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Parameters in High Performance Boars**

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Influence of Ejaculation Time on Sperm Quality Parameters in High Performance Boars

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Abstract

The aim of this study was to evaluate the influence of ejaculation time on sperm quality parameters in high performance boars. 447 fresh semen samples were used from 9 boars. The samples were collected by the gloved hand method and the duration of ejaculation in seconds was determined. The ejaculates obtained were divided into three groups based on the ejaculation time and into two categories based on the age of the animals. The sperm volume (using graduated bottles), total number of sperm using a Neubauer chamber, motility and intensity of motion using phase contrast microscopy and spermatid morphology using wet preparation with formaldehyde-sodium citrate solution were all evaluated. All variables were compared regarding the ejaculation time and animal age, and the correlation between these variables and the ejaculation time was also evaluated. Animals with higher ejaculation times were found to have a higher semen volume ($P<0.05$). A positive correlation (0.56; $P<0.001$) was observed between ejaculation time and the seminal volume, but correlations were low ($P>0.05$) with regards to other variables. Animals younger than 15 months old had lower semen volumes and higher sperm counts, as well as altered spermatid morphology ($P<0.05$) compared with older animals. The ejaculation time was found to be associated with the seminal volume, with little effect on the other variables. In conclusion, an adequate seminal analysis should always be performed to evaluate the fresh boar ejaculate, especially in possible semen donor animals.

Key words: Boar, libido, reproduction, spermatid alterations

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Introduction

The monitoring and analysis of the semen quality and quantity produced by boar is of great economic importance to swine producers, becoming a critical issue for the success of artificial insemination (AI) programs (Vyt *et al.*, 2008; Smital, 2009; Tsakmakidis *et al.*, 2010; Broekhuijse *et al.*, 2011; López Rodríguez *et al.*, 2011). An ejaculate with a large number of high quality sperm is expected from each semen collection. However, this optimal situation does not always occur (Spessatto *et al.*, 2007).

The boar semen can be collected in three parts, a pre-sperm fraction, where sperm and seminal vesicle secretions are absent, a sperm-rich fraction containing approximately 70-80% of the total sperm, and the post-sperm fraction, which consists mainly of accessory gland secretions (Glover & Mann, 1954; Murgas & Zangeronimo, 2004). Despite the fact that the sperm-rich fraction contains the greatest number of sperm, the collection of the post-sperm fraction does not significantly affect the ejaculate quality. According to the same authors, the characteristics of ejaculate/fresh semen are the initial step in quality control of the AI process, irrespective of the portion collected. Ultimately, these are the characteristics that define the suitability of ejaculates for the production of semen doses of a required quality (Oberlender *et al.*, 2012).

The impact of the boar on the reproductive performance of the herd is high, especially if the male serves many females (Smital, 2009). In turn, the economic gain from an AI Center primarily depends on the ability of the boar to produce viable sperm during his lifetime (Robinson & Buhr, 2005), and this production is limited by the testis capacity, libido and physical integrity, including hooves, aplombs and column (Murgas & Zangeronimo, 2004; Spessatto *et al.*, 2007). Several studies have indicated that the reproductive performance and the sperm quality of a boar depends on the following factors: heritability, testicular size, nutrition, age, breed, sexual exploration intensity, collection method, handling of semen post-collection, temperature, photoperiod, social environment, sexual behavior

and systemic diseases (Marchev *et al.*, 2003; Chenoweth, 2005; Smital, 2009).

With respect to sexual behavior, the duration of time between entering the collection pen and mounting the dummy, the number of mounts before ejaculation started, and the duration of time ejaculating are some of the variables evaluated with respect to their effects on sperm characteristics (Cameron, 1985; Levis & Reicks, 2005). On the other hand, despite the numerous possibilities of evaluation methods, few groups have studied the influence of sexual behavior on sperm quality parameters in boars (Alonso *et al.*, 2011). According to Levis and Reicks (2005), despite the studies already performed, the current knowledge of the influence of sexual behavior on the reproductive performance of boars is far less than the knowledge regarding the physiology of sperm production.

