal of Veterinary Science 26(4): 695–704. https://doi.org/10.24425/pjvs.2023.148289

Kondracki S, Wysokińska A, Kania M and Górski K, 2017. Application of two staining methods for sperm morphometric evaluation in domestic pigs. Journal of Veterinary Research 61: 345-349. https://doi.org/10.1515/jvetres-2017-0045

Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ and Roldan ERS, 2006. Sperm design and sperm function. Biology Letters 2: 246–249. http://dx.doi.org/10.1098/rsbl.2006.0449

Maroto-Morales A, García-Álvarez O, Ramón M, Martínez-Pastor F, Fernández-Santos MR, Soler AJ and Garde JJ, 2016. Current status and potential of morphometric sperm analysis. Asian Journal of Andrology 18: 863–870. http://dx.doi.org/10.4103/1008-682X.187581

Mircu C, Cernescu H, Igna V, Knop R, Frunză I, Ardelean V, Bonca GH, Otava G, Zarcula S, Korodi G and Ardelean A, 2008. Boar semen evaluation using CASA and its relation to fertility. Lucrări Stiintifice Medicină Veterinară 12: 203-212.

Nagy A, Polichronopoulos T, Gáspárdy A, Solti G and Cseh S, 2015. Correlation between bull fertility and sperm cell velocity parameters generated by computer-assisted semen analysis. Acta Veterinaria Hungarica 63(3): 370–381. http://dx.doi.org/10.1556/004.2015.035

Ngcauzele A, 2018. Seasonal differences in semen characteristics and sperm functionality in Tankwa goats. Masters of Science, Medical Bioscience. University of the Western Cape. South Africa.

Ntanjana N, 2014. Hyperactivation in human semen and sperm subpopulations by selected calcium modulators, University of the Western Cape, South Africa., pp: 1-213.

Savić R, Radojković D, Gogić M, Popovac M, Petrović A and Radovic C, 2022. Do Motility and Sperm Dose Count Affect In Vivo Fertility in Boar? Chemistry Proceedings 10(10): 1-5. https://doi.org/10.3390/IOCAG2022-12213

Schmidt H and Kamp G, 2004. Induced hyperactivity in boar spermatozoa and its evaluation by computer-assisted sperm analysis. Reproduction 128: 171–179. https://doi.org/10.1530/rep.1.00153

Sutkevičienė N and Žilinskas H, 2004. Sperm morphology and fertility in artificial insemination boars. Veterinarija ir Zootechnika 26(48): 11-13.

Tremoen NH, Gaustad AH, Andersen-Ranberg I, van Son M, Zeremichael TT, Frydenlund K, Grindflek E, Våge DI and Myromslien FD, 2018. Relationship between sperm motility characteristics and ATP concentrations, and association with fertility in two different pig breeds. Animal Reproduction Science 193: 226-234. https://doi.org/10.1016/j.anireprosci.2018.04.075

Valverde A, Castro-Morales O, Madrigal-Valverde M and Soler C, 2019. Sperm kinematics and morphometric subpopulations analysis with CASA systems: a review. Revista de Biología Tropical 67(6): 1473-1487. http://dx.doi.org/10.15517/rbt.v67i6.35151

van der Horst G and Maree L, 2009. SpermBlue®: A new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. Biotechnic Histochemistry 84: 299-308. https://doi.org/10.1080/10520290902984274

Vijayaraghavan S, 2003. Sperm motility: Patterns and regulation, In: Introduction to Mammalian Reproduction. Springer, Boston, MA, USA, pp: 79–91.

Waheed MM, Ghoneim IM and Abdou MSS, 2015. Morphometric characteristics of spermatozoa in the Arabian horse with regard to season, age, sperm concentration, and fertility. Journal of Equine Veterinary Science 35: 244–249. http://dx.doi.org/10.1016/j.jevs.2015.01.005