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## Semen characteristics of the three genetic types of boars reared in Benin

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## ABSTRACT

**Objective:** To characterize the semen of three genetic types of boars (local, improved and Large White) reared in Benin.**Methods:** Semen of local, improved and Large White boars reared in Benin were collected using the gloved hand method and analyzed to determine volume, pH, concentration, mobility, motility, and morphology. The effect of the genetic type of boar on semen characteristics was also studied.**Results:** Duration of ejaculation and semen volume of Large White boar were significantly higher than those of local and improved boars ( $P < 0.05$ ). The semen of improved boars had a higher motility score than that of Large White and local boars ( $P < 0.001$ ). The semen of local boars was more concentrated in the spermatozoa than that of improved and Large White boars ( $P < 0.05$ ). The proportion of spermatozoa of improved boars with normal morphology (93.6%) was significantly higher than that of local (82.2%) and Large White boars (81.6%) ( $P < 0.001$ ). The proportion of spermatozoa with folded tails in the semen of Large White boars (9.2%) was significantly higher than that observed in improved (1.8%) and local (5.0%) boars ( $P < 0.001$ ). The proportion of spermatozoa with proximal cytoplasmic droplets in semen of improved boars (2.7%) was significantly lower than that in Large White (6.8%) and local (9.7%) boars ( $P < 0.001$ ). The local (1.5%) and Large White boars (1.1%) showed more spermatozoa with distal cytoplasmic droplets in their semen compared to the improved boars (0.4%).**Conclusions:** The semen characteristics of pigs reared in Benin vary from one genetic type to another. Each genetic type has a strong point. The Large White boar produces larger semen, the local boar produces more concentrated semen and the improved boar produces spermatozoa that are morphologically better. The semen of these

three genetic types can be used in artificial insemination but the improved boar's semen is more recommended.

**KEYWORDS:** Boars; Semen; Local pigs; Benin; Semen characteristics; Genetic types

## 1. Introduction

Pork is widely consumed by the Beninese population, especially the southern population. Unfortunately, this livestock population is not sufficient to support the population's expressed need for pig meat due to low animal productivity resulting from difficulties in breeding techniques in general and genetic improvement and health monitoring in particular [1–4]. To find a solution to these bottlenecks limiting the development of this sector, studies have been carried out on feeding practices, farming methods, habitats, health monitoring and reproductive management in the farms and suggestions for improvement have been made [4–7]. Thus, the

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Beninese government imported exotic Large White pigs through development projects[8]. As for the farmers, they have organized themselves into associations and cooperatives to solve the problems of the sector[9]. These efforts have contributed to improving national production, but the level of improvement is often not equal to the efforts made[9]. This situation is mainly due to difficulties related to the diffusion of genetic material and diseases such as African swine fever and scabies[1,10–12]. Animals imported by the government and the best reproducers selected by the breeders are generally unable to satisfy producer demand given the method of multiplication and distribution of these reproducers, which is exclusively by natural breeding[4]. In terms of health, the inadequacy of biosecurity practices on farms favors the entry of diseases such as African swine fever and these diseases neutralize the efforts made by farmers[10]. For better livestock management, it is necessary to use biotechnology to significantly increase the national level of pig meat production. Among these technologies, artificial insemination is the most appropriate for the farming method used by farmers. It can be used to increase the fertility of reproducers, increase the genetic potential of animals, reduce working time and preserve animal health[13].

The artificial insemination technique may be used to disseminate imported or selected genitors in the farms so that several farms can be served at once by a single boar which in natural mating shall serve only one farm. It is also a biosecurity tool, as its implementation makes it possible to limit the introduction of new diseases into farms and to prevent the spread of diseases already present in the farm[13]. In addition, it will limit contact between males and females. The advantages of this biotechnology make it used in more than 90% of pig farms in Europe[14,15] and its introduction into Benin's livestock farms will be very easy because livestock associations are very interested in innovations that will improve the productivity of their livestock[9]. For the use of this tool to be effective, it must be done with locally produced semen, avoiding the import and storage costs of semen of foreign breeds.