The ejaculation time is an example of a sexual behavior parameter that is able to demonstrate the libido of the boars (Poto *et al.*, 2000). Males that have high ejaculation times can be considered to have good libido, but not necessarily a high quality of semen (Cameron, 1985). The longer the duration of semen collection, the more time is spent on all subsequent processes of analysis and manipulation of the ejaculate. So, in this case, there is a greater chance of sperm undergoing external damage, as a result of environmental temperature, bacterial contamination by the environment or the animal itself, or other injuries, thus leading to possible damage of the sperm quality (Murgas & Zangeronimo, 2004). It is essential that the sperm quality is evaluated in order to verify whether a longer ejaculation time exerts any influence on the sperm characteristics.

Therefore, this study aims to evaluate the influence and relationship of the ejaculation time on parameters of semen quality of high performance boars, including volume, sperm count, motility, intensity of motion and morphological alterations.

Materials and Methods

Place and Animals

This study was performed at the Experimental Center of Porcine of the Department of Veterinary

Medicine and Animal Science of the Federal University of Lavras, Minas Gerais, Brazil. A total of 447 samples of fresh semen were analyzed from 9 high performance Pietran breed boars, aged between seven and 25 months. The collection protocol was started from seven months of age when males weighed approximately 115.0kg. The animals were housed in individual pens located in a shed with a concrete floor and a tiled roof made of asbestos cement. They were fed with 2.5 to 3.5kg of feed per day and given water *ad libitum* through manual drinking fountains like nipples.

Semen Sampling

The semen samples were collected by one trained and experienced person using the gloved hand method, with the help of a fixed dummy. The ejaculates were collected in either a graduated container, with a capacity of 500 mL, which was pre-heated to 37°C and protected by and isothermal container (thermal cup collection) (Hancock & Howell, 1959). The ejaculation time was determined from the time when the animal jumped on the dummy and the collection of semen began until the completion of the process, which corresponded to the end of the ejaculation with posterior penile retraction. The samples were collected weekly, and the time of its duration was recorded in seconds.

Quality of the Ejaculates

The ejaculates were collected in their entirety and evaluated for the routine characteristics of seminal quality, as detailed below. Samples were evaluated macroscopically with regards to volume using the graduated container, smell, by looking for the presence of urine, blood or other contaminations and appearance (watery, milky or milky serum). The motility and intensity of motion, spermatic count and morphology were evaluated microscopically.

To assess motility, three subsamples of each semen sample were placed on warm glass slides (37°C). The slides were examined under a phase contrast microscope at a magnification of 400 times. A total of 10 microscopic fields were assessed to determine the percentage of spermatozoa with rapid progressive motility and

the intensity of motion. Spermatic motility was expressed as a progressive motility percentage and the intensity was classified on a 0–5 scale, with 5 being the maximum intensity of motion. The spermatic count was evaluated using a Neubauer chamber and the total number of sperm in the entire ejaculate was determined ($\times 10^9$ sperm).

For the evaluation of sperm morphology the conventional wet preparation technique was used, which utilizes a 2.95% formaldehyde-citrate solution. In this evaluation, about five drops of fresh semen from each ejaculate were added to 1.0mL of citrate-formaldehyde solution in a microtube. Next, approximately 10 μ L of this solution was placed between the slide and the coverslip, and observed using phase contrast microscopy at 1,000 times magnification. The differential count of 100 spermatic cells was evaluated, and the percentages of normal sperm and those with abnormalities were calculated (Scheid 1993).

Evaluated Groups

The ejaculates collected were first evaluated as a whole, including all samples from all animals. Afterwards, based on the study of Alonso *et al.* (2011) the ejaculates were divided into three groups, according to the duration of the semen collections: Group I (G1) = ejaculation time less than 360 seconds, Group II (G2) = ejaculation time between 360 and 420 seconds, and Group III (G3) = time above 420 seconds. The ejaculates were also grouped into two categories based on the age of animals in months: Category I (C1) = animals under 15 months old (young sexually) and Category II (C2) = animals over 15 months old (adults sexually).

