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ORIGINAL ARTICLE

Effects of age, season, breed, and sperm counting chamber on boar semen quality variables in tropical conditions

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ABSTRACT. In the porcine industry, male sperm quality plays a relevant role in the productivity and profitability of the productive system in the tropics. Understanding the factors affecting semen quality is important for optimizing male reproductive efficiency. The present study aimed to evaluate the seminal quality associated with season, breed, age, and sperm counting chamber in boar ejaculates. A total of 22 sexually mature and healthy boars from maternal and sire commercial breeds were utilized as semen donors, with an average age (mean ± standard deviation) of 21.0 ± 7.2 months. The boars were housed individually in well-ventilated pens and fed a standard breeder mix. The boars were grouped according to age into three categories: <12 months, 12-24 months, and >24 months, and semen doses were collected from each boar during the dry and rainy seasons. Ejaculates with at least 75 % morphologically normal sperm and more than 8.5 x 109 total sperm per ejaculate were used. The semen doses were analyzed using Computer-Assisted Semen Analysis (CASA; ISAS® v1). The results showed that younger boars (<12 months) exhibited higher progressive and total sperm motility and faster swimming patterns than older boars. The estimation of total motility and fast spermatozoa increased during the rainy season. The kinematic variables showed significant differences ($P < 0.05$) between the sperm counting chambers. The Duroc and Landrace breeds presented spermatozoa with greater total motility, whereas the terminal sire line breeds showed accelerated linear progressiveness ($P < 0.05$). Overall, the impact of various factors on seminal and kinematic variables in boar ejaculates, including age, season, genetic breed composition, and sperm counting chamber, enables us to better understand semen quality in boars. This emphasizes the importance of optimizing swine reproductive management practices in sexually active boars.

Keywords: spermatozoa; swine; reproduction; CASA-systems; motility

Introduction

Advancements in artificial insemination (AI) within the swine industry have led to enhancements in the quantitative assessment of boar-produced spermatozoa quality, aiding the prediction of seminal dose fertility potential (Waberski *et al.*, 2008). Enhancing sperm quality has economic advantages for insemination centers (Krupa *et al.*, 2020) and contributes to the profitability of the swine production industry (Gonzalez-Pena *et al.*, 2016). The consistent production of high-quality semen by genetically superior animals is a critical determinant of success for AI centers (Gonzalez-Peña *et al.*, 2014; Okere *et al.*, 2005). In contemporary swine management practices, elimination of breeding boars showing inferior semen quality is routine to maintain optimal reproductive outcomes.

Semen analyses may determine semen quality, male fertility potential, and potential causes of infertility using conventional methods such as spermatozoa counts (Sevilla *et al.*, 2023) and assessing sperm swimming patterns (Calderón-Calderón *et al.*, 2022) such as total and progressive motility and sperm kinematics (Barquero, Roldan *et al.*, 2021; Barquero *et al.*, 2021; Fair & Romero-Aguirregomezcorta, 2019) using CASA systems (Madrigal-Valverde *et al.*, 2020; Yeste *et al.*, 2018). The importance of these analyses lies in the fact that motility and kinematic parameters may have a predictive capacity for fertility variables (Barquero *et al.*, 2021) and could contribute to the productive performance of pig farms.

Sexual maturity in boars can be affected by factors such as breed and environment (Huang *et al.*, 2010; Kumaresan *et al.*, 2011; Kunavongkrit *et al.*, 2005; Yeste *et al.*, 2010). Studies indicate that maximum semen quality is typically achieved in boars aged 24–29 months in temperate zones (Kennedy & Wilkins, 1984). Age or seasonal variations influence in hormone levels and have been observed to affect

boar semen quality (Huang *et al*., 2010; Kumaresan *et al*., 2011; Tsakmakidis *et al*., 2012) and sperm production (Park & Yi, 2002). Some studies on farm animals have explored and highlighted the effect of male age on semen characteristics (Carreira *et al*., 2017; Hallap *et al*., 2006; Hoflack *et al*., 2007; Long *et al*., 2010), which could influence semen quality. For example, older males often show reduced sperm motility in canine semen (Goericke-Pesch & Failing, 2013). Similarly, in older bulls, age has been correlated with decreases in ejaculate volume, semen concentration, and total sperm production (Kelso *et al*., 1997; Murphy *et al*., 2018).

Advanced male age is consistently linked to a notable decline in various semen parameters, including semen volume, concentration, motility, morphology, and viability (Kipper *et al*., 2017; Winkle *et al*., 2009). Among the mechanisms proposed to understand how sperm motility varies include dysfunction of the accessory sex glands and epididymis leading to impaired semen motility (Dowsett & Knott, 1996). Collectively, these factors contribute to impaired spermatogenesis in older animals, potentially resulting in swimming pattern abnormalities in their sperm (Mandal *et al*., 2010). Age and seasonal changes significantly impact the quality of boar semen (Huang *et al*., 2010; Tsakmakidis *et al*., 2012; Zasiadczyk *et al*., 2015). Reproductive seasonality, influenced by factors such as photoperiod and temperature, profoundly affects boar semen quality. Numerous studies have explored the impact of seasonal variations on boar semen quality (Ciereszko *et al*., 2000; Flowers, 2008; Fraser *et al*., 2016; Knecht *et al*., 2014, 2017; Savic *et al*., 2013).

The CASA systems use sperm counting chambers for assessments (Bompart *et al*., 2018). However, variations in results that may lead to errors, such as using different types of sperm counting chambers for analysis (Gloria *et al*., 2013; Hoogewijs *et al*., 2012; Lenz *et al*., 2011; Soler *et al*., 2018). There are various types of counting sperm chamber available for use with CASA systems (Bompart *et al*., 2019; Nanni Peng & Li, 2015; Valverde & Madrigal-Valverde, 2019). Additionally, there is variability in the design, shape, and size of the chambers (Del Gallego *et al*., 2017; Soler *et al*., 2018). These instruments may influence the eventual semen analysis, affecting sperm dynamics in different ways, leading to divergent output values.