Farmers have three genetic types of pigs: local pigs, improved pigs and Large White[8], capable of providing semen for insemination. The improved genetic type is large in size with the coat mostly white in colour with various designs. The head had a generally concave profile and ended with a short muzzle. Its ears are mostly erect and forward facing[7,8]. Local pigs are of small size. The head has a mostly straight facial profile. The back line was mostly straight. The coat is white or black with uniform designs. The ears are mainly erect and pointing upwards[7,8]. Unfortunately, no scientific work has been done in Benin on the semen characteristics of these genetic types. However, knowledge of these characteristics is indispensable for the production of the doses, as they make it possible to assess the quality of the semen in order to prepare the insemination doses.

Therefore, the aim of this study was to determine the semen characteristics of these three genetic types of boars (local, improved and Large White boars) reared in Benin.

## 2. Materials and methods

### 2.1. Study area

The study was carried out at the Laboratory of Animal Biotechnology and Meat Technology (LBTA) of the University of Abomey-Calavi (Benin), from July 2018 to June 2019. The laboratory is located in the municipality of Abomey-Calavi.

### 2.2. Animal

The animal material used consisted of 3 Large White boars, 2 improved boars and 2 local boars. These three genetic types belong to the swine species (*Sus domesticus*). The improved pigs were results of the uncontrolled crossbreeding performed by the breeders between exotics breeds. These pigs were widely used in farms and the potential breeds involved in these crossings were Large White and Landrace[7,8]. The improved and local pigs were described by Youssao *et al*[8] and their zootechnical performance was assessed by Dotche *et al*[7]. The animals were purchased at 2 months of age and reared at the LBTA's Artificial Insemination Center. They were of approximately the same age at purchase (all born in the same week). The Large Whites of parents from France were purchased from the Kpinnou Livestock Farm, the improved boars from the "Foyer Berger" Farm in Abomey-Calavi and the local pigs from a private farm in Zè. On the day of arrival, they received oral anti-stress and vitamin supplements (Stress vitam<sup>®</sup>, Vetoquinol, France). They were then placed in quarantine for two weeks. After the quarantine, each male was transferred to his loge. The data collection started at 6 months of age. They were fed twice a day with the complete feed of the Vêto Service Group (GVS, Cotonou, Benin). This feed was composed of corn and cereal products, palm kernel meals, butylated hydroxytoluene, amino acids, Grobel toxin bind, corn bran and rice bran. They were dewormed every month by levamisole.

### 2.3. Semen collection

The collection technique used was the gloved hand method described by Maes *et al*[14]. The boars were trained to ride for 1 month to familiarize them with the mannequin. Thus, the boar was introduced into the collection room where there was a mannequin. When the boar mounted on the mannequin, the preputial diverticulum was emptied of its contents. Once the penis came out, it was grasped strongly at its corkscrew-shaped end. The penis was tightened strongly without pulling it until full erection. The first drop was left on the ground and the collection container with a bag to collect the semen and a gauze filter to filter and retain the gelatinous fraction which was then placed. Collection was performed at different frequencies: twice a week (February, May, July, October), 3 times in two weeks (March, June, September, December) and once

a week (January, April, August, November). The collection was performed for 12 months at the same monthly collection frequency for the three genetic types. The duration of the ejaculate was taken by a stopwatch. In the 8th month of collection, one improved boar fell ill and was removed from the study. The total number of ejaculate collected was 238 (80 for one boar, 79 the second, 79 for the third ) in Large White boars, 129 (80 for one boar and 49 for the second) in improved boars and 33 (17 for one boar and 16 for the second) in local boars.

The variation in the number of ejaculate between boars was due to ejaculate losses (2 ejaculates were lost in the Large White breed and 1 ejaculate in local pigs) by the breaking of the bags that contained the semen after ejaculation. The variation in the improved breed was due to the removal of one boar from the study. The number of ejaculate of local boars was lower than those of other boars because the collection had not been regular in this genetic type.

#### 2.4. Semen analysis

The collected semen was immediately sent to the laboratory for analysis. The analyses performed were macroscopic and microscopic. Macroscopic analysis included volume, color, concentration and odor. Thus, the semen volume and the weight of the gelatinous fraction (fraction from bulbo-urethral glands) were taken by using an electronic load cell of the brand OHAUS (USA) with a capacity of 2 kg and a graduated cylinder with a capacity of one liter. The color and odor were detected by sense organs. The semen concentration was taken by a photometer SDM1, model 12300/0100 (Minitube International, Germany). The microscopic analysis focused on individual motility, mobility, percentage of normal spermatozoa and percentage of abnormal spermatozoa using the following techniques.

