Austral Journal of Veterinary Sciences

Austral J Vet Sci 58, e5802 (2026)
https://doi.org/10.4206/ajvs.58.2

ORIGINAL ARTICLE

Influence of age, breed lines and season on boar sperm motility and morphology under tropical conditions

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Article History
Received: 18.08.2025
Accepted: 01.12.2025
Published: 27.02.2026

ABSTRACT. Sperm morphology assessment is essential for maintaining reproductive efficiency and profitability on farms. In tropical countries and smaller-scale farms, the assessment of sperm morphology of the doses used in artificial insemination is not routinely carried out; therefore, its analysis and standardization are necessary. This study evaluated the influence of age, season, and genetic line on sperm morphology in breeding boars under tropical conditions. The study was conducted on commercial farms in the northern region of Costa Rica from 2019 to 2023, with sampling during both the dry and rainy seasons. Sixty-five boars and 312 ejaculates representing Duroc, Landrace, Pietrain, and Pietrain × Duroc genetic lines were analyzed. Animals were classified by age at collection into three groups according to age at the time of collection:< 24 months (G1, n = 19), 24–48 months (G2, n = 26), and > 48 months (G3, n = 33); some animals were analyzed and classified into different groups. Morphology was assessed by Trumorph® fixation and visual examination using a light microscope at 400× magnification. The percentage of sperm morphological abnormalities varied significantly by year, season, and genetic line ($P < 0.05$). The Pietrain line showed the highest average abnormality frequency, whereas the rainy season exhibited the greatest proportion of abnormal sperm ($P < 0.05$). In conclusion, season and genetic line significantly influenced sperm morphology in breeding boars. Standardization and validation of morphological assessment are necessary in semen production centers and commercial pig farms.

Keywords: animal reproduction; semen quality; reproductive biology; pigs; swine

INTRODUCTION

Semen evaluation is critically important for swine production systems that rely on artificial insemination with cooled semen; it underpins reproductive management on farms and is directly linked to both reproductive efficiency and profitability (Barquero *et al*., 2021). Assessing semen quality improves the selection of both sires and superior ejaculates (Valverde *et al*., 2020). A wide range of intrinsic and extrinsic factors affect boar semen quality (Flowers, 2015; Lopez-Rodriguez *et al*., 2017). These factors include seasonality of semen collection (Fraser *et al*., 2016), animal age (Knecht *et al*., 2017), nutrition (Calderón-Calderón *et al*., 2022), and genetic lines (Sevilla *et al*., 2025), which can alter normal sperm morphology (Kamanova *et al*., 2021), resulting in the culling of boars that have a high incidence of sperm morphoanomalies (Rocha *et al*., 2021). These factors can also hinder the selection of breeding males that do not meet the minimum acceptable threshold for normal sperm morphology (Gatimel *et al*., 2017).

Sperm morphology assessment involves identifying normal and abnormal cell forms, considering defects in the head, acrosome, midpiece, and tail (Kondracki *et al*., 2020). These anomalies can be classified as either uncompensable defects, which affect embryonic development, or compensable defects, which may restrict motility or viability without impairing later embryogenesis. They are also categorized as primary or secondary morphological defects (Banaszewska & Andraszek, 2021). Morphological abnormalities may, in turn, influence other semen quality parameters, such as motility (Hook & Fisher, 2020), volume (Górski *et al*., 2017), and sperm concentration (Górski *et al*., 2018; Kondracki *et al*., 2020), as well as overall fertility and subsequent embryo development (Fitzpatrick & Lüpold, 2014).

Some systems, such as computer-assisted semen analysis (CASA), can evaluate sperm morphology (Suzuki *et al*., 2002) by capturing multiple images, which provide a more precise measure of sperm shape and size; however, they use a great diversity of fixation and staining, which could alter cell morphology and dimensions. Trumorph® systems use a pressure- and temperature-based fixation method that does not permeabilize spermatozoa and is a robust method for analyzing sperm morphology (Soler

et al., 2015). These results can be further optimized using neural network approaches for automated morphology identification (Keller *et al*., 2024). The data produced by these systems help predict relationships with reproductive parameters in sows (Barquero *et al*., 2021), suggesting avenues for improving reproductive and production indices on farms (Rocha *et al*., 2021). Differences in the applied protocols, such as fixation (Górski *et al*., 2017) and sample preparation methods (Gatimel *et al*., 2017) may affect the obtained results. Therefore, proper standardization of these assessments (Gatimel *et al*., 2017) and their automation (Hao *et al*., 2025) is needed.

Currently, various research teams are promoting more objective semen evaluations at production facilities, where such analyses are either not performed or are performed subjectively (Hao *et al*., 2025). Internal and external factors associated with boars could influence the percentage of normal sperm morphology (Hackerova *et al*., 2025). Consequently, the aim of this study was to analyze sperm morphology and motility using a fixation method without wet staining and to determine the influence of age, season, and genetic line of boars under tropical conditions.