Statistical Analysis

All data obtained were represented as means and standard deviations. A randomized block design was used on the experimental boars in this study to compare the data. Two different treatment sets were used. The first involved three different treatments: time of ejaculation – Groups I, II and III, and the second included two treatments: age of the animals – Categories I and II.

For data analysis in these two treatment sets, all variables, such as age, seminal volume, sperm count, sperm motility, sperm intensity of motion and sperm morphology, were submitted to a normality analysis using the Kolmogorov-Smirnov test. The data was not found to be normally distributed ($P < 0.05$), even after data transformation using the square root option. The treatments in each set were compared using the Mann-Whitney test, when there was significance using the Kruskal-Wallis test ($P < 0.05$). The Spearman's correlation test among was performed for all sperm quality variables compared to the ejaculation times. All statistical analyses were performed using the statistical package *SPSS for Windows* version 17.0 (SPSS Statistics 17.0, Rel. 17.0.1. 2008, SPSS Inc, Chicago, IL.).

Results and Discussion

The data obtained (mean \pm standard deviation – SD) on the quality parameters of fresh semen for each boar as well as the overall mean of all ejaculates evaluated are shown in Table 1. For all of the analyzed variables, the mean values obtained showed considerable variation between animals.

The classification of the ejaculates from 9 animals is displayed in Table 2. The data are subdivided according to the ejaculation time and the total numbers of ejaculates per group and per boar are also shown. It was observed that in the total number of ejaculates evaluated, the majority of these (60.18%) were classified as G1. G2 accounted for only 15.21% of total ejaculates evaluated, and G3 comprised 24.61% of the ejaculates.

The variables of volume, motility and intensity of motion were different ($P < 0.05$) for the three evaluated groups. It was observed that the ejaculates from the G3 presented a higher semen volume compared with the other groups, but with respect to the motility and intensity of motion, Group III presented the lowest values compare with the Group I. For the other variables of sperm count and morphology there were no differences ($P > 0.05$) between the groups (Table 3).

With regards to age, the boars classified in Category I (C1) had a mean age of 11.92 ± 2.42 months, and C2 had an age of 20.49 ± 2.21 months.

It was also observed that the average seminal volumes and sperm motility for animals over 15 months of age (C2) was significantly higher ($P < 0.05$) than for animals below this age (C1). On the other hand, the animals under 15 months of age presented superior values for sperm count and morphology variables ($P < 0.05$) when compared with animals above this age. There was no difference ($P > 0.05$) in the sperm intensity of motion between the evaluated categories (Table 4).

A positive correlation was obtained between the duration of ejaculation and age ($r = 0.23$; $P < 0.01$), seminal volume ($r = 0.56$; $P < 0.01$) and sperm count ($r = 0.121$; $P = 0.01$). With regards to sperm motility and intensity of motion, the correlation with the ejaculate time was negative ($r = -0.163$; $P = 0.001$) and ($r = -0.152$; $P = 0.001$), respectively. Conversely, the sperm morphology was not significantly correlated with ejaculation time ($r = 0.090$; $P = 0.057$) (Table 5). For the variables of sperm count, motility and intensity of motion, the correlation with ejaculation time was low.

In this study, all of the mean values obtained for each analyzed variable in the 9 animals were within the standards recommended for swine (Fonseca *et al.*, 1992; Corrêa *et al.*, 2001). The range of data obtained between the animals is a common feature found in the semen of boars and a characteristic also demonstrated in other studies (Thiengtham, 1992). According to Petrunkina *et al.* (2005) there are higher spermatic variations between ejaculates of the same animal, which implies that a greater number of ejaculates should always be used for a reliable evaluation of the data. Additionally, the fertility and sperm quality of the same boar can change over time (Bernardi, 2008).