For motility and mobility assessment, after collection, a drop of pure semen was placed on a slide and placed on the heating plate (38 °C) of the Novex B-Series microscope (Euromex, Netherlands) and observed at a 400× magnification. The measurement of motility was made according to the Bishop scores[16]. These scores were : score 0 if the spermatozoa did not move; score 1 if the spermatozoa had a very slow movement or no movement ( tremor with tail oscillations); score 2 for slow movement, tremor, unorganized movements, some spermatozoa move quickly; score 3 for curvilinear movement without tremor; score 4 for rapid displacement, some cells with a straight trajectory, others with a curved trajectory and score 5 for straight and rapid spermatozoa movement[16]. The percentage of mobile spermatozoa was estimated by the observer.

The percentages of normal and abnormal spermatozoa were determined by microscopic counts of eosine/nigrosin stained semen. A slide smear was made with a mixture of a drop of semen and eosine/nigrosin. After drying at room temperature for a few seconds, the slide was placed on the microscope stage and observed at 100×

magnification (immersion in oil) for spermatozoa counting. In total, 100 spermatozoa were counted per sample and the percentages of normal spermatozoa and different abnormalities were determined. The different anomalies searched were those recommended for the classical method used[15] which were: 1) Spermatozoa with detached head: spermatozoa without tail; 2) Spermatozoa with an abnormal head: spermatozoa with an abnormality in the head (abnormal acrosome, small or narrow head, enlarged pear-shaped head, etc.); 3) Spermatozoa with folded tail: spermatozoa with an abnormality in the flagellum; 4) Spermatozoa with proximal cytoplasmic droplet; 5) Spermatozoa with distal cytoplasmic droplet.

#### 2.5. Statistical analysis

The data were recorded in a database designed on Epidata and analyzed with SAS9.4 software (SAS Institute Inc., Cary, NC, USA[17]. The procedure of the generalized linear models (Proc GLM) was used for the analysis of variance and the Fischer test specified the significance of the genetic type effect on the studied variables. For a more appropriate assessment of the genetic type effect on sperm characteristics, a linear fixed-effect model was adjusted to the data and included the fixed effects of the genetic type and month of collection.

The means were compared by the Student's *t* test. The correlations between the semen parameters were then studied with the PROC CORR procedure of the SAS. This procedure was used to calculate the correlation between the semen parameters by genetic type. Finally, The PROC CORRESP procedure of SAS was used to visualize the correlations through the principal component analysis.

#### 2.6. Ethics statement

The experimental design was approved by the Ethics Committee of the Department of Animal Production and Health through approval number N 252/PSA/EPAC/UAC of April 12, 2018.

### 3. Results

#### 3.1. Characteristics of sperm of genetic types of boars reared in Benin

The live weights of boar at the start and end of collections of Large White boars were significantly higher than those of improved and local boars ( $P < 0.001$ ). Improved boars also had a higher weight than local boars ( $P < 0.001$ ). The sperm of boars reared in Benin was whitish in color without odor. The duration of ejaculation, semen volume, pH, weight of gelatinous fraction, proportion of gelatinous fractions, concentration of ejaculate, number of spermatozoa in the ejaculate and mobility varied significantly from one genetic type to

another (Table 1). The duration of Large White boars' ejaculation was significantly longer than that of improved boars ( $P<0.05$ ). The ejaculation time of local boars was shorter than that of improved and Large White boars ( $P<0.05$ ) (Table 1). The volume of ejaculate and semen produced by the Large White boars was significantly superior than those of improved boars ( $P<0.05$ ). These volumes of local boars' sperm were significantly lower than that of improved and Large White boars ( $P<0.05$ ). The same finding was made for the weight of the gelatinous fraction (Table 1). On the other hand, the proportion of the gelatinous fraction of local boars' ejaculate was significantly higher than that of Large White and improved boars. Local boars' semen was more concentrated in spermatozoa than that of improved and Large White boars ( $P<0.001$ ) (Table 1). The concentration of spermatozoa in Large White boars' semen was significantly higher than that in improved boars ( $P<0.05$ ). The number of spermatozoa in the ejaculate of Large White boars was higher than that of improved boars and local boars ( $P<0.001$ ) (Table 1). Improved boars' semen contained more spermatozoa than local boars' semen ( $P<0.05$ ). The percentage of mobile spermatozoa in Large White and improved boars' semen (approximately 84%) was significantly higher than that of local boars (80.8%). Individual spermatozoa motility did not vary significantly from one genetic type to another. It was 3.9 for improved boars, 3.7 for local boars and 3.8 for Large White boars. The motility score of four was the most frequently encountered for the three genetic types (85.3% in improved boars, 72.7% in Large White and Local boars). The score 2 wasn't reported in the improved and local boars. The semen pH