To improve understanding of the physiological characteristics of sperm, and swimming patterns of spermatozoa, the effect of breed, genetic lines, seasonal changes (Peña *et al*., 2019a) and intrinsic boar traits is essential for optimizing reproductive efficiency and perpetuating the most favorable traits in semen quality (Žaja *et al*., 2016). Therefore, the aim of this study was to evaluate the seminal quality associated with age, season, breed, and sperm counting chamber in boar ejaculates.

MATERIAL AND METHODS

Study site

The experiment was conducted on two commercial pig farms located in the northwest region of Costa Rica in the provinces of Alajuela (Agropecuaria Los Sagitarios S.A., Río Cuarto, 10°20-32" N, 84°12'55" W) and Heredia (Mejoramiento Porcino S.A., San José de la Montaña, Barva 10°3-0'' N, 84°7-0'' W). In this area, the rainy season is from May to October and the dry season is from November to April. The meteorological conditions for the rainy season; a high temperature mean of 26.45±0.75°C, a rainfall mean of 313.67±51.11 mm, and a humidity mean of 86.17±2.32 %. For the dry season, the conditions are a high temperature mean of 27.9±1.61°C, a rainfall mean of 55.83±50.43 mm, and a humidity mean of 76.17±6.58%.

At the time of research, both farms had a valid veterinary operation certificate (CVO), and animal health was monitored through vaccinations and deworming treatments. Comprehensive production records were maintained for all animals. The study was conducted from June 2022 to June 2023.

Animals

In this study, 22 sexually mature and healthy boars of the Duroc (n = 5), Landrace (n = 2), Pietrain (n = 6), and Yorkshire (n = 1) breeds, as well as boars from a commercial sire line (LT: Duroc x Pietrain; n = 8), with an average age of 21.0 ± 7.2 months at the start of the experiment and known fertility, were used as semen donors. At least two ejaculates per boar were utilized (collected), totaling 45 ejaculates. The boars were grouped according to age into three categories: < 12 months (n = 5), 12-24 months (n = 8), and > 24 months (n = 9).

Animals were individually housed in well-ventilated pens with an average temperature range of 17.5 to 23.6 °C throughout the experiment. They were fed a standard breeder mix containing soybean meal, corn, mineral mix, and common salt as ingredients to meet the nutrient requirements of swine (National Research Council, 2012). Boars were provided with water *ad libitum*.

Semen collection and processing

Semen doses were collected using the "double-glove" technique (Hancock & Hovell, 1959) and diluted 1:1 (v:v) with Androstar Plus®, commercial diluent (Minitübe GmbH, Germany) after each extraction. Earlier each ejaculation, boars were stimulated by taking them to a separate extraction pen, which contained the extraction dummy, and semen was obtained by manual manipulation of the penis after the boar mounted the extraction dummy. The last three fractions of semen from each ejaculation were collected in graduated semen collection containers and filtered through three layers of sterilized gauze to separate the bulbourethral gland secretions from the other semen constituents. After each semen collection, a routine macroscopic evaluation was performed (volume, color, and consistency). A spectrophotometer was used for sperm concentration measurement following established protocols (Sevilla *et al*., 2023). An aliquot of less than 1 ml of diluted semen (1:1, v:v) was taken using a Pasteur pipette and placed

in a micro cuvette before measurement. After this evaluation, the filtered fraction was placed in a water bath (37°C), where the doses remained during the packaging and semen density determination period. Following the sperm concentration evaluation, the ejaculate was processed to prepare semen doses. One seminal dose from each ejaculate was transported to the laboratory under refrigeration conditions (17°C) for analysis in a Dometic® cooler (Lane Manufacturing Inc, Denver, CO, USA) and without exposure to light. Upon arrival at the laboratory, samples were stored horizontally for 24 h at 17°C. Subsequently, the samples were to acclimate for 30 min in room laboratory (24°C), then placed in a 1.5 ml microtube (Eppendorf®, Germany) on a heating plate at 37°C for 30 min. The sperm analysis was then performed.

Semen evaluation

Seminal doses from each ejaculation were assessed to determine motility and morphology, and only ejaculates with >75% morphologically normal spermatozoa were utilized. The evaluation of sperm morphology was conducted by the same technician, following strict criteria outlined by the World Health Organization (2021). For the analysis, 200 spermatozoa per slide were evaluated, with 100 spermatozoa assessed from each of the two different areas on the slide. If the variation morphology between the two areas was 5% or less, the mean value was calculated. The samples were prepared using a 10 μL aliquot, which was placed on a glass slide and covered with a coverslip. The Trumorph® system was utilized following the conditions and methodology previously established by Calderón-Calderón *et al.* (2022).

Sperm motility analysis was performed using disposable counting chambers: Leja® (Microoptic, Barcelona, Spain), 20 μm deep with four counting areas, and the reusable chamber; Makler® (Sefi-Medical Instruments Ltd., Israel), 10 μm deep; both preheated (37°C). After thorough mixing of diluted semen doses, in the Leja® chamber, semen volume (2.7 μL) was capillary distributed along the counting chamber fields until completely filled, while in the Makler® chamber, the diluted semen was drop-dispersed with an equivalent volume of 2.7 μL. Semen evaluations were conducted using CASA-Mot (ISAS® v1 system, Integrated Semen Analysis System, Proiser I+D company, Paterna, Spain) equipped with a video camera (Proiser 782M, Proiser I+D), capturing 25 frames per field at a frame rate of 50 Hz and a final resolution of 768 x 576 pixels. The camera was connected to a UB203 microscope (UOP/Proiser R+D) with a 1x eyepiece and a negative phase contrast objective of 10x (AN 0.25), and an integrated heated stage maintained at a constant temperature of 37.0 ± 0.5°C.