The higher seminal volume ejaculated by animals of G3 in comparison with the other groups can be explained by the fact that these animals spend more time on the dummy, and, consequently, have a higher probability of ejaculating a greater volume of semen.

As well as for the ejaculation time, the fact that animals of C1 (age less than 15 months) present a lower ejaculate volume is due to the fact that these animals are still in the pre-pubertal phase and do not yet have a defined or relatively constant ejaculate volume. Therefore, this fact is observed

through the occurrence of longer ejaculations with a small semen volume, or the complete opposite. Conversely, C2 animals have already reached complete sexual maturity and semen volumes have evolved in comparison with the animals that still

were in the pre-pubertal phase. These results are consistent with those seen by Jankeviciute and Zilinskas (2002), who reported an increase in the seminal volume ejaculate with the advancing of age.

Table 1: Data (mean ± SD) of fresh semen quality parameters for each boar evaluated and the overall mean, standard deviation, minimum and maximum value and variation coefficient obtained of the all animals^a

| Boar | N ^b | Duration of ejaculation (seconds) | Seminal volume (mL) | Sperm count (x10 ⁹ sperm) | Sperm motility (%) | Sperm intensity of motion (1-5) | Sperm morphology (%) |
|-----------|----------------|-----------------------------------|---------------------|--------------------------------------|--------------------|---------------------------------|----------------------|
| 1 | 62 | 519.61 ± 298.98 | 305.40 ± 98.01 | 95.14 ± 59.91 | 77.98 ± 15.77 | 2.97 ± 0.77 | 21.42 ± 14.94 |
| 2 | 58 | 309.95 ± 81.03 | 198.62 ± 66.54 | 76.65 ± 58.94 | 83.19 ± 12.76 | 3.33 ± 0.71 | 11.91 ± 8.03 |
| 3 | 50 | 353.20 ± 151.46 | 269.50 ± 68.26 | 93.52 ± 64.85 | 77.90 ± 17.00 | 3.04 ± 0.78 | 20.08 ± 15.34 |
| 4 | 50 | 304.46 ± 87.37 | 244.30 ± 65.35 | 103.22 ± 61.05 | 84.40 ± 7.19 | 3.36 ± 0.56 | 8.54 ± 6.45 |
| 5 | 48 | 313.21 ± 62.35 | 200.42 ± 47.99 | 72.44 ± 37.75 | 84.79 ± 6.10 | 3.21 ± 0.71 | 25.67 ± 13.03 |
| 6 | 42 | 360.36 ± 91.00 | 247.14 ± 61.44 | 71.04 ± 60.30 | 81.90 ± 16.53 | 3.24 ± 0.76 | 18.12 ± 9.15 |
| 7 | 44 | 502.05 ± 284.44 | 286.14 ± 93.39 | 97.87 ± 67.10 | 79.77 ± 14.14 | 3.05 ± 0.68 | 22.11 ± 16.29 |
| 8 | 43 | 391.60 ± 149.59 | 240.81 ± 74.50 | 88.93 ± 70.79 | 78.26 ± 14.26 | 3.09 ± 0.68 | 22.77 ± 15.56 |
| 9 | 50 | 337.18 ± 102.62 | 264.70 ± 79.10 | 98.11 ± 65.09 | 84.60 ± 13.40 | 3.42 ± 0.73 | 10.50 ± 7.29 |
| Mean ± SD | | 378.15 ± 186.89 | 251.29 ± 82.11 | 88.77 ± 61.56 | 81.41 ± 13.69 | 3.19 ± 0.72 | 17.72 ± 13.51 |
| Minimum | | 126 | 40 | 9.75 | 10 | 0 | 1 |
| Maximum | | 1860 | 500 | 437.90 | 95 | 4 | 60 |
| VC (%) | | 49.42 | 32.68 | 69.35 | 16.82 | 22.57 | 76.24 |

^aValues represent the means for n = 447 ejaculates.

^bNumber of ejaculates per boar.