MATERIALS AND METHODS

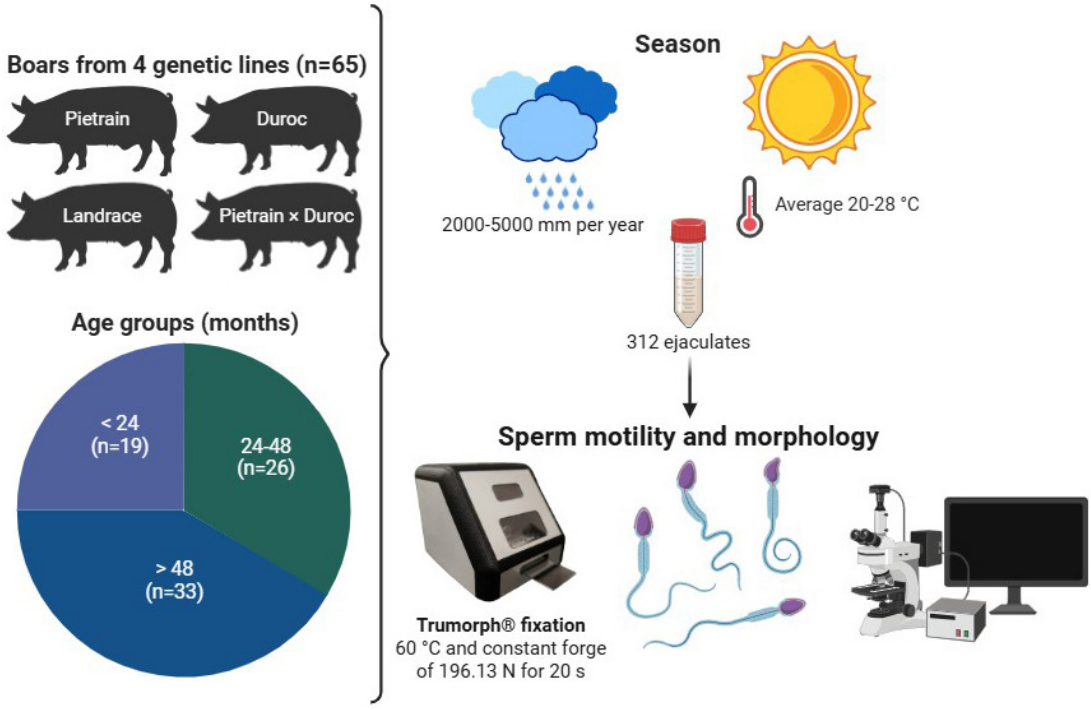
Study site

The study was conducted on commercial farms in northern Costa Rica, a tropical region with two seasons: dry from November to April and rainy from May to October. The average rainfall is from 2,000 to 5,000 mm/year, and the average temperature oscillates between 20 °C and 28 °C. Sperm morphology analyses were performed at the Animal Reproduction Laboratory, School of Agronomy, Costa Rica Institute of Technology, San Carlos Campus, Costa Rica. Sampling was conducted in both seasons between 2019 and 2023.

Animals

We evaluated breeding boars from four genetic lines, comprising 65 sires and 312 ejaculates, for this study. Each boar contributed at least two ejaculates as follows: Duroc (n = 61 ejaculates), Landrace (n = 14 ejaculates), Pietrain (n = 70 ejaculates), and Pietrain × Duroc (n = 167 ejaculates). Ejaculates were collected in the morning using the double-glove (hand-glove) technique; only the sperm-rich fraction was retained and stored at 37 °C. Each ejaculate was diluted 1:1 (v/v) with a commercial extender (long-term). Samples were transported to the Animal Reproduction Laboratory within two hours under optimal cooling at approximately 17 °C. Upon arrival, the samples were stored at the same temperature for 18 h before analysis. The boars were housed individually, fed a standard diet, and provided water *ad libitum*. At each collection, the animals were classified by age into three groups: < 24 months (n = 19 boars and 56 ejaculates), 24–48 months (G2, n = 26 boars and 110 ejaculates), and > 48 months (n = 33 boars and 146 ejaculates) (Figure 1).

Figure 1.
Graphical abstract for experimental design and analysis of boar sperm motility and morphology. Created in biorender.com.



Sperm motility and morphology analysis

Each semen dose was removed from storage (17 °C) and allowed to equilibrate for 30 min at room temperature (25 °C). Two 1 mL aliquots of each ejaculate were transferred into Eppendorf® tubes (Sigma-Aldrich, St. Louis, MO, USA). Total motility (TM) and progressive motility (PM) were analyzed using CASA-Mot systems (ISAS, Integrated Semen Analysis, Proiser R+D, Paterna, Spain) in a Makler® chamber. The CASA system was integrated with a video camera (Proiser 782M, Proiser R + D) with a final resolution of 768 × 576 pixels. The system used a microscope UB203 (UOP/Proiser R + D), 1x eyepiece, and an objective of 10 x magnification (negative phase, AN 0.25). The samples were maintained at 37 ± 0.5 °C using an integrated heating stage. A 2.7 µL aliquot was pipetted and deposited in the Makler® chamber for analysis. Total motility refers to the percentage (%) of sperm that are moving, and progressive motility is the percentage (%) of sperm that are moving quickly and in a straight line.

Sample fixation for morphological analysis was performed using the Trumorph® system (Proiser R+D, Paterna, Spain). A 3 µL aliquot of each ejaculate was placed on a microscope slide, and a coverslip was gently applied at a 45° angle to evenly spread the drop. The slide was inserted into the Trumorph® device, which applied a constant pressure of 196.13 N and exposed the sample to 60–65 °C for 5 s; the sample reached 45 °C, thereby fixing the spermatozoa for morphological evaluation. Sperm morphology was assessed under a B-383Phi microscope (Optika, Italy) equipped with a 1× eyepiece and a 40× negative phase-contrast objective. A total of 200 cells were examined for each ejaculate.

Eight categories of abnormalities were evaluated in the sperm head, midpiece, and tail: proximal cytoplasmic droplets, distal cytoplasmic droplets, coiled tails, tightly coiled tails, bent tails, micro-head spermatozoa, pyriform heads, and head- or tail-detached cells. During the assessment, representative photographs of each defect were taken at 400× and 1000× magnifications using a B-383Phi microscope (Optika, Italy) fitted with an HDMI C-HP digital camera (Optika, Italy).