The percentage of total motile cells (TM) was defined as the percentage of motile cells exhibiting a curvilinear velocity (VCL) >10 $\mu m \cdot s^{-1}$ within the sample. Progressive motility (PM, %) corresponded to spermatozoa exhibiting a fast forward swimming pattern in a straight line. Progressive motility was defined as: straightness (STR, linearity index) ≥45%, and average path velocity (VAP) ≥25 $\mu m \cdot s^{-1}$, defined as the mean velocity along the smoothed cell path. Non-progressive motile sperms (NPM) were motile spermatozoa with a prevalence of circular movements. Additionally, the percentages of static spermatozoa were determined. Progressive movement was defined as the percentage of spermatozoa exhibiting movement with a straightness index (STR) ≥75% within the sample. Static spermatozoa corresponded to cells exhibiting a curvilinear velocity (VCL) <10 $\mu m \cdot s^{-1}$. Within motile spermatozoa, the percentage of cells with movement classified as fast, medium, and slow was determined according to the curvilinear velocity criterion ($\mu m \cdot s^{-1}$): 10 < slow < 25; 25 < medium < 45; and > 45 fast. Seminal analyses were performed in the Animal Reproduction Laboratory of the Center for Research and Development in Sustainable Agriculture for the Humid Tropics (CIDASTH), School of Agronomy at San Carlos Local Campus, Costa Rica Institute of Technology, Alajuela, Costa Rica.

Analysis of sperm kinematics variables using a CASA-Mot system.

Sperm kinetic analysis was conducted by acquiring seven microscopic fields along the sperm counting chambers to obtain an average of 600 spermatozoa per field. The variables evaluated by CASA-Mot system were: straight-line velocity (VSL , $\mu m \cdot s^{-1}$), corresponding to the speed of the sperm head along a straight line from the start to the end of the detected cell position; curvilinear velocity (VCL, $\mu m \cdot s^{-1}$), measured along the actual, point-by-point trajectory followed by the spermatozoon; average path velocity (VAP, $\mu m \cdot s^{-1}$), the average velocity along the smoothed cell path calculated as an interpolation between points corresponding to the VCL trajectory; lateral head displacement amplitude (ALH, μm), defined as the maximum (or mean) height of the head oscillation while the spermatozoon moves in a curvilinear trajectory; beat-cross frequency (BCF, Hz), defined as the frequency at which the actual (curvilinear) trajectory crosses the smoothed linear trajectory in any direction; motility (%), defined as the percentage of total mobile cells; and progressive motility (%), corresponding to spermatozoa rapidly moving forward in a straight line. Three progression ratios, expressed as percentages, were calculated from the velocity measurements described above: linearity of forward progression [LIN = (VSL/VCL) ×100], straightness index [STR = (VSL/VAP) ×100], and wobble index [WOB = (VAP/VCL) ×100]. The CASA software configuration was adjusted for boar sperm analysis, with a particle area ranging from 10 to 80 μm^2 for the sperm head area and a connectivity of 11 μm.

Statistical analysis

The assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene tests, respectively. Normal probability plots were used to evaluate the normal distribution of all analyzed sperm variables. Analysis of variance (ANOVA) and generalized linear models were employed to ascertain the impact of age, genetic composi-

tion, and type of sperm counting chamber on sperm quality variables. The total sample space comprised 22 experimental units. Mean differences for the effects of age, genetic composition, and type of sperm counting chamber were analyzed using the Bonferroni test ($P < 0.05$). Results were expressed as mean ± standard error of the mean (SEM). Data analysis was performed using IBM SPSS version 23.0 for Windows (SPSS Inc., Chicago, IL, USA).

Landrace and Yorkshire (boars: 15-16). However, maternal line boar 3 is an exception, showing higher values than other boars from both sire and maternal lines, excluding sire line boars 1 and 2 (Figure 2).

Differences in total sperm motility and swimming patterns of boar age were observed ($P < 0.05$) (Table 1). The ejaculates of the youngest boars under 12 months old had greater total motility and progressive motility, as well as the proportion of

