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J Vet Res 61, 121-125, 2017
DOI:10.1515/jvetres-2017-0015

Ejaculate traits and sperm morphology depending on ejaculate volume in Duroc boars

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Received: October 17, 2016 *Accepted: March 6, 2017*

Abstract

Introduction: The aim of this study was to evaluate the dependence between ejaculate traits, sperm morphology, and ejaculate volume in Duroc boars. **Material and Methods:** The analysis involved 121 ejaculates collected from 12 Duroc boars kept in three artificial insemination centres located in central Poland. Ejaculates were collected manually at one-month intervals, over a period of 10 months. At least 10 ejaculates were collected from each boar. The material was divided by ejaculate volume and each ejaculate was assigned to one of three volume groups: 160 mL and lower, 161–200 mL, and 201 mL and higher. The ejaculates were assessed to identify the basic physical traits and determine the incidence of morphological abnormalities in the spermatozoa, specifying major and minor abnormalities. Furthermore, the morphological structure indices for the spermatozoa were also calculated. **Results:** In large-volume ejaculates, spermatozoa were more elongated in shape, their heads were more elongated and had the largest flagella. With an increase in the ejaculate volume, sperm concentration in the ejaculate decreased. Moreover, while the total number of sperm in the ejaculate increased, the number of insemination doses obtained from a single ejaculate were higher. **Conclusion:** The volume of ejaculate has little impact on the occurrence of morphological abnormalities and the size of sperm cells. Ejaculate volume is important for the shape of the sperm cells.

Keywords: boar, ejaculate volume, sperm morphology.

Introduction

Rapid progress and constantly increasing number of artificial insemination (AI) procedures require proper examination of the semen of sires used for reproduction. The quality of semen obtained from insemination males depends on both genetic and environmental factors. These include breed (25), age of sire (2), use intensity (12), and the season of the year when semen is collected (17). It has been demonstrated that porcine semen traits are influenced by air temperature and humidity, as well as by atmospheric pressure (16). Boars of various breeds may produce ejaculates varying in volume, sperm concentration, and sperm motility (5). It has also been found that ejaculate quality depends on boar libido (10, 30).

The normal structure of the sperm is a prerequisite of its ability to penetrate the layers covering the ovum and to successfully fertilise. The fraction of sperm with morphological abnormalities and its type reflects the degree of disturbances in the process of spermatogenesis (28, 29). Morphological examination of sperm enables objective evaluation of semen quality and represents the basis for the evaluation of boar's fertility (14). The ability of particular spermatozoa to penetrate into the ovum depends on the size and shape of the sperm cell. Sperm dimensions are highly variable even among the males within the same population (19, 27). It is proven that there is a relationship between the dimensions, shape of sperm cells, and ejaculate characteristics (17).

Basic ejaculate characteristics, routinely evaluated at AI centres, depend on the breed of the boar. Duroc boars feature as a model of sexual development that is different from that of boars of other breeds. Duroc boars undergo intensive sexual maturation until age of 21–22 months; however, the pace of changes is slower compared to other breeds (15). The boars produce ejaculates of smaller volumes, though of higher sperm concentrations. Many authors claim that this trait is

genetically fixed in this breed (15, 22, 26). The aim of this study was to evaluate the dependence between ejaculate traits, sperm morphology, and ejaculate volume in Duroc boars.

Material and Methods

The analysis involved 121 ejaculates collected from 12 Duroc boars managed in three AI centres located in central Poland. The boars selected were young, just beginning their insemination use. The boars were managed in accordance with the rules of animal welfare (21). Ejaculates were collected manually (9), at one-month intervals, for 10 months. At least 10 ejaculates were collected from each boar. The ejaculates were grouped according to volume, forming the following groups: Group I: ejaculates of a volume below 160 mL; Group II: ejaculates of a volume between 161 and 200 mL; Group III: ejaculates of a volume above 201 mL.

Freshly collected ejaculates were analysed for ejaculate volume (mL), sperm concentration ($\times 10^6$/mL), sperm motility (%), total number of spermatozoa ($\times 10^9$), and number of insemination doses per ejaculate (n). Ejaculate volume was determined immediately after collection. Gel was removed by filtering the ejaculate through gauze. The volume of gel-free fraction was measured by weight using electronic scale. Sperm concentration in the ejaculates was measured using the colorimetric assay, using a spectrophotometer (IMV Technologies, France). Sperm motility was estimated with a microscopic examination, as a fraction of sperm cells showing forward progression, using Nikon Eclipse 50i light microscope equipped with a heated stage. The total number of motile spermatozoa and the number of insemination doses per ejaculate were calculated using SYSTEM SUL (v. 6.35; Gogosystem, Poland) software package.