Statistical analysis

The results were compiled using Microsoft Excel spreadsheets. Each record was meta-tagged by season, month, and year of collection, animal ID, genetic line, and age category. Percentages were calculated for total motility, progressive motility, normal morphology, overall abnormalities, and each specific abnormality. The data obtained for the analysis of all sperm variables were first assessed for normality and homoscedasticity using the Shapiro-Wilk and Levene tests. A normal probability plot was used to assess the normal distribution.

The statistical model was as follows:

$Yijklmnopqr = \mu + A_i + Ej(A)_j + Ag_k + S_l + L_m + Y_n + (Ag\times S)_o + (Ag\times L)_p + (S\times L)_q + (Y\times S)_r + eijklmnopqrs$ in which Y is the variable ; µ = overall mean, A_i = *i*-th random effect of animal ; $Ej(A)_j$= *j*-th random effect of ejaculate nested within animal; Ag_k= *k*-th age effect; S_l= *l*-th season effect; L_m= *m*-th genetic-line effect; Y_n= *n*-th year effect; $(Ag\times S)_o$ = *o*-th age × season interaction effect; $(Ag\times L)_p$= *p*-th age × genetic-line interaction effect; $(S\times L)_q$= *q*-th season × genetic-line interaction effect; $(Y\times S)_r$= *r*-th year × season interaction effect; e: experimental error. Residual (co)variances were modeled to account for the repeated-measures structure, and the model with the lowest Bayesian information criterion (BIC) and Akaike information criterion (AIC) values was selected as the best fit.

An analysis of variance (ANOVA) was performed to assess the influence of age, season, genetic line, year, and month of collection and their interactions on the percentages of total motility, progressive motility, normal sperm, and abnormalities. A random residual term was included to account for the correlations among ejaculates from the same boar. When significant effects were detected, the means ± standard error of the mean (SEM) were compared using the Bonferroni test. Statistical significance was set at $P < 0.05$. All analyses were conducted using IBM SPSS Statistics for Windows, version 29.0 (IBM Corp., Armonk, NY, USA). Pearson's correlation was analyzed for total motility, progressive motility, and normal morphology, with statistical significance set at $P < 0.01$.

RESULTS

Analysis of sperm motility and morphology

Age group was significantly associated with total motility (TM). Boars aged < 24 months had the highest mean TM ($P < 0.05$), whereas season and breed did not significantly affect this parameter. However, season and breed were significant ($P < 0.05$) for progressive motility (PM), and the highest mean PM was observed in Pietrain × Duroc and the dry season (Table 1).

A moderate positive correlation was observed between total motility and normal morphology (r = 0.36; $P < 0.01$). This suggests that higher total motility contributes to higher normal sperm values. In addition, a weak positive correlation was observed between progressive motility and normal morphology (r = 0.19; $P < 0.01$).

External effects on sperm morphology analysis

Interannual variation in the percentage of sperm abnormalities was observed in boars from 2020 to 2022 ($P < 0.05$). The highest mean incidence occurred in 2020 (15.34 ± 1.36 %; coefficient of variation (CV) = 75.60), significantly exceeding the values recorded in 2022 ($P < 0.05$). In contrast, 2022 showed the lowest mean abnormality rate (9.15 ± 2.70%; CV = 72.98), although its coefficient of variation was similar to that of 2020. The highest individual abnormality value (61%) was also recorded in 2020 (Table 2).

Monthly analysis of sperm morphology revealed a significant difference between February and June, July, August, September, and November ($P < 0.05$). In addition, December revealed lower percentages of sperm abnor-

Table 1.
Influence of season, breed and age group variables on total motility and progressive motility in boars from 2019 to 2023 in northern Costa Rica.

Variable	Total motility (%) (Mean ± SEM)	Progressive motility (%) (Mean ± SEM)
Rainy (n=41, *185)	76.94 ± 1.56	60.72 ± 1.85^{x}
Dry (n=56, *127)	72.85 ± 1.93	40.47 ± 2.29^{y}
Duroc (n=13, *61)	75.63 ± 2.08	50.79 ± 2.46^{ab}
Landrace (n=7, *14)	78.34 ± 1.45	57.58 ± 1.72^{a}
Pietrain (n=22, *70)	69.85 ± 5.12	34.04 ± 6.07^{b}
Pietrain x Duroc (n=23, *167)	73.40 ± 1.92	51.09 ± 2.27^{ab}
< 24 months (n=19, *56)	81.23 ± 2.34^{r}	48.77 ± 2.77
24–48 months (n=26, *110)	70.19 ± 1.80^{s}	52.26 ± 2.13
> 48 months (n=33, *146)	73.35 ± 2.51^{rs}	47.63 ± 2.97

n= Number of semen donors analyzed for each variable. Some boars were analyzed in both seasons. *Indicates the number of ejaculates for each variable. SEM: standard error of the mean.
^{x,y} Different superscripts in the same column indicate significant differences between seasons ($P < 0.05$).
^{a,b} Different superscripts in the same column indicate significant differences between breeds ($P < 0.05$).
^{r,s} Different superscripts in the same column indicate significant differences between the age groups ($P < 0.05$).