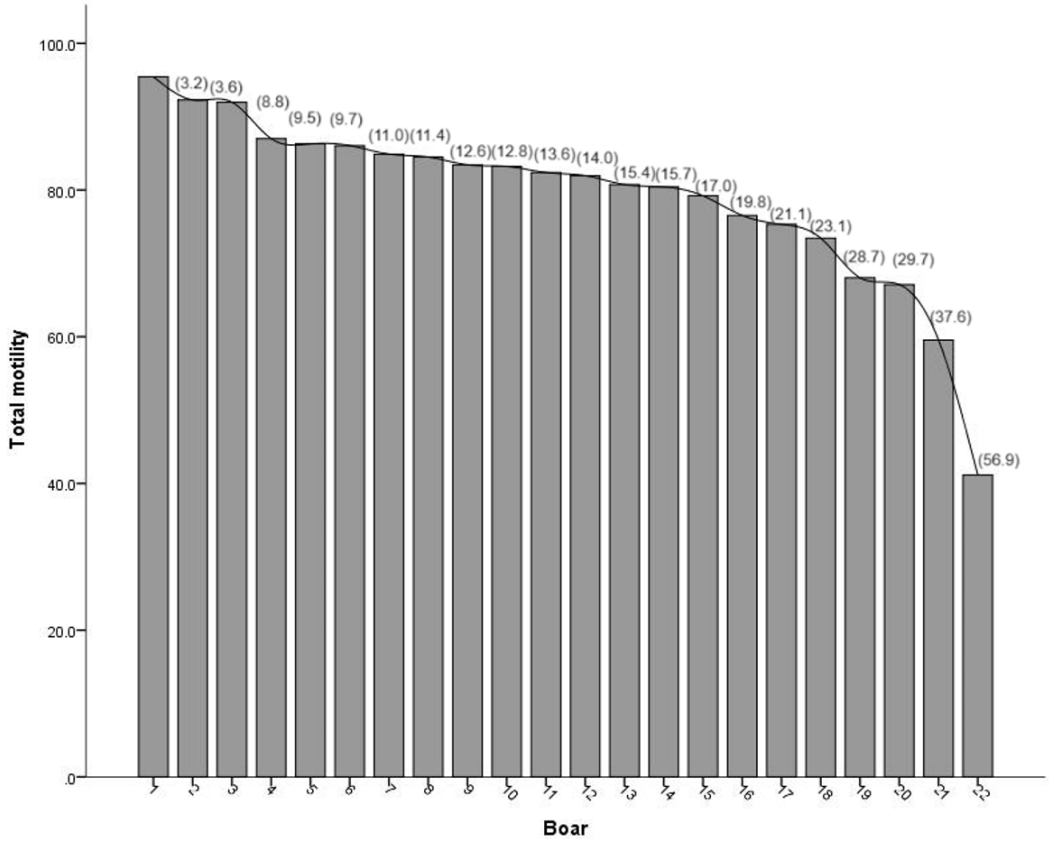

Figure 1.
Total motility (% of mean) of boar ejaculates, in order from the upper mean value to the lower mean value. The percentage in brackets indicates the absolute deviation between boars analyzed with respect to the total motility value of Boar 1.

RESULTS

Motile and swimming patterns of spermatozoa

The total motility of boar ejaculates of different breeds is shown in Figure 1. The highest values corresponded mainly to boars of the terminal sire lines (Duroc, Pietrain; boars: 1-7, 9-16, 19-22) compared to the maternal line males (Landrace and Yorkshire; boars: 8, 17, 18).

The progressive sperm motility values showed how younger boars had higher values than older boars (Table 1). In addition, boars from sire lines, such as Duroc, Pietrain and LT (boars: 1, 2, 4-14, 17-22) had higher progressive motility values ($P < 0.05$) than boars from maternal lines, such as spermatozoa with fast movement, than in older ages. Regarding the swimming patterns categories, boars aged under 12 months old exhibited the highest percentage of fast sperm (76.37 ± 1.47%). However, as age increased, the value (%) of total and progressive motility decreased (Table 1).

Total sperm motility and swimming patterns were affected by the season of the year ($P < 0.05$). In the rainy season boar ejaculates had a greater total motility and proportion of spermatozoa classified with fast movement. No differences were found ($P > 0.05$) between dry or rainy seasons or age for progressive motility (Table 1).

There was an effect of the sperm counting chamber on the swimming patterns ($P < 0.05$) (Supplementary data

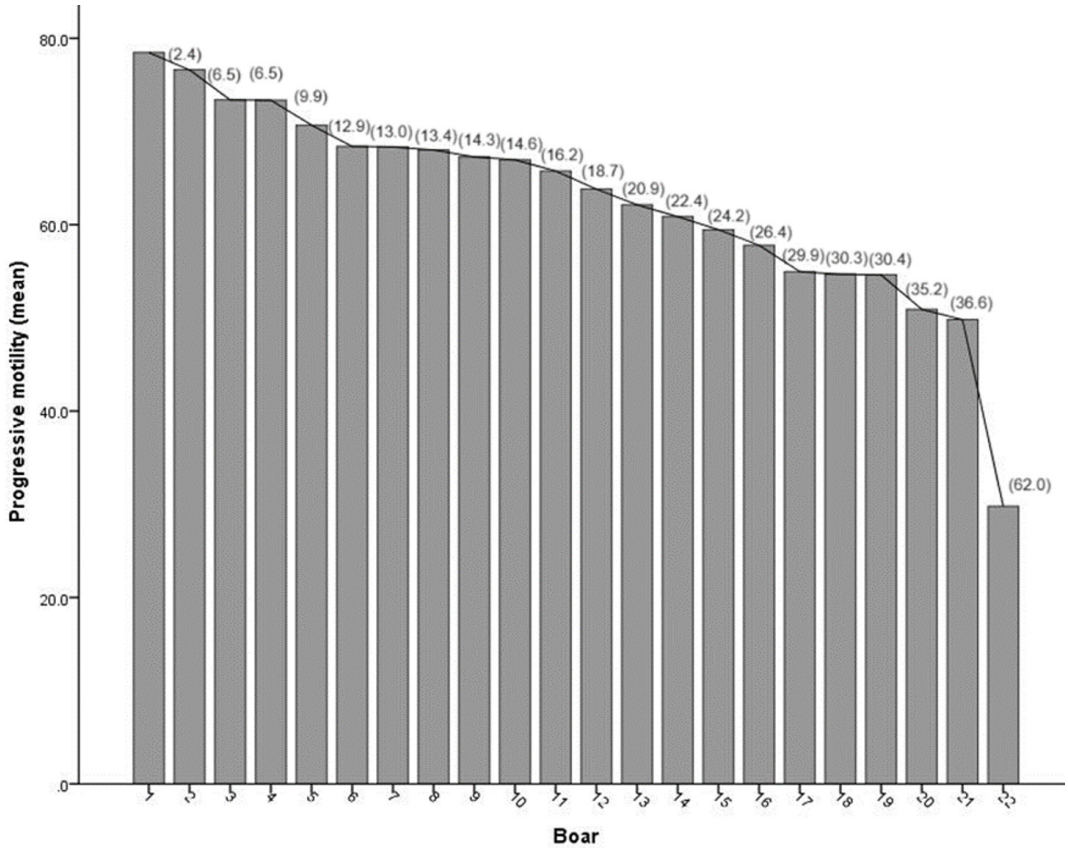

Figure 2.
Progressive motility (% of mean) of boar ejaculates, in order from the upper mean value to the lower mean value. Percentage in brackets absolute deviation between boars analyzed respect the progressive motility value of the Boar 1. Maternal line (Boar 3, Boar 15, Boar 16) and the other boars were terminal sire lines.