of the improved boars (7.5) was significantly higher than that of the Large White (7.4) and local boars (7.3) ( $P<0.05$ ).

### 3.2. Morphological characteristics of semen of the genetic types of boars reared in Benin

The majority of the counted spermatozoa showed a normal morphology. However, the percentage of normal spermatozoa counted in improved boars semen was significantly higher than that in Large White and local boars ( $P<0.001$ ) (Table 2). The abnormalities reported were abnormalities of the head (abnormal head and detached head), flagellum (folded tail) and cytoplasmic droplets (proximal and distal). Head abnormalities did not vary significantly from one genetic type to another (Table 2). In contrast, the percentage of Large White boars' spermatozoa with folded tails was significantly higher than that of improved boars and local boars ( $P<0.001$ ). The percentage of spermatozoa in local boars with proximal cytoplasmic droplets was significantly higher than that in improved and Large White boars ( $P<0.001$ ). The percentage of Large White spermatozoa with proximal cytoplasmic droplets was significantly higher than that of improved boars ( $P<0.05$ ). The percentage of local and Large White boars' sperm with distal cytoplasmic droplets was significantly higher than that of improved boars ( $P<0.05$ ). The percentage of coloured spermatozoa in semen samples of local boars was significantly superior ( $P<0.001$ ) than those of improved and Large White boars.

**Table 1.** Characteristics of semen of Large White, local and improved boars reared in Benin.

Variables	Improved boars (n=129)	Local boars (n=33)	Large White boars (n=238)
Initial live weight (kg)	125.5±0.7 <sup>a</sup>	32.5±0.7 <sup>b</sup>	188.7±10.1 <sup>c</sup>
Final live weight (kg)	137.0±3.5 <sup>a</sup>	55.0±1.4 <sup>b</sup>	202.0±4.0 <sup>c</sup>
Duration of ejaculation (mn)	4.1±1.1 <sup>a</sup>	2.1±0.4 <sup>b</sup>	5.9±1.2 <sup>c</sup>
Ejaculate volume (g)	135.7±51.5 <sup>a</sup>	27.9±11.3 <sup>b</sup>	245.6±52.4 <sup>c</sup>
Semen volume (mL)	103.5±39.6 <sup>a</sup>	16.9±8.4 <sup>b</sup>	181.2±40.0 <sup>c</sup>
Weight of gelatinous fraction (g)	32.1±11.9 <sup>a</sup>	7.8±3.8 <sup>b</sup>	64.4±21.7 <sup>c</sup>
Proportion of gelatinous fraction (%)	23.9±10.6 <sup>a</sup>	37.9±6.7 <sup>b</sup>	26.4±6.2 <sup>c</sup>
Concentration (10 <sup>6</sup> spermatozoa/mL)	279.5±107.6 <sup>a</sup>	407.7±147.8 <sup>b</sup>	376.7±122.7 <sup>c</sup>
Number of spermatozoa in the ejaculate (10 <sup>9</sup> )	28.0±12.9 <sup>a</sup>	6.2±3.6 <sup>b</sup>	66.9±26.4 <sup>c</sup>
Mobility (%)	84.4±5.5 <sup>a</sup>	80.8±6.3 <sup>b</sup>	83.7±0.4 <sup>a</sup>
Individual motility	3.9±0.4 <sup>a</sup>	3.7±0.5 <sup>a</sup>	3.8±0.5 <sup>a</sup>
pH	7.5±0.2 <sup>a</sup>	7.3±0.1 <sup>b</sup>	7.4±0.2 <sup>c</sup>

n: number of ejaculates. The different superscripts (a, b, c) in the same row differ significantly at the threshold of 5%.