Table 2.
Descriptive values and mean comparison test of sperm morphological anomalies (%) in boars by year from 2019 to 2023 in northern Costa Rica

Year (n)	Mean ± SEM	SD	CV	Min	Max
2019 (n=18)	12.82 ± 1.56^{ab}	6.97	54.14	4.00	35.00
2020 (n=14)	15.34 ± 1.36^{a}	11.41	75.60	3.00	61.00
2021 (n=12)	12.76 ± 1.49^{ab}	10.10	79.17	2.00	44.00
2022 (n=10)	9.15 ± 2.70^{b}	6.37	72.98	2.50	25.00
2023 (n=33)	12.02 ± 1.02^{ab}	5.53	49.50	1.00	27.50

n= Number of semen donors per year; some boars were collected in more than one year. SEM, standard error of the mean; SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value.
a,b Different superscripts in the same column indicate significant differences between years ($P < 0.05$).

malities than June and July ($P < 0.05$). No other comparisons among the remaining months were significant. The highest rates of sperm morphoanomalies occurred in June (16.54%) and July (16.12%), whereas the lowest rates were recorded in February (7.75%), April (10.41%), and December (9.75%) (Figure 2).

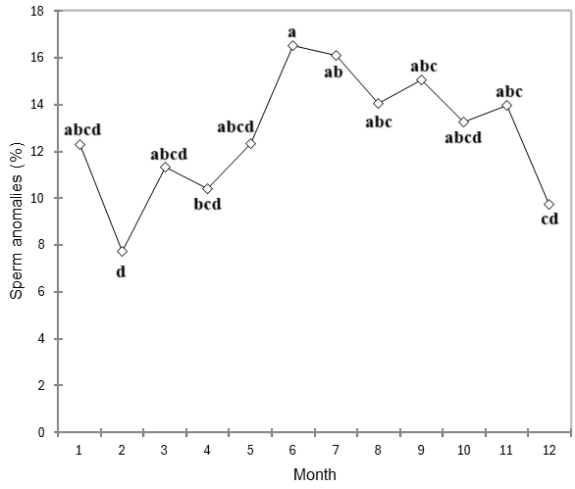


Figure 2.
Percentages of sperm morphological anomalies by month in boars in northern Costa Rica between 2019 and 2023.
a-d Different letters indicate significant differences between months ($P < 0.05$).

Season also significantly affected sperm morphology ($P < 0.05$). The rainy season showed the highest mean proportion of sperm abnormalities (13.68 ± 1.03%; CV = 70.00), whereas the dry season showed lower values (11.15 ± 1.20%; CV = 61.33). Similarly, the maximum individual incidence of morphoanomalies occurred during the rainy season (61%), compared with 42% during the dry season (Table 3).

Table 3.
Descriptive values and mean comparison test of sperm morphological anomalies (%) in boars by season from 2019 to 2023 in northern Costa Rica

Season	Mean ± SEM	SD	CV	Min	Max
Rainy (n=41, *185)	13.68 ± 1.03^{a}	9.93	70.00	2.00	61.00
Dry (n=56, *127)	11.15 ± 1.20^{b}	6.71	61.33	1.00	42.00

n= Number of semen donors analyzed in each season, some boars were analyzed in both seasons. *Indicate number of ejaculates per season. SEM, standard error of the mean; SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value.
a,b Different superscripts in the same column indicate significant differences between seasons ($P < 0.05$).

Influence of genetics lines and age on sperm morphology

Significant differences in the proportion of sperm abnormalities were detected among the genetic lines ($P < 0.05$). Pietrain boars exhibited the highest mean incidence ($P < 0.05$) of sperm abnormalities (14.56 ± 1.22%; CV = 65.85) surpassing Pietrain × Duroc (10.08 ± 0.90%; CV = 72.02). The maximum individual abnormality rates were recorded in Pietrain (60%) and Pietrain × Duroc (61%) ejaculates, but the lowest mean value was recorded in Pietrain × Duroc (Table 4).

Age did not significantly affect sperm morphology in this study ($P > 0.05$). Comparisons of age groups did not reveal any significant differences between the groups. Groups 2 and 3 showed the maximum values of sperm abnormalities (Table 5).

Interaction analysis results

Analyses of the possible interactions between season, breed, age, and year were performed. Interactions were tested for the effect of *season × year* and *season × breed* ($P < 0.05$). No interaction effect was observed between *age × breed* and *age × season* ($P > 0.05$). Figure 3 shows the relevant variation in normal sperm morphology in 2020 during the dry and rainy seasons (Figure 3A). When analyzing the interaction between season and breed, the Pietrain breed showed a significant decrease in normal sperm morphology during the rainy season. The interaction effect between Duroc and Duroc × Pietrain showed less variation during the dry and rainy seasons. In addition, the Landrace breed showed a different seasonal influence in the rainy season, increasing the value of normal sperm morphology (Figure 3B).

The most prevalent sperm abnormalities were proximal cytoplasmic droplets, tightly coiled tails/Dag defects, and tail coiling. In contrast, the least frequent defects were micro-head spermatozoa, pyriform heads, and distal cytoplasmic droplets. The greatest variability occurred in micro-head sperm (CV = 1 046.16%) and distal droplet (CV = 223.64 %), indicating substantial dispersion within the population (Table 6). At the 95th percentile, the highest values were observed for proximal droplets (P95 = 16%) and tightly coiled tails (P95 = 9%), suggesting that at least 5% of the evaluated boars displayed markedly elevated levels of these anomalies.

Identification of boar sperm morphology

Standardized reference images of boar spermatozoa exhibiting normal morphology are shown below, and the subsequent figures depict the standardized sperm morphoanomaly types listed in Table 6. Figure 4 shows representative boar spermatozoa with normal morphology in commercial semen doses.