Table 1.
Overall changes in chilled boar sperm swimming characteristics (means ± SEM) at different ages and season.

	Age (months)				Season	
Variable (%)	<12	12-24	>24	Overall[α]	Dry	Rainy
TM	84.74±1.55[a]	77.28±1.80[b]	78.61±1.33[b]	77.81±18.09	75.43±1.06[y]	79.16±0.80[x]
PM	64.73±1.43[a]	62.47±1.65[a]	61.05±1.22[a]	62.16±16.84	61.35±0.99[x]	62.63±0.75[x]
NPM	20.01±0.49[a]	14.80±0.56[c]	17.56±0.41[b]	16.64±7.25	14.08±0.42[y]	16.54±0.32[x]
Fast spermatozoa*	76.37±1.47[a]	63.78±1.14[b]	76.91±1.51[a]	67.32±19.76	56.02±3.55[y]	70.48±1.02[x]
Medium speed spermatozoa*	19.26±1.09[b]	28.76±0.85[a]	18.59±1.12[b]	26.01±14.87	39.67±2.80[x]	23.90±0.80[y]
Slow speed spermatozoa	4.37±0.62[b]	7.45±0.48[a]	4.50±0.64[b]	6.66±7.58	4.32±1.15[x]	5.62±0.33[x]

TM, total motility; PM, progressive motility; NPM, non-progressive motility.
* Spermatozoa with movement categorized as fast (> 45 $\mu m \cdot s^{-1}$), medium (25 ≤ x < 45 $\mu m \cdot s^{-1}$), slow (10 < y < 25 $\mu m \cdot s^{-1}$).
a-c Different letters inside rows indicate differences ($P < 0.05$) between boar ages.
x-y Different letters inside rows indicate differences ($P < 0.05$) among seasons.
SEM, standard error of the mean.
α SD, mean ± standard deviation.

1). Semen samples analyzed with the Makler® chamber presented a greater total motility (%) compared with the semen samples analyzed with the Leja® counting chamber. The progressive motility was higher in the Makler® chamber (63.31 ± 1.21%) than in Leja® Chamber (61.57 ± 1.09 %). Non-progressive motility was also higher in the Makler® chamber (20.00% ± 0.41) than in the Leja® chamber (15.53% ± 0.37). There were no significant differences between the two chambers for fast, average, and slow sperm swimming patterns.

An effect of breed composition on seminal characteristics was observed ($P < 0.05$). The Duroc and Landrace boars presented a greater total motility rate compared with the other breeds (Table 2). The total motility was lower in the Yorkshire breed than in Duroc breed and Landrace breed ($P < 0.05$). The sperm swimming characteristics indicated a higher proportion of fast spermatozoon in terminal sire breeds (Duroc and Pietrain) in comparison to maternal lines (Landrace and Yorkshire). No differences were found ($P > 0.05$) between boar breeds for the progressive motility of the ejaculates. There was a 28.4 % difference in non-progressive motility of breed Duroc in relation to the Yorkshire breed.

Table 2.
Overall changes in chilled boar sperm motility parameters (means ± SEM) at different breeds.

	Breed composition				
Variable	Duroc	Pietrain	Landrace	Yorkshire	LT
TM	84.75±1.75^{a}	78.44±1.38^{b}	80.40±2.54^{ab}	73.43±3.30^{c}	78.23±1.28^{b}
PM	62.91±1.62^{a}	62.47±1.27^{a}	66.61±2.3^{a}	57.79±3.03^{a}	59.87±1.18^{a}
NPM	21.85±0.55^{a}	15.97±0.43^{c}	13.79±0.79^{c}	15.64±1.03^{bc}	18.36±0.40^{b}
Fast spermatozoa*	75.92±1.71^{a}	74.75±1.21^{a}	57.14±2.39^{c}	65.93±3.13^{b}	69.94±1.22^{b}
Medium speed spermatozoa*	21.04±1.27^{b}	19.20±0.90^{b}	32.14±1.77^{a}	27.50±2.32^{a}	24.13±0.90^{b}
Slow speed spermatozoa*	3.04±0.72^{c}	6.05±0.51^{b}	10.71±1.01^{a}	6.57±1.32^{b}	5.92±0.51^{b}

LT, commercial sire line Duroc x Pietrain.
TM, total motility; PM, progressive motility; NPM, non-progressive motility.
* Spermatozoa with movement categorized as fast (> 45 $\mu m \cdot s^{-1}$), medium (25 ≤ x < 45 $\mu m \cdot s^{-1}$), slow (10 < y < 25 $\mu m \cdot s^{-1}$).
$^{a-c}$ Different letters within rows indicate differences ($P < 0.05$) between genetic breed composition of boars.
SEM, standard error of the mean.