**Table 2.** Morphological characteristics of sperm of genetic types of boars reared in Benin (%).

Variables	Improved boars (n=129)	Local boars (n=33)	Large White boars (n=238)
Normal	93.6±5.6 <sup>a</sup>	82.2±6.6 <sup>b</sup>	81.6±12.3 <sup>b</sup>
Abnormal head	1.2±2.5 <sup>a</sup>	1.3±1.1 <sup>a</sup>	0.9±1.5 <sup>a</sup>
Detached head	0.3±0.9 <sup>a</sup>	0.3±0.5 <sup>a</sup>	0.4±1.5 <sup>a</sup>
Folded tail	1.8±2.3 <sup>a</sup>	5.0±2.5 <sup>a</sup>	9.2±4.5 <sup>b</sup>
Proximal cytoplasmic droplet	2.7±2.1 <sup>a</sup>	9.7±5.3 <sup>b</sup>	6.8±2.4 <sup>c</sup>
Distal cytoplasmic droplet	0.4±0.7 <sup>a</sup>	1.5±1.2 <sup>b</sup>	1.1±1.5 <sup>b</sup>
Coloured	2.0±1.6 <sup>a</sup>	4.3±2.7 <sup>b</sup>	2.4±1.5 <sup>a</sup>

n: number of ejaculates; The different superscripts (a, b, c) in the same row differ significantly at the threshold of 5%.

### 3.3. Correlations between the semen parameters of boars reared in Benin

Mobility and motility were negatively but not significantly correlated with semen volume in improved boars (Table 3). The same finding was made for the correlation between these parameters and the weight of gelatinous fraction but the correlation with motility was significant. For Large White boars, these correlations were also negative, but significant. Thus, in this breed, the correlation between semen volume and mobility was  $-0.26$  ( $P<0.001$ ) and the correlation between semen volume and motility was  $-0.14$  ( $P=0.03$ ) (Table 4). As opposed to improved and Large White boars, mobility and motility were positively but not significantly ( $P>0.05$ ) correlated with semen volume ( $r=0.15$ ) and the gelatinous fraction weight ( $r=0.06$  for mobility and  $r=0.03$  for motility) of the local boars' semen (Table 5).

The duration of improved boars ejaculation was highly and significantly correlated ( $0.6\leq r\leq 0.9$ ;  $P<0.001$ ) with semen volume and gelatinous fraction weight. The same finding was obtained for Large White boars ( $r<0.3$ ;  $P<0.001$ ) (Table 4). As for Large White and improved boars, the correlation between ejaculation duration and semen volume was significant in local boars ( $r=0.46$ ;  $P<0.01$ ). However, the correlation between ejaculation duration and gelatinous fraction weight was not significant in local boars ( $r=0.13$ ;  $P=0.49$ ) in contrast to those of the two other genetic types (Table 5).

Semen volume of all three genetic types was negatively and significantly correlated with concentration except in local boars where this correlation was not significant (Tables 3, 4 and 5). Correlations between the concentration and weight of the gelatinous fraction of the local boars ( $r=0.24$ ) and Large White ( $r=0.12$ ) were not significant. In contrast, in improved boars, this

correlation was significant ( $r=-0.19$ ;  $P<0.05$ ).

The number of spermatozoa in semen was positively correlated with semen volume and concentration for each genetic type ( $r=0.60$  and  $P<0.001$  for improved boars;  $r=0.58$  and  $P<0.001$  for Large White boars;  $r=0.43$  and  $P<0.05$  for local boars) (Tables 3, 4 and 5).

The correlations between the concentration and mobility of spermatozoa of the three genetic types were significant. This correlation was  $0.42$  ( $P<0.001$ ) for Large White boars;  $0.33$  ( $P<0.001$ ) for improved boars and  $0.45$  ( $P<0.001$ ) for local boars. The correlation between concentration and motility of spermatozoa of the three genetic types were also significant. Similarly, semen mobility was significantly correlated with motility in each of the three genetic types (Tables 3, 4 and 5).