Table 4.
Descriptive values and mean comparison test of sperm morphological anomalies (%) in boars by breed from 2019 to 2023 in northern Costa Rica

Breed	Mean ± SEM	SD	CV	Min	Max
Duroc (n=13, *61)	12.66 ± 1.28^{ab}	8.15	64.68	2.00	35.00
Landrace (n=7, *14)	12.36 ± 2.65^{ab}	5.01	43.39	5.00	23.50
Pietrain (n=22, *70)	14.56 ± 1.22^{a}	10.36	65.85	4.00	60.00
Pietrain x Duroc (n=23, *167)	10.08 ± 0.90^{b}	8.57	72.02	1.00	61.00

n= number of semen donors per breed. *Indicate number of ejaculates analyzed per breed. *Indicate number of ejaculates per breed. SEM, standard error of the mean; SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value.
a,b Different superscripts in the same column indicate significant differences between breeds ($P < 0.05$).

Table 5.
Descriptive values in percentage and mean comparison test of sperm morphological anomalies (%) in boars by age from 2019 to 2023 in northern Costa Rica

Age Group	Mean ± SEM	SD	CV	Min	Max
< 24 months (n=19, *56)	11.61 ± 1.63	6.71	61.78	1.00	42.00
24–48 months (n=26, *110)	12.97 ± 1.12	10.29	76.21	3.00	60.00
> 48 months (n=33, *146)	12.67 ± 1.63	8.45	64.17	2.00	61.00

n= Number of semen donors per age group analyzed. *Indicate number of ejaculates per age group. SEM, standard error of the mean; SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value.

Table 6.
Descriptive values of sperm morphological anomalies (%) in boars from 2019 to 2023 in northern Costa Rica

Sperm anomalies	Mean	SD	CV	Min	Max
Proximal droplet	4.04	5.36	132.65	0.00	44.00
Distal droplet	0.87	2.03	223.64	0.00	15.00
Coiled tail	1.96	2.44	124.49	0.00	19.00
Tightly coiled tail	2.34	3.56	152.22	0.00	25.00
Micro-head	0.07	0.72	1,054.75	0.00	12.50
Bent tail	1.92	1.97	102.28	0.00	15.00
Pyriform head	0.55	1.01	182.66	0.00	8.50
Loose head	1.12	1.51	134.95	0.00	13.00

n= 312 ejaculates in total were analyzed. SD, standard deviation; CV, coefficient of variation; Min, minimum value; Max, maximum value.

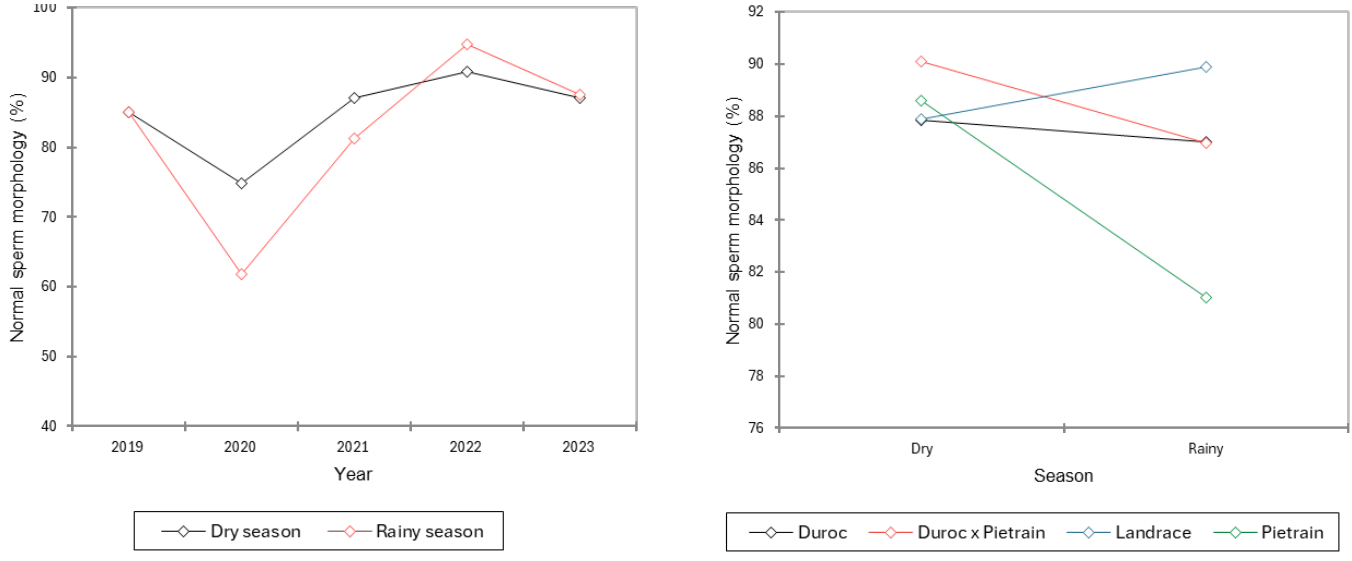


Figure 3.
Interaction between season × year (A) and season × breed (B) for the normal sperm morphology (%) using a confidence interval of 95% (IC 95 %).