Overall kinematic variables

There were differences ($P < 0.05$) in the pairwise comparison by age category on these kinematic variables. Also, the interaction for breed-season-age was significant ($P < 0.05$) for kinematics boar semen variables. The velocity variables (curvilinear velocity and average path velocity) were different ($P < 0.05$) in all age groups (Table 3). For curvilinear velocity, boars with > 24 months age presented higher values than the other ages groups analyzed, and for linearity (LIN), boars in the < 12 months age group exhibited the lowest value (44.00 ± 0.08 %) in relation to the boar with > 24 months age (49.32 ± 0.08 %) and boars with 12-24 months (59.15 ± 0.18 %). Percentage of straightness index (STR) was higher in the 12-24 months group compared to other age groups < 12 and > 24 months old. There were differences between age groups for the WOB (sperm oscillation, %), where intermediate age group (12-24 months) was greater than < 12 months (73.72 ± 0.13 %; 61.70 ± 0.05 % respectively). For the amplitude of the sperm head (ALH) the intermediate age groups presented lower values compared to the other groups of age. The crossover frequency (BCF), the boar group with more than 24 months presented lower values of Hz, compared to the group less than 12 months and intermediate age group although these differences were not considered biologically relevant. Found an effect of season on kinematics sperm variables analyzed ($P < 0.05$). In the rainy season boar ejaculates had a lower ($P < 0.05$) velocities, progressiveness, and oscillation of spermatozoa.

There was an effect of the sperm counting chamber on the kinematic variables (Supplementary data 2). The sperm analyzed with the Makler® chamber showcased the highest

Table 3.
Effect of age and season on kinematics semen variables (mean ± SEM) of boar ejaculates.

	Age (months)			Season	
Variable (%)	<12	12-24	>24	Dry	Rainy
VCL	73.39±0.11[a]	54.83±0.28[c]	67.66±0.12[b]	73.74±0.13[x]	70.75±0.08[y]
VSL	31.46±0.07[a]	31.64±0.18[a]	32.68±0.08[c]	33.62±0.08[x]	28.99±0.05[y]
VAP	44.80±0.08[a]	40.07±0.19[c]	44.64±0.08[b]	48.98±0.09[x]	41.79±0.05[y]
LIN	44.00±0.08[c]	59.15±0.18[a]	49.32±0.08[b]	47.93±0.09[x]	42.32±0.06[y]
STR	67.73±0.08[c]	77.70±0.21[a]	70.40±0.10[b]	68.84±0.10[x]	66.32±0.06[y]
WOB	61.70±0.05[c]	73.72±0.13[a]	66.83±0.06[b]	67.45±0.07[x]	60.33±0.04[y]
ALH	2.88±0.00[a]	1.99±0.01[c]	2.60±0.01[b]	2.49±0.05[x]	2.94±0.03[y]
BCF	7.12±0.01[b]	7.47±0.02[a]	7.06±0.01[c]	8.42±0.01[x]	6.55±0.01[y]

VCL, curvilinear velocity, $\mu m \cdot s^{-1}$; VSL, straight-line velocity, $\mu m \cdot s^{-1}$; VAP, average path velocity, $\mu m \cdot s^{-1}$; LIN, linearity of forward progression, %; STR, straightness index, %; WOB, wobble, %; ALH, amplitude of lateral head displacement, µm; BCF, beat-cross frequency, Hz.
[a-c] Different letters indicate differences ($P < 0.05$) between age groups of boars.
[x-y] Different letters within rows indicate differences ($P < 0.05$) among seasons.
SEM, standard error of the mean.

VCL (69.73 ± 0.12 $\mu m \cdot s^{-1}$) compared to the Leja® chamber (63.90 ± 0.13 $\mu m \cdot s^{-1}$). For VSL there were no differences ($P > 0.05$) between sperm counting chamber. A superior average path velocity with the Makler® chamber was determined for VAP kinematic variable compared to the Leja® chamber (45.54 ± 0.08 $\mu m \cdot s^{-1}$; 42.00 ± 0.09 $\mu m \cdot s^{-1}$, respectively). For linearity (LIN), the Leja® chamber exhibited the highest value (51.98 ± 0.08 %) compared to the Makler® chamber (47.40 ± 0.08 %). The straightness index (% of STR) was higher in the sperm analyzed with Leja® chamber (74.05 ± 0.10 %) compared to Duroc breed (65.75 ± 0.10 %). There were differences between all sperm counting chambers for the sperm oscillation (WOB), the amplitude of the sperm head (ALH), and BCF, where the higher values were determined with the Makler® sperm counting chamber.

There was an effect of genetic breed composition on the kinematic variables (Table 4). Sire line breed Duroc x Pietrain (LT) showcased the highest VCL (72.80 ± 0.10 $\mu m \cdot s^{-1}$), followed closely by Duroc (71.50 ± 0.13 $\mu m \cdot s^{-1}$). The Landrace breed recorded the lowest VCL (55.96 ± 0.32 $\mu m \cdot s^{-1}$), with Pietrain and Yorkshire manifesting intermediate velocities.

For VSL and VAP kinematic variables, the Pietrain breed exhibited the most accelerated linear progression (36.23 ± 0.10 $\mu m \cdot s^{-1}$ and 48.50 ± 0.10 $\mu m \cdot s^{-1}$, respectively). By contrast, Duroc displayed the most sluggish values of VSL and VAP (28.01 ± 0.08 $\mu m \cdot s^{-1}$ and 41.60 ± 0.09 $\mu m \cdot s^{-1}$, respectively). For linearity (LIN), the Landrace boars exhibited the highest value (64.35 ± 0.21 %). Duroc breed presented the least linear progression (40.87 ± 0.08 %), while LT and Yorkshire breeds presented intermediary linearity indices. The straightness index (STR) was higher in the Landrace breed (80.20 ± 0.24 %) compared to Duroc breed (65.75 ± 0.10 %). There were differences between all boar breeds for the sperm oscillation (WOB), where boar breeds for the sperm oscillation (WOB), where the Landrace breed was greater than Duroc (77.86 ± 0.15 %; 59.31 ± 0.06 %, respectively). For the amplitude of the sperm head (ALH) the Duroc breed presented higher values than the other breeds.