Two axes were chosen for the interpretation of the results of the principal component analysis (Figure 1). The contribution to the total inertia of the two axes was 100% (75.4% on the first axis and 24.6% on the second axis). The variables ejaculation duration, semen volume, gelatinous fraction weight, gelatinous fraction proportion, total ejaculate weight, mobility and local breed were very well represented in the first axis. The variables pH, concentration, motility, number of spermatozoa in the ejaculate and the individuals of improved and Large White boar genetic types contributed to the construction of the second axis. The local breed was characterized by the concentration of spermatozoa in the semen and the proportion of gelatinous fraction of the ejaculate while the improved boars were characterized by the pH of the semen, mobility and motility of the spermatozoa (Figure 1). Large White boars were characterized by higher semen volume, long ejaculation duration, higher gelatinous fraction weight, higher ejaculate weight and number of spermatozoa in the semen (Figure 1).

**Table 3.** Correlation between the semen parameters of the improved boars.

Parameters	Duration of ejaculation	Semen volume	Proportion of gelatinous fraction	Weight of gelatinous fraction	Concentration	Number of spermatozoa in the ejaculate	Mobility	Motility
Duration of ejaculation	1	0.61***	-0.03 <sup>NS</sup>	0.58***	-0.08 <sup>NS</sup>	0.40***	-0.09 <sup>NS</sup>	0.08 <sup>NS</sup>
Semen volume		1	-0.25**	0.90***	-0.18*	0.64***	-0.16 <sup>NS</sup>	-0.04 <sup>NS</sup>
Proportion of gelatinous fraction			1	-0.16 <sup>NS</sup>	-0.07 <sup>NS</sup>	-0.21*	-0.13 <sup>NS</sup>	-0.07 <sup>NS</sup>
Weight of the gelatinous fraction				1	-0.19*	0.57***	-0.19*	-0.07 <sup>NS</sup>
Concentration					1	0.60***	0.42***	0.18*
Number of spermatozoa in the ejaculate						1	0.23*	0.10 <sup>NS</sup>
Mobility							1	0.43***
Motility								1

NS: Not significant; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ .

**Table 4.** Correlation between the semen parameters of the Large White boars.

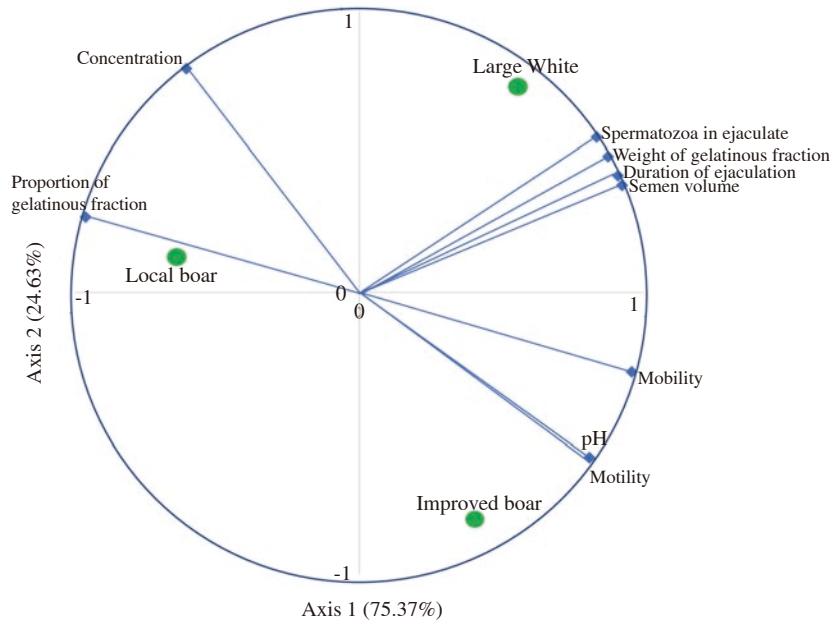
Parameters	Duration of ejaculation	Semen volume	Proportion of gelatinous fraction	Weight of gelatinous fraction	Concentration	Number of spermatozoa in the ejaculate	Mobility	Motility
Duration of ejaculation	1	0.27***	0.08 <sup>NS</sup>	0.28***	-0.10 <sup>NS</sup>	0.18**	-0.21**	-0.15*
Semen volume		1	-0.30***	0.56***	-0.15*	0.68***	-0.26***	-0.14*
Proportion of gelatinous fraction			1	0.56***	0.30***	-0.01 <sup>NS</sup>	-0.03 <sup>NS</sup>	-0.08 <sup>NS</sup>
Weight of the gelatinous fraction				1	0.12 <sup>NS</sup>	0.56***	-0.28***	-0.25***
Concentration					1	0.58***	0.33***	0.22***
Number of spermatozoa in the ejaculate						1	-0.02 <sup>NS</sup>	-0.02 <sup>NS</sup>
Mobility							1	0.74***
Motility								1