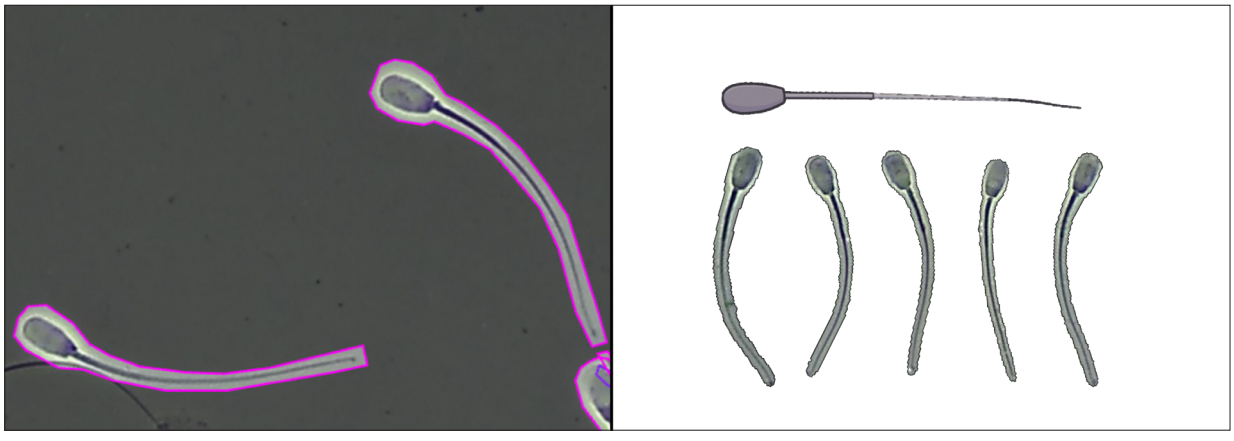


Figure 4.
Boar spermatozoa with normal morphology at 400× magnification, fixed using the Trumorph® system.

Proximal and distal cytoplasmic droplets appear as irregular swellings or "drops" on the sperm midpiece and are classified as proximal or distal according to their position relative to the sperm head. Figure 5 shows spermatozoa bearing distal cytoplasmic droplets (Figure 5A) and proximal cytoplasmic droplets (Figure 5B).

Tail defects included slight and moderate coiling (Figure 6A), tightly coiled tails, or Dag defects (Figure 6B), in which the tail loops into a moderately pronounced knot. Additional abnormalities, such as a fractured or torsion-like appearance of the tail (i.e., bent tails), were also observed (Figure 6C).

Finally, head-level abnormalities were observed. These included shape defects, such as pyriform heads, which widen at the basal region and resemble a pear (Figure 7A). Heads detached from the midpiece or tail were also observed (Figure 7B).

DISCUSSION

Assessing sperm morphology contributes to the determination of ejaculate quality in livestock animals (Gatimel *et al*., 2017), thereby aiding sire selection or culling and ultimately optimizing semen dose production (Broekhuijse *et al*., 2015). The morphology of spermatozoa also indicates physiological disturbances in the animal (Rocha *et al*., 2021), which may provoke problems in subsequent generations because certain defects are heritable (Toner *et al*., 1995). Additional studies have linked morphology with other semen parameters, such as motility and kinematics (Soler *et al*., 2016), concentration (Kondracki *et al*., 2020), and even fertility in inseminated sows (Hook & Fisher, 2020).

The absence of morphological assessment or its standardization seriously limits semen quality analysis (Gatimel *et al*., 2017) and species; therefore, rigorous technical validation within each facility is essential (Brito *et al*., 2025). Staining protocols can alter morphological evaluations, particularly by affecting sperm dimensions (Szablicka *et al*., 2022). Although staining procedures have been extensively studied, they introduce variability by damaging spermatozoa; therefore, devices such as Trumorph®, which minimize semen manipulation prior to morphological assessment, may enhance the validation and standardization of morphological analysis (Soler *et al*., 2015). Simplified fixation methods with fewer procedural steps may reduce variation (Soler *et al*., 2015) and further automate sperm morphology evaluation (Keller *et al*., 2024).

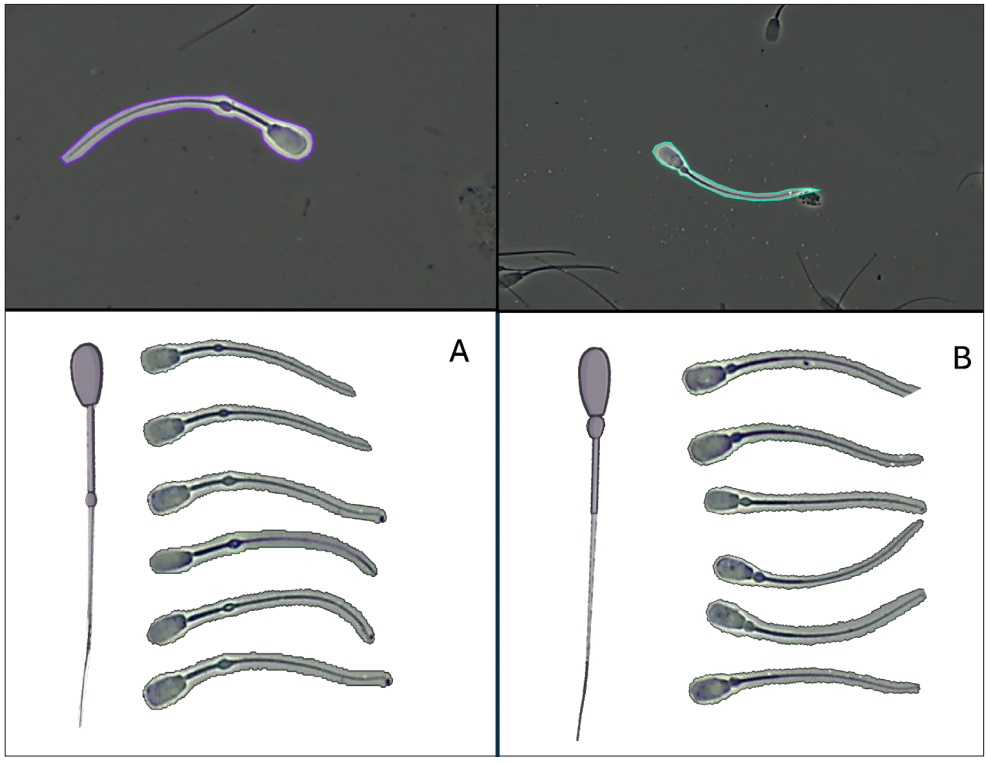


Figure 5.
Cytoplasmic droplets observed in boar semen doses. A) Distal cytoplasmic droplet; B) proximal cytoplasmic droplet. Viewed at 400× magnification.