DISCUSSION

In our study, we estimated the swimming patterns for multiple breeds (commercial dam and sire breeds) in different age cohorts, seasons and two sperm counting chambers. Studies of age on sperm quality in boar studs have covered a short timeframe, spanning from 8 months to 3 years of age, and generally, boars are replaced (Tsakmakidis *et al*., 2010, 2012). The high turnover rate of boars is attributed to various factors including the necessity for genetic diversity, suboptimal semen quality, foot and leg issues, as well as diminished health and libido beyond the age of three-years-old (Huang *et al*., 2010). From our previous studies on the evaluation of boar breed composition on semen traits, it was found that there were no remarkable differences in the boar breeds. It is important to note that when studying the impact of male age on sperm production and quality in boars, several environmental variables such as season, breed, and nutrition can influence the results. Numerous studies, such as those by Banaszewska and Kondracki (2012)

Table 4.
Effect of genetic breed composition on kinematics semen variables (mean ± SEM) of boar ejaculates.

	Breed composition				
Variable	Duroc	Pietrain	Landrace	Yorkshire	LT
VCL	71.50±0.13[b]	68.18±0.15[c]	55.96±0.32[e]	59.76±0.35[d]	72.80±0.10[a]
VSL	28.01±0.08[d]	36.23±0.10[ab]	36.50±0.20[a]	30.94±.22[b]	30.32±0.06[c]
VAP	41.60±0.09[c]	48.50±0.10[a]	43.82±0.22[b]	41.18±0.24[d]	43.54±0.07[b]
LIN	40.87±0.08[e]	53.57±0.10[b]	64.35±0.21[a]	51.72±0.24[c]	43.36±0.06[d]
STR	65.75±0.10[d]	72.74±0.12[b]	80.20±0.24[a]	72.66±0.27[b]	67.41±0.08[c]
WOB	59.31±0.06[e]	71.18±0.07[b]	77.86±0.15[a]	68.52±0.17[c]	61.08±0.04[d]
ALH	2.92±0.00[a]	2.50±0.001[c]	1.95±0.01[e]	2.34±0.01[d]	2.84±0.001[b]
BCF	6.96±0.01[d]	7.52±0.02[a]	7.12±0.03[c]	6.93±0.03[d]	7.24±0.01[b]

VCL, curvilinear velocity ($\mu m \cdot s^{-1}$); VSL, straight-line velocity ($\mu m \cdot s^{-1}$); VAP, average path velocity ($\mu m \cdot s^{-1}$); LIN, linearity of forward progression (%); STR, straightness index (%); WOB, wobble (%); ALH, amplitude of lateral head displacement (μm); BCF, beat-cross frequency (Hz).
[a-e] Different letters within rows indicate differences ($P < 0.05$) between genetic breed composition of boars.

and Czubaszek *et al*. (2020) have attempted to correlate these factors with sperm quality across different age groups. However, these studies often overlook the interaction between age and other influencing factors.

The seasonal effect is a major problem in pig production systems, limiting the production and quality of boar semen (Petrocelli *et al*., 2015). Some studies have shown that the time of year affects the size and volume of boar testicles (Madrigal-Valverde *et al*., 2020), which influences variables such as sperm concentration or ejaculate volume (Knecht *et al*., 2014). These characteristics determine the number of seminal doses that can be produced per ejaculate (Calderón-Calderón *et al*., 2022). The response to changes in semen production and potential quality can be identified in direct impacts on the sperm formation and maturation processes (Flowers, 2022). Our results go further, since the effect on swimming patterns and progressiveness of sperm movement was determined, which allows us to identify that despite presenting faster sperm in the dry season, the higher progressiveness is seen in the rainy season. These results are contrary to kinematic patterns reported in other regions with tropical climates (Peña *et al*., 2019b), where the decline is seen with the rains; however, the differences may be due to more extreme changes between one season and another in relation to the Central American tropics. In this same sense, during the dry season the temperature and relative humidity tend to be higher than in the rainy season, which affects the thermal stress of the animals. The total motility and the presence of fast spermatozoa was higher during the rainy season, which suggests a potential effect of thermal stress on the animals. It has been shown that thermal stress affects the motility and kinematics of boar sperm (Sui *et al*., 2022), although the lack of uniformity (speed variables) in the behavior of the results of our study could be due to a degree of tolerance of the breeds that were analyzed.

The CASA-mot system has enabled a comprehensive exploration of the motility characteristics of boar sperm and the tools used in the evaluation of semen in the laboratory such as the sperm counting chambers under investigation (Gallagher *et al*., 2018; Yániz *et al*., 2018; Yeste *et al*., 2018). Several works have performed a detailed boar semen evaluation and artifacts such as frame rate (Valverde *et al*., 2019b), video capture time (Valverde *et al*., 2019c) and sperm counting chamber height (Soler *et al*., 2018; Valverde & Madrigal-Valverde, 2019) analyzed by CASA systems. It was found that there were remarkable differences in the semen parameters of commercial breeds. This fact highlights the importance of defining optimal conditions for boar semen analysis in the laboratory. The findings regarding total and progressive motile spermatozoa in boars seem to be inconsistent, as various studies have reported either no significant difference concerning the age of the boar or higher sperm motility in young or middle-aged boars (Kondracki *et al*., 2005; Wolf & Smital, 2009). Our work has demonstrated that in younger boars, sperm motility is better than in older animals; however, when analyzing sperm kinematics, boars of intermediate ages (12-24 months) exhibit better values of progressiveness than younger or older animals over 24 months of age.