Each ejaculate was sampled for microscopic slides, which were prepared and stained according to method of microscopic slide preparation described by Kondracki *et al.* (12). The morphology of 500 sperm cells was examined, turning to normal and morphologically altered sperm cells. The latter were divided into groups with major and minor abnormalities, according to classification by Blom (1). Sperm microscopic examinations were performed using an immersion objective, at a 1000× magnification, under the Nikon Eclipse-50i light microscope.

The sperm cells were also measured morphometrically. Each slide was used to measure 15 randomly selected sperm cells with a normal morphological structure. The measurements were performed using the computer image analysis system Screen Measurement v. 4.1, according to methodology developed by Kondracki *et al.* (11). The following measurements were taken: head length, head width, head area, head perimeter, flagellum length, and total sperm length. These were next used to calculate the following morphological parameters: head width/head length ratio; head length/total sperm length ratio; head length/flagellum length ratio; flagellum length/total sperm length ratio; head perimeter/total sperm length ratio; head area/total sperm length ratio; head length x head width/total sperm length ratio.

Experimental data were analysed using Statistica 10 PL (StatSoft, USA). All results were expressed as mean ± standard deviation (SD). The obtained data were statistically analysed according to the following model: $Y_{ij} = \mu + a_i + e_{ij}$, where: Y_{ij} - trait level, μ - population mean, a_i - effect of ejaculate volume, e_{ij} - error. The significance of the differences between the groups was assessed with the Tukey's test at $P \leq 0.05$ and $P \leq 0.01$.

Results

The data obtained allow to conclude that physical characteristics of ejaculate and morphological characteristics of sperm cells depend on ejaculate volume. Namely, differences in quantitative traits of ejaculates and sperm morphology were found between ejaculates of different volume. Table 1 presents the physical characteristics of Duroc boar ejaculates in relation to their volume.

With an increase in the volume, total sperm count in ejaculate also increased, which was additionally connected with a higher number of insemination doses produced per ejaculate. The magnitude of this increase was statistically significant ($P \leq 0.01$).

Table 1. Basic characteristics (means ±SD) of ejaculates related to the ejaculate volume of Duroc boars

Variable	Ejaculate volume (mL)		
	Group I <160	Group II 161-200	Group III >201
Number of ejaculates (n)	36	43	42
Sperm concentration (x10^6/mL)	$545.36^a \pm 161.31$	$510.86^a \pm 88.72$	$496.69^a \pm 84.94$
Percentage of spermatozoa with progressive motility (%)	$79.16^a \pm 2.8$	$79.06^a \pm 2.93$	$78.81^a \pm 2.27$
Total number of spermatozoa (x10^9)	$62.65^A \pm 20.4$	$80.12^B \pm 17.55$	$98.39^C \pm 20.38$
Number of insemination doses per ejaculate (n)	$18.11^A \pm 4.78$	$24.09^B \pm 3.73$	$31.24^C \pm 6.18$

* Different superscripts mean significant differences among means within particular rows; lower-case letters: $P \leq 0.05$; upper-case letters: $P \leq 0.01$

With an increase in ejaculate volume, however, sperm concentration and motility showed a slightly decreasing trend, although these relationships were insignificant. The data in Table 1 also showed that group I, which comprised ejaculates of smallest volume, exhibited the highest sperm cell concentration. The concentration in this group reached on average 545.36×10^6/mL and was by 34.5×10^6/mL higher than in group II, and nearly by 48.7×10^6/mL higher than in group III, with the largest volume of ejaculate. The differences, however, were not significant.

The data in Table 1 demonstrated that with an increase in ejaculate volume, a decreasing trend of sperm motility was observed. These changes in motility, however, were very low and statistically insignificant. From the practical point of view it is important that a higher number of sperm cells in an ejaculate translate to more insemination doses produced per ejaculate. Data presented in Table 1 revealed that there is a direct proportional dependency between sperm count per ejaculate, number of insemination doses produced from ejaculate, and the volume of obtained ejaculates. The highest number of sperm cells in an ejaculate was found in ejaculates of the largest volume (group III). Ejaculates in this group had more than 98 billion sperm cells, over 18 billion cell more ($P \leq 0.01$) than in group II ejaculates, and by about 36 billion cells more ($P \leq 0.01$) than in ejaculates of group I. Since the number of insemination doses per ejaculate depends on sperm cell count, the highest number of doses was obtained from ejaculates of the largest volume (group III). These gave more than 31 insemination doses, *i.e.* about 7.1 doses more compared to group II and over 13 doses more than from ejaculates of group I ($P \leq 0.01$).