SD: Standard deviation

VC: Variation coefficient

Table 2: Classification of the ejaculates of 9 boars subdivided into three groups according to the total time duration of the ejaculation^a

| Boar | Classification of Groups ^b | | | Total ^c |
|--------------|---------------------------------------|---------------|----------------|--------------------|
| | Group I (G1) | Group II (G2) | Group III (G3) | |
| 1 | 23 | 10 | 29 | 62 |
| 2 | 43 | 9 | 6 | 58 |
| 3 | 29 | 9 | 12 | 50 |
| 4 | 41 | 4 | 5 | 50 |
| 5 | 39 | 7 | 2 | 48 |
| 6 | 25 | 6 | 11 | 42 |
| 7 | 16 | 7 | 21 | 44 |
| 8 | 23 | 6 | 14 | 43 |
| 9 | 30 | 10 | 10 | 50 |
| Total Groups | 269 | 68 | 110 | 447 |

^aValues represent the means for n = 447 ejaculates.

^bGroup I (G1) = ejaculation time less than 360 seconds; Group II (G2) = ejaculation time between 360 and 420 seconds; Group III (G3) = ejaculation time over 420 seconds.

^cTotal number of ejaculates per boar.

In this study, the seminal volume average obtained in across 447 samples from 9 animals was in the physiological range, with an average that was lower than those found by other groups.

Henao-Restrepo *et al.* (2004) worked with 244 ejaculates from 10 boars aged between one and two years and Alonso *et al.* (2011) worked with animals between 15 and 18 months old. However,

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the seminal volume obtained was greater than the values found by [Poto *et al.* \(2000\)](#) working with

five Chato Murciano boars aged between 10 months and 12 years.

Table 3: Data (mean \pm SD) for the sperm quality variables of the ejaculates collected from 9 boars divided into three groups according to ejaculation time^a

| Sperm quality parameters | Classification of Groups ^b | | | P value |
|--------------------------------------|---------------------------------------|----------------------|----------------------|--------------------|
| | Group I (G1) | Group II (G2) | Group III (G3) | |
| Semen volume (mL) | 217.81 \pm 67.38 c | 268.16 \pm 51.16 b | 322.73 \pm 81.98 a | <0.01 ^c |
| Sperm count (x10 ⁹ sperm) | 91.38 \pm 67.03 | 85.65 \pm 73.18 | 84.31 \pm 33.52 | 0.167 ^d |
| Sperm motility (%) | 82.57 \pm 12.95 a | 82.35 \pm 6.94 ab | 78.00 \pm 17.60 b | 0.003 ^c |
| Sperm intensity of motion (1-5) | 3.26 \pm 0.72 a | 3.21 \pm 0.51 ab | 2.99 \pm 0.82 b | 0.001 ^c |
| Sperm morphology (%) | 17.44 \pm 13.96 | 19.44 \pm 13.09 | 17.33 \pm 12.64 | 0.295 ^d |

^aValues represent the means for n = 447 ejaculates.

^bGroup I (G1) = ejaculation time less than 360 seconds; Group II (G2) = ejaculation time between 360 and 420 seconds; Group III (G3) = ejaculation time over 420 seconds.

^cMeans followed by different letters in the line differ by Mann-Whitney test (P<0.05).

^dNot significant with the Kruskal-Wallis test (P>0.05).

Table 4: Data (mean \pm SD) for the sperm quality parameters of the ejaculates collected from 9 boars divided into two categories according to age^a

| Sperm quality Parameters | Classification of Category ^b | | Mean | P value |
|--------------------------------------|---|----------------------|--------------------|--------------------|
| | Category I (C1) | Category II (C2) | | |
| Semen volume (mL) | 210.66 \pm 57.95 b | 275.52 \pm 84.87 a | 251.29 \pm 82.11 | <0.01 ^c |
| Sperm count (x10 ⁹ sperm) | 110.19 \pm 82.38 a | 75.99 \pm 39.76 b | 88.77 \pm 61.56 | <0.01 ^c |
| Sperm motility (%) | 79.82 \pm 15.41 b | 82.36 \pm 12.49 a | 81.41 \pm 13.69 | 0.001 ^c |
| Sperm intensity of motion (1-5) | 3.19 \pm 0.72 | 3.19 \pm 0.73 | 3.19 \pm 0.72 | 0.956 ^d |
| Sperm morphology (%) | 21.04 \pm 16.36 a | 15.74 \pm 11.04 b | 17.72 \pm 13.51 | 0.001 ^c |

^aValues represent the means for n = 447 ejaculates.