Several studies highlighted that assessing sperm motil-

ity remains a crucial aspect in determining sperm fertility potential (De Ambrogi *et al.*, 2006; Holt *et al.*, 1997; Maes *et al.*, 2011; Tardif *et al.*, 1999; Vyt *et al.*, 2004, 2008). Our study unveiled that advanced age correlated with diminished sperm motility, contrasting the observed in younger counterparts. The assessment of spermatozoa progression and velocity offers precise insights into progressively rapid sperm, widely recognized as a key indicator when using sperm motility to predict fertilizing capacity (Barquero, Roldan, *et al.*, 2021; Barquero, Soler, *et al.*, 2021; Gadea *et al.*, 2004; Santolaria *et al.*, 2015; Tremoen *et al.*, 2018). Furthermore, the analysis of sperm kinematic characteristics underscores the potential for objectively evaluating the quality profile of boar spermatozoa in different treatments and conditions (Boe-Hansen & Satake, 2019; Holt *et al.*, 1997). Regarding sperm kinematics, significant differences were observed, particularly in velocity parameters (VCL, VSL, VAP) and progressiveness (LIN, STR), albeit primarily in younger boars regarding older boars. Sperm kinematics were higher in boar ejaculates of sire lines than maternal sire lines, with additional effects attributed to the age of boars used. These motility outcomes align with prior studies on the impact of different laboratory conditions on boar sperm analysis (Barquero, Soler *et al.*, 2021; Schulze *et al.*, 2013).

Additionally, elder boars exhibited compromised swimming patterns, evidenced by low levels of fast spermatozoa. While these findings may indicate the sperm quality of the boar reduces with age, differences could be attributed to variations in semen microbiota (Li *et al.*, 2023). Recent studies in microbiota analysis have unveiled that semen is not sterile, hinting at potential variations in semen microbiota across boars of varying reproductive ages (Baud *et al.*, 2019; Dalmutt *et al.*, 2020). These microbial variances carry the potential to influence semen antioxidant capacity, thereby impacting semen quality (Li *et al.*, 2023; Zhou *et al.*, 2015). Some studies have demonstrated the presence of detrimental bacteria in the semen of aging boars, contributing to the observed decline in semen antioxidant capacity and overall semen quality, consequently impairing the fertility of the semen dose (Fraczek *et al.*, 2007; McAnally *et al.*, 2023; Jones *et al.*, 2012).

Most disposable sperm counting chambers available in the market are based on the principle of capillary action loading and commonly include a sliding cover that is fixed with different types of adhesives (Bompart *et al.*, 2018). Other chambers are based on the principle of drop dispersion (Mrkun *et al.*, 2007). In our study, we evaluated both types of counting sperm chambers and determined that motile and kinematics were higher when the Makler® chamber (drop dispersion) was used than the Leja® chamber (capillary action). However, when analyzing sperm progressive motility, higher values were observed with the Leja® analysis chamber. Several instances have demonstrated improved analysis outcomes using drop-displacement and capillary chambers, as seen in studies conducted on rams (Palacín *et al.*, 2013) and goats (Del Gallego *et al.*, 2017). These studies noted that drop-displacement chambers yielded better results in terms of motile parameters compared to capillary chambers (Del Gallego *et al.*, 2017; Palacín *et al.*, 2013) which coincides with results obtained in our study in boar semen. Regarding kinematics variables, our study has reported variation for drop- or capillarity-loaded chambers across the microscopic fields. Conversely, some studies have not reported significant differences between the two types of counting chambers, such as in boars (Gączarzewicz, 2015), fish (Gallego & Asturiano, 2018) and eels (Caldeira *et al.*, 2019; Gallego *et al.*, 2013).

When CASA analysis is utilized, typically advocates for selecting the determination of semen quality that yields the highest sperm motility, kinematics, or straightest movement (Lenz *et al.*, 2011). Given that motility remains the primary criterion for assessing sperm quality in production facilities, we emphasize the importance of recognizing the dependency of CASA output on the type of viewing chamber (Nanni Peng & Li, 2015; Valverde *et al.*, 2019a). If different chambers produce different values for the same sample, the spermatozoa movement may be somewhat inhibited by the chamber with lower values (Ibănescu *et al.*, 2016). Based on this premise and considering the two chambers we assessed, the Makler® chamber yields superior results immediately after filling. However, for longer duration examinations on progressiveness or those conducted a few minutes after filling, the Leja® chambers are recommended. Therefore, it is advisable for examiners to standardize their methods for the species they are evaluating before assessing semen samples.

A positive effect of younger age of boars was observed on the semen parameters of motility and faster swimming patterns, while seasonal variations also influenced motility positively. Duroc and Landrace breeds showed higher sperm motility, with sire line breeds displaying accelerated linear progression. The advanced age correlated with diminished sperm motility, highlighting the need for thorough evaluation, and monitoring of boar semen quality over time. The standardization of laboratory practices is necessary, particularly in assessing motility and kinematic variables using different sperm counting chambers.

DECLARATIONS

Competing interests statement

The authors declare that they have no competing interests

Ethics statement

The study was conducted in compliance with laws and regulations for conducting experiments on live animals in Costa Rica. In this investigation, animals were handled with care to avoid any unnecessary stress and conformed with the animal welfare guidelines of the Costa Rica Institute of Technology. This study was conducted following ethical principles and with the approval of the Committee of the Research and Development Center for Sustainable Agriculture in the Humid Tropics of the Costa Rica Institute of Technology (CIDASTH-ITCR) Section 22/2022, Article 6.0, DAGSC-262-2022. All experiments were conducted in accordance with relevant guidelines and regulations. The study was carried out in compliance with the ARRIVE guidelines (https://arriveguidelines.org/).

Author contributions

Conceptualization, A.V., M.A.S; methodology, F.S., L.M., A.V.; software, F.S., L.M., I.A.Z.; validation, F.S, A.V.; formal analysis, A.V.; investigation, F.S., L.M., I.A.Z.; resources, A.V.; data curation, L.M., F.S., I.A.Z; writing—original draft preparation, A.V., F.S.; writing—review and editing, A.V., F.S., A.S., M.A.S. B.V.; visualization, A.V.; supervision, A.V.; project administration, A.V.; funding acquisition, A.V., M.A.S.. All authors have read and agreed to the published version of the manuscript.

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