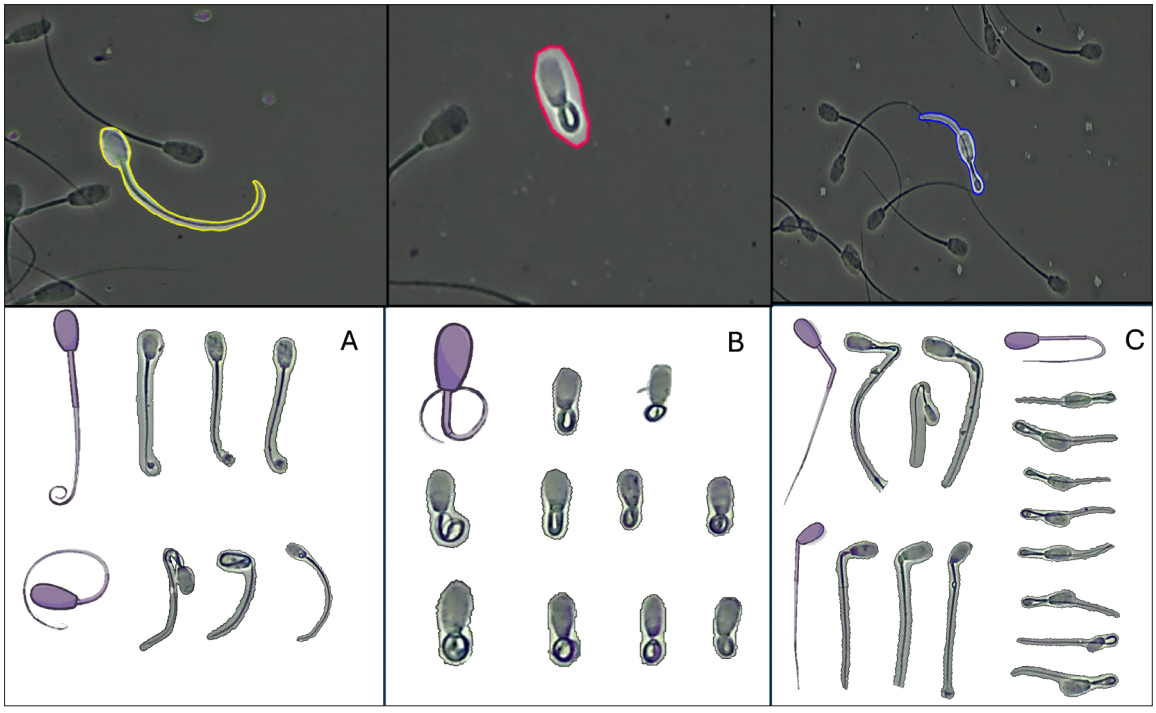


Figure 6.
Midpiece and tail defects in boar semen doses at 400× magnification. A) Coiled tails; B) tightly coiled tail (Dag defect); C) bent tail or midpiece.

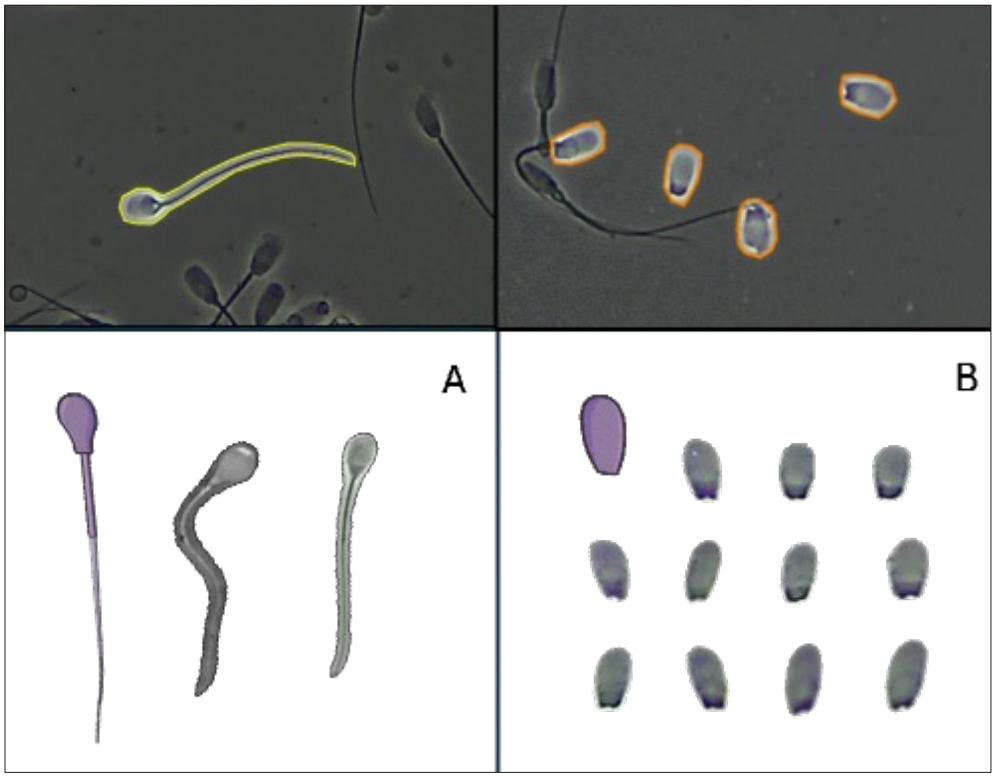


Figure 7.
Head morphological defects in boar semen. A) pyriform head; B) detached tail and midpiece. Viewed at 400× magnification.