^bCategory I (C1) = animals under 15 months old and Category II (C2) = animals above 15 months old.

^cMeans followed by different letters in the line differ by Mann-Whitney test (P<0.05).

^dNot significant for the Kruskal-Wallis test (P>0.05).

Table 5: Spearman's rank correlation coefficients (r) between the duration of ejaculation and sperm quality parameters of the 9 high performance boars^a

| Variable compared | Age | Seminal volume | Sperm count | Sperm motility | Sperm intensity of motion | Sperm morphology |
|-------------------------|---------------------------------|---------------------------------|----------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Duration of ejaculation | r = 0.23 ^b P<0.01 | r = 0.56 ^b P<0.01 | r = 0.121 ^c P=0.01 | r = -0.163 ^b P=0.001 | r = -0.152 ^b P=0.001 | r = 0.090 ^{NS} P=0.057 |

^aValues represent the means for n = 447 ejaculates.

^bSpearman's correlation is significant to the level P=0.01.

^cSpearman's correlation is significant to the level P=0.05.

NS: Non-significant

According to [Corrêa *et al.* \(2001\)](#), the normal volume of ejaculate in boars can vary from 50 to 500 mL, with an average volume close to 200mL. Therefore, the results from this study are in the physiological range of swine species, for animals of G1, G2 and G3, as well as those from C1 and C2. The volume of the ejaculate is characteristic of each species and also of each breed within the same species ([Jasko, 1992](#); [Colenbrander *et al.*, 1993](#)). The volume appears to

have no association with fertility, and affects only the total number of sperm and doses produced per ejaculate ([Tardif *et al.*, 1999](#)). Although there are differences between breeds, in this study, the animals were of the same breed, meaning that the differences in the seminal volume between the animals cannot be evaluated for this variable.

The seminal volume presented a high variation among all evaluated ejaculates as well as among ejaculates from the same animal, making these

variations similar to those observed in other studies (Singleton & Shelby, 1972; Wettermann *et al.*, 1976; Mazzarri & Fuentes, 1978; Trudeau & Sanford, 1986; Henao-Restrepo *et al.*, 2004). The variation in semen volume is mainly due to individual variation in the size of accessory glands, and is also related to the amount of sexual stimulation prior to the collection process (Cooper, 1980). According to Alonso *et al.* (2004), the marked variation in semen volume can be attributed to factors inherent to the animal or the environment, such as nutrition, genetics, breed and management. The seasons also have significant effects on ejaculate volume, since the production of semen in the winter is comparatively higher than in the summer in general (Corrêa *et al.*, 2001). In this study, the volume variability between boars can be explained by the individual variation that exists among animals, by the stimulation time before the collection process that was different in each collection for each animal, and also due to the varying ejaculation time between the animals, in addition to the factors already discussed above.

The correlation analysis between ejaculation time, which is an independent variable, and the seminal volume, a dependent variable, showed that when there is an increase in the ejaculation time, the semen volume also tends to increase ($r = 0.56$). This correlation can be considered moderate for this variable, and these data are similar to those obtained by Kuciel *et al.* (1983) cited by Levis and Reicks (2005), who reported a significant correlation between the duration of ejaculation with volume of ejaculation ($r = 0.58$). On the other hand, these data differ from those of Alonso *et al.* (2011) who found an r value of 0.822, which was higher than the coefficients obtained in this study. The difference in comparison with the current study may be explained due to the age of the animals, as this study evaluated young boars aged 15 to 18 months with little variation in age. Also, another factor to be taken into consideration is the different breeds of animals evaluated, which may result in differences between the sperm characteristics (Alonso *et al.*, 2004). However, the ejaculation time was found to have a direct effect on the volume of ejaculate collected despite this difference.