NS: Not significant; \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ .

**Table 5.** Correlation between semen parameters of the local boars.

Parameters	Duration of ejaculation	Semen volume	Proportion of gelatinous fraction	Weight of gelatinous fraction	Concentration	Number of spermatozoa in the ejaculate	Mobility	Motility
Duration of ejaculation	1	0.46**	-0.43*	0.13 <sup>NS</sup>	0.17 <sup>NS</sup>	0.44*	0.51**	0.40*
Semen volume		1	-0.73***	0.11 <sup>NS</sup>	-0.06 <sup>NS</sup>	0.84***	0.15 <sup>NS</sup>	0.15 <sup>NS</sup>
Proportion of gelatinous fraction			1	0.51**	0.22 <sup>NS</sup>	-0.53**	-0.01 <sup>NS</sup>	-0.04 <sup>NS</sup>
Weight of the gelatinous fraction				1	0.24 <sup>NS</sup>	0.27 <sup>NS</sup>	0.06 <sup>NS</sup>	0.03 <sup>NS</sup>
Concentration					1	0.43*	0.45**	0.39*
Number of spermatozoa in the ejaculate						1	0.38*	0.32 <sup>NS</sup>
Mobility							1	0.75***
Motility								1

NS: Not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 1.** Presentation of variables by genetic type on the two principal axes of the principal component analysis.

### 4. Discussion

The quantity of semen produced varied from one genetic type to another and the Large White boars produced the highest and the local boars the lowest. This genetic effect on semen volume of boars was reported between Large White boars, local boars, and crossbreeds (Large White × local) in Nigeria[18] and European breeds (Large White, Landrace, Duroc, Hampshire and Pietrain)[19,20]. Apart from the genetic effect, the semen quantity produced by the boar was proportional to its weight[21,22] and this weight effect may also justify the difference between the volumes of the three genetic types because the Large White boars were heavier than improved boars which in turn were heavier than the local boars.

This effect is mainly expressed in the testis weight, because the semen production of the boars is strongly correlated to the testis weight[23,24]. The selection of boars with large testis size for production showed a 10% increase over normal production[24]. The duration of ejaculation was longer in the Large White boar because this boar produces more semen than improved boar and local boar, explaining the observed strong correlation between semen volume and the duration of ejaculation in improved boar. In Large White and local boars, the correlations were also positive and showed that there

is a relationship between volume and collection time even if these correlations were low. This effect of the duration of ejaculation on volume has been demonstrated by Oberlender *et al*[25] in the Pietrain boars in Brazil.

The semen concentration has varied from one genetic type to another and semen of local boars was more concentrated. This finding is due to the fact that the local boars produced a less voluminous semen. However, the more voluminous the semen is, the less concentrated it is[21,22,26]. This low volume of local boars' semen is also related to the short duration of ejaculation, which means that the rich fraction dominates production. In fact, the ejaculate of a boar contains three fractions: the pre-sperm, the rich fraction in spermatozoa and the poor fraction[13,14,27]. The poor fraction representing the major part of the semen (40% to 60%) is secreted at the end of ejaculation and is clear and fluid[14,27]. The rich fraction contains 80% to 90% of the spermatozoa of semen[14,28]. The effect of genetic type on concentration has also been reported by Schulze *et al*[20] in European boars (Large White, Landrace, Piétrain, Duroc and crossbreeds). The number of sperm in the Large White boar ejaculate was higher than that of improved and local boars because the semen quantity produced by the Large White boars was higher than that of local and improved boars. The effect of volume on the

number of spermatozoa in semen has been reported in Pietrain and Duroc boars in Poland[26,29]. Motility (approximately 4 scores) and mobility (81%-84%) reported in these three genetic types are within the standards of at least 70% for mobility and 3 scores for motility[27]. The spermatozoa of the improved and Large White boars were more mobile than those of the local boars, a finding that is linked to the difficulties encountered in collecting semen in local boars, which would lead to maturation anomalies and the death of some spermatozoa.