The present study demonstrated a genetic line influence on the sperm morphology. Pietrain boars showed the highest abnormality rates compared to Pietrain × Duroc. Previous comparisons between Pietrain and Duroc sires revealed no difference in the abnormality percentage, but studies have detected disparities in sperm size and shape (Kondracki *et al*., 2012), which are likely of genetic origin (Wysokińska & Kondracki, 2019). A breed influence on morphology has also been reported when Colombian Creole boars were compared with Duroc and Pietrain sires (Suárez-Mesa *et al*., 2021). Therefore, the selection of boars for use as maternal, paternal, or terminal lines may influence sperm morphology. In addition, this study showed that the crossbred boars (Pietrain × Duroc) showed fewer morphological sperm anomalies than the sperm of boars of the parent breeds. Previously, the effect of heterosis on membrane integrity in boars has been analyzed (Wysokińska & Szablicka, 2021). This could indicate the relevance of frequency in the analysis of boar cell morphology according to breed.

Boar age did not significantly affect the sperm morphology. However, in humans, the incidence of morphological anomalies increases with age (Sharma *et al*., 2015). Similarly, our study showed that the maximum values of abnormality percentage occurred in boars older than 24 months (24–48 months) and those > 48 months. Age-related variations in boar sperm morphology and functionality have also been reported, with improvements between 20 and 26 months of age (Czubaszek *et al*., 2020). Age and sexual development further influence semen dose preparation (Banaszewska & Kondracki, 2012), underscoring the importance of boar age as a critical selection criterion.

When seasonal effects were examined, a significant difference was observed between the rainy and dry periods. Previous studies have documented a reduction in the percentage of morphologically normal sperm during certain summer months (Gonzalez- Castro *et al*., 2022). This differs from the current findings because of the different climatic conditions and the effect of relative humidity on tropical conditions during the rainy season. Monthly analysis revealed that peak abnormality rates in June, July, and September are likely attributable to higher ambient relative humidity, which may have damaged spermatozoa (Li *et al*., 2023). Temperature-induced stress has been shown to alter sperm morphology, thereby affecting fertility indicators, such as the number of piglets born and born alive (Schulze *et al*., 2021).

The study of the interaction of season and breed was significant, while the interaction with age was not significant. These findings suggest that in tropical conditions, external factors associated with the season (humidity and temperature) and internal factors such as breed could affect sperm quality, whereas age may not influence sperm morphology. Previous studies have suggested that tropical conditions are challenging for pig production and that fluctuations in temperature pose significant challenges in these regions (Kemoi *et al*., 2025). In addition, previous studies have found that tropical regions may not have very marked seasons and that the effects on boar fertility may not be detectable in all locations (Knox, 2024).

In this study, proximal cytoplasmic droplets (4.01%) and Dag defects (2.29%) showed the highest incidence among morphoanomalies. These figures were lower than those reported in other investigations (Kondracki *et al*., 2020), which found 1.70% Dag defects and 1.31% proximal cytoplasmic droplets; however, both studies agree that these are the most frequently observed non-compensable abnormalities. This finding suggests that genetic selection for boars with superior sperm morphology, despite the trait's generally low to moderate heritability, could still promote genetic progress in this characteristic (Zhao *et al*., 2019).

In conclusion, variations in semen quality are determined by several internal and external factors. This study demonstrated that the genetic line of breeding boars clearly influences sperm morphology, while the season also affects morphological profiles, especially during the rainy season. The spectrum of sperm abnormalities in pigs varies according to genetics, sire age, and time of collection, highlighting the need to implement appropriate breeding boar selection programs. Therefore, rigorous standardization and validation of sperm morphology assessment are essential to produce high-quality semen doses in both semen production centers and commercial pig farms.

DECLARATIONS

Competing interests' statement

The authors declare no competing interest. The funding entities played no role in the study design, data collection, analysis, decision to publish, or manuscript preparation.

Ethics statement

This study was conducted in accordance with ethical principles and was approved by the Research Committee of the Center for Research and Development in Sustainable Tropical Agriculture (CIDASTH) at the Costa Rica Institute of Technology, according to Section 20-2023, Article 1.0, DAGSC-188-2023, and CIE-206-2023. This investigation also adhered to the ARRIVE guidelines (https://arriveguidelines.org/). The farms held a valid Veterinary Operation Certificate that verified and ensured the requirements for guaranteeing respect for animals and the environment.

Author contribution

Conceptualization, A.V., F.S., M.A.S.; methodology, F.S. and I.A.-Z.; software, F.S., I.A.Z., A.V.; validation, F.S., A.V., I.A.Z.; formal analysis, F.S., A.V.; investigation, F.S., I.A.Z., A.V., resources, A.V.; data curation, F.S., A.V., original draft preparation, F.S., A.V.; review and editing, FS, AV, IA-Z, M.A.S., A.H., A.S.-C.; F.S., A.V., P.C., A.V.; visualization, A.V., M.A.S.; supervision, A,V., M.A.S.; project administration, A.V., M.A.S.; funding acquisition, A.V., M.A.S. All authors have read and agreed to the final version of the manuscript.

Acknowledgments

The authors thank the Costa Rica Institute of Technology for funding this study. This work was part of the research project VIE-2151-083, "Optimización de la conservación y búsqueda de parámetros de la fertilidad en espermatozoides de animales de interés productivo". F.S. and I.A.Z. thank the Postgraduate Office of the Costa Rica Institute of Technology.

Data Availability Statement
The raw data supporting the conclusions of this article will be made available by the authors without undue reservation.

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