In addition to that of seminal volume, the correlation between ejaculation time and the age of the animals was moderate. The longer time that the older animals spend on the dummy is due to the fact that the seminal volume and spermatid production increases with age, so more time is needed for the ejaculation process to reach completion.

The averages sperm count observed was 88.77 ± 61.56 ($\times 10^9$ sperm per ejaculate), which is higher than that found by Alonso *et al.* (2011), lower than that found by Henao-Restrepo *et al.* (2004) and similar to results found by Del Toro and Diéguez (1997) and Fernández *et al.* (1996). The sperm count is indicative of the ability to produce gametes in the seminiferous tubules and does not appear to be related to fertility rates, being associated with seminal dilution rate and production rate of insemination doses from the ejaculate (Tardif *et al.*, 1999). This is a highly variable characteristic between animals and depends on many factors, including age, environment, breed and the season (Rodríguez-Martínez & Wallgren, 2000; Alonso *et al.*, 2011). In the current study, the average sperm count obtained is in agreement with the recommended values for swine (Corrêa *et al.*, 2001).

Although no statistically significant difference was observed for sperm count between the different groups (G1, G2 and G3), a numerical difference was observed. The highest number of sperm in the ejaculates of G1 can be justified, as the animals in G2 and G3 had longer ejaculation times, and also produced higher semen volumes, leading to a greater degree of sperm dilution/ejaculate. The consequence of this was a reduced sperm count in these groups compared with Group I.

The higher sperm count in the animals of Category I can be explained by the fact that the seminal volume of these animals was lower and the sperm production was either equal or superior to the C2 animals. Therefore, the higher seminal volume results in a decreased sperm concentration, leading to a lower sperm count, which is associated with a lower spermatid production in older animals (C2). These results are consistent with Jankeviciute

and Zilinskas (2002), who reported a decrease of this variable with advancing age.

The correlation analysis between ejaculation time and sperm count showed that when there is an increase in the ejaculation time, the sperm count also tends to increase ($r = 0.121$). This result was lower than the obtained by Thiengtham (1992), who observed an r value of 0.38 for the correlation between ejaculation time and the total number of sperm in the ejaculate. On the other hand, the results in the current study were similar to those obtained by [Alonso *et al.* \(2011\)](#) who found an r of 0.2148, meaning that both correlations are considered low. Although this study did not assess the effect of the seasonality in the seminal characteristics, according to Kunavongkit and Proteep (1990) and Sánchez (1991) sperm production is seasonal, meaning that in the months of higher temperatures there is a decrease in sperm production and an adverse effect on the characteristics of boar semen.

The values obtained for sperm motility are considered acceptable for fresh semen, especially for those ejaculates which will later be used for the preparation of insemination doses, which must have a minimum of 70% motility ([Corrêa *et al.*, \(2001\)](#)). The results of this study were higher than those obtained by [Alonso *et al.* \(2011\)](#) but lower than in [Henao-Restrepo *et al.* \(2004\)](#), who also studied the relationship between ejaculation time and sperm motility. According to [Tardif *et al.* \(1999\)](#) and [Silva *et al.* \(2012\)](#) motility is one of the most indicative characteristics of the fertilizing capacity of boar and bull semen, and there are negative effects on female fertility when percentages are low. In the present study, all animals presented spermatic values of motility higher than 77%.

The highest values of sperm motility observed in Groups I and II may have occurred because the animals of these groups remained on the dummy for less time (close to 100 seconds less compared with G3), and the ejaculates obtained were evaluated more rapidly after the seminal collection process compared with the other group. Consequently, it is possible to suggest that spending less time under the influence of external factors are favorable to sperm motility.