The proportion of normal spermatozoa in improved boars' semen is higher than that of Large White boars' semen. This difference could be explained by the influence of temperature on spermatozoa maturation. Indeed, improved boars have been reared for a long time by breeders while the Large White boars used have been introduced recently and are therefore less acclimatized[8]. Temperature also affects spermatogenesis[24] and may be responsible for the high anomaly rate recorded in Large White boars. The nature of the anomalies observed makes it possible to rule out this hypothesis, because the anomalies generated in the case of a spermatogenesis problem are mainly head anomalies[27] which were less observed and did not vary between genetic type. On the other hand, the anomalies observed in the case of insufficient maturation of the spermatozoa (cytoplasmic droplets and the folded tail)[27,28] were more recorded. The influence of temperature on the anomaly rate was demonstrated in Large White boars imported into Nigeria with an anomaly rate greater than 33% in boars exposed to the sun[30,31]. This influence of temperature on spermatogenesis can be corrected by scrotal isolation[32]. However, local boars had a higher rate of cytoplasmic droplets than Large White boars. In contrast to Large White boars, semen collection has encountered some difficulties (refusal sometimes of the mounting of the model by boars) that sometimes make it irregular and these irregularities justify the increase in the rate of cytoplasmic droplets in the local boars' ejaculate, as cytoplasmic droplets should be absent in the semen and their presence indicates a lack of maturity in relation to a condition, young age of the boar, stress and insufficient collection[27]. This effect of insufficient collection on semen quality has been reported in the literature but deserves to be further investigated by scientific work. The insufficient semen collection and the stress caused by the technique better explain their presence in our context. More frequent collection can generate the same anomalies in addition to head anomalies and for good semen quality it is recommended, 2 collections per week[28]. For this reason, it is necessary to improve the collection technique in local breeds in order to improve the quality of semen. Concerning insufficient collection, it has been shown that boar spermatozoa loses cytoplasmic droplets during ejaculation and the number of spermatozoa with these droplets increases with collection frequency[33,34]

This study made it possible to have a repertory of semen parameters of boars reared in Benin such as concentration, mobility, motility, weight, color, pH and morphology. Unfortunately, it didn't allow us to determine the parameters of the spermatozoa travel and the integrity of the acrosome which can only be determined by CASA

or flux cytometry[35]. The CASA system permits an automatic, repetitive and accurate analysis of a semen sample according to the following parameters: motility, concentration, morphology, levels of DNA fragmentation, vitality, acrosome and leukocyte reactions. The high cost of the CASA system and its absence in our country explains the non-use of this technique. However, the parameters determined are sufficient to assess the quality of the semen and to realize the doses for artificial insemination.

In conclusion, the study on the semen characteristics of boars reared in Benin showed that Large White boar produces more semen than local and improved boars. The semen of the local boar is more concentrated in spermatozoa than that of the improved and Large White boars. The spermatozoa present in the semen of the boars in the study are normal. In summary, the semen of pigs reared in Benin is of good quality and could be used for artificial insemination and the improved boar semen is more recommended.

### Conflict of interest statement

The authors declare that there is no conflict of interest.

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### Authors' contributions

Ignace O. Dotché, Issaka Youssao Abdou, Aïchatou Gakou and Pierre Thilmant conceived the study design. Ignace O. Dotché, Constant Boris O.B. Bankolé, and Aïchatou Gakou collected the data (semen collection and analysis). Isidore Houaga, Benoît G. Koutinhouin, Constant Boris O.B. Bankolé and Issaka Youssao Abdou analyzed the data. Ignace O. Dotché, Aïchatou Gakou and Mahamadou Dahouda wrote the manuscript. Nicolas Antoine-Moussiaux, Jean Paul Dehoux, Pierre Thilmant and Issaka Youssao Abdou corrected the manuscript. All authors read and approved the final manuscript.

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