The correlation analysis between ejaculation time and sperm motility showed that when there is an increase in the ejaculation time, the seminal volume tends to decrease ($r = -0.163$). These results are different to those found by [Alonso *et al.* \(2011\)](#) who obtained an r of 0.2148 for motility in relation to ejaculation time. Despite this difference, both studies showed that the correlation between these two variables is low.

As well as motility, the intensity of motion was normal for the boar semen. According to [Corrêa *et al.* \(2001\)](#) the minimum amount of intensity of motion for boar fresh semen that can be used in AI is 3.0. The higher values of the intensity of motion obtained for the Group I can be explained due to the fact that G1 animals remained on the dummy for a shorter time, and consequently the sperm also remained in a hostile and harmful environment for a shorter time. This resulted in the maintenance of a high quality of sperm.

The correlation analysis between ejaculation time and intensity of motion also showed similar results to motility, with a low correlation ($r = -0.152$) being obtained between these variables. Regarding motility and intensity of motion, correlations exist between these variables and ejaculation time, but they are not high, which can be explained because the sperm may have greater feasibility and be minimally affected by a longer ejaculation time even if the ejaculation time is high.

The average results for morphological changes obtained from the 447 ejaculates are in agreement with [Corrêa *et al.* \(2001\)](#) and [Fonseca *et al.* \(1992\)](#), who described that the total morphological abnormalities allowed in boar semen is 20%. The results are lower than those reported by [Henao-Restrepo *et al.* \(2004\)](#), who discovered very high values of morphological defects in animals housed in a humid tropical climate. However, the mean morphological alterations in some individual boars exceeded 20% of changes. Despite the fact that these values are higher than those suggested by some authors, they remain within the standards established by [Barth and Oko \(1989\)](#) who consider a viable ejaculate to include up to 30% of changes.

The higher percentage of morphological changes in C1 animals can be explained by the fact that the animals are not yet completely mature

sexually (prepubertal). The occurrence of alternated ejaculations with very different morphological changes often leading high values of alterations in the ejaculate is common in this group (Murgas & Zangeronimo, 2004). Despite this fact, with the advancing of age and with the development of the boar (puberty), this characteristic tends to normalize. Thus, the percentage of alterations in the ejaculate of a healthy pubertal animal is within acceptable limits. The results obtained are similar to those recorded by Jankeviciute and Zilinskas (2002), who demonstrated that the spermatic morphology is one of the variables affected by the age of the animals.

The insignificant correlation analysis between ejaculation time and morphology is due to the extreme variability that exists among animals as well as between the same animal (Petrunkina *et al.*, 2005). Furthermore, according to Murgas and Zangeronimo (2004) the spermatic morphology may be influenced by many factors. However, the ejaculation time showed no significant effect on the sperm morphology in this study.

Despite the fact that all of the animals evaluated did not present with genital diseases, the evaluation of sperm morphology is a parameter which allows for the identification of males who suffer from these pathologies (Henao-Restrepo *et al.*, 2004). The results generate in this study are not able to relate morphological defects with semen fertility. However, according to Rodríguez-Martínez and Eriksson (2000), the identification of boars that produce semen of poor quality could be used to identify possible animal semen donors for AI programs.

From the results obtained in this study relating ejaculation time with the parameters of volume, sperm count, motility, intensity of motion and morphology of high performance boars, it can be concluded that the collection time has a major effect on the variable ejaculate volume. The other semen quality variables are poorly influenced by ejaculation time, but there is a set of factors that can affect these variables. Therefore, even animals presenting with a good libido and longer ejaculation times, the semen analysis must be performed properly to avoid any influence on the results. Considering the animals destined for AI

programs, these analyses are essential, as all of the characteristics of semen quality that have been evaluated are influenced by numerous factors and not just by the ejaculation time.

The current knowledge of the sexual behavior influence of boars, such as the ejaculation time, and the reproductive parameter is small in comparison with information about factors such as the spermatic production physiology. Therefore, more research should be conducted with the use of a greater number of animals of different breeds, with the aim of studying factors that may influence the correlation between sexual behavior and seminal characteristics.

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