Table 2 presents the results of the analysis of incidence of morphological changes in sperm cells in relation to ejaculate volume. The average fraction of normal sperm cells ranged from 90.96% to 94.97%. The lowest percentage of morphologically normal sperm was found in ejaculates of the largest volume (group III). These showed 90.96% normal sperm, approx. 4% less compared to ejaculates of group II ($P \leq 0.01$) and 2.56% less than in ejaculates of group I.

Table 2 shows that the quality of Duroc boars' semen is very high. The average fraction of spermatozoa with major morphological abnormalities did not exceed 2.09%, and the differences between the groups were low and insignificant. The fraction of sperm with minor abnormalities was higher. Most spermatozoa with these morphological changes were found in ejaculates of the largest volume (group III), which exhibited on average 6.85% of such sperm cells. The volume of ejaculate, however, had little effect on the fraction of sperm with minor abnormalities, and differences between the groups were statistically insignificant. The data in Table 2 demonstrated that the frequency of morphological abnormalities in the semen of Duroc boars to a very low degree depends on the volume of discharged semen.

The morphometrics of spermatozoa are presented in Table 3. The data revealed that the dimensions of sperm cells to little extent depended on ejaculate volume.

Some trends may be observed in the measurements of the head and tail of sperm. Sperm cells in ejaculates of the lowest volume (group I) had heads of slightly higher length and width, as compared with sperm of middle- or largest-volume ejaculates (groups II and III). Head dimensions decreased with an increase in ejaculate volume. Sperm cells from the largest-volume ejaculates (group III) had also smallest average head areas. On the other hand, sperm cells of this group featured the longest flagella. The tails were on average by 0.73 μm longer compared to those of spermatozoa in group II, and by 0.71 μm longer than those in group I. Total sperm length was also the greatest in group III sperm cells, which resulted from the length of the flagella.

Table 4 presents the parameters for evaluation of differences in the shape of spermatozoa in ejaculates differing in volume.

Table 2. Frequency of occurrence of normal and abnormal spermatozoa (means ±SD) related to the ejaculate volume of Duroc boars

Variable (%)	Ejaculate volume (mL)		
	Group I <160	Group II 161-200	Group III >201
Percentage of normal spermatozoa	$93.52^{ab} \pm 4.48$	$94.97^{a} \pm 4.64$	$90.96^{b} \pm 12.57$
Sperm with major abnormalities	$2.09^{a} \pm 3.46$	$1.47^{a} \pm 1.87$	$1.99^{a} \pm 3.19$
Sperm with minor abnormalities	$4.38^{a} \pm 3.57$	$3.54^{a} \pm 3.71$	$6.85^{a} \pm 12.26$

*Different superscripts mean significant differences among means within particular rows; lower-case letters: $P \leq 0.05$

Table 3. Morphometric characteristics (means ±SD) of spermatozoa related to the ejaculate volume of Duroc boars

Variable	Ejaculate volume (mL)		
	Group I <160	Group II 161-200	Group III >201
Head length (μm)	$9.39^{a} \pm 0.36$	$9.36^{a} \pm 0.26$	$9.30^{a} \pm 0.37$
Head width (μm)	$4.74^{a} \pm 0.27$	$4.72^{a} \pm 0.29$	$4.66^{a} \pm 0.38$
Perimeter of the head (μm)	$23.90^{a} \pm 0.93$	$23.72^{a} \pm 0.82$	$23.80^{a} \pm 0.96$
Head area (μm^2)	$40.43^{a} \pm 1.76$	$40.41^{a} \pm 1.77$	$40.03^{a} \pm 1.1$
Flagellum length (μm)	$44.14^{a} \pm 1.17$	$44.13^{a} \pm 2.02$	$44.86^{a} \pm 1.71$
Total length (μm)	$53.53^{a} \pm 1.36$	$53.49^{a} \pm 2.13$	$54.16^{a} \pm 1.86$

Table 4. Morphometric indices (means ±SD) of spermatozoa related to the ejaculate volume of Duroc boars

Variable (%)	Ejaculate volume (mL)		
	Group I <160	Group II 161-200	Group III >201
Head width/head length	50.47^{a} ±1.86	50.46^{a} ±2.57	50.02^{a} ±1.07
Head length/total length	17.54^{B} ±0.52	17.51^{B} ±0.65	17.18^{A} ±0.64
Head length/flagellum length	21.27^{B} ±0.76	21.24^{B} ±0.97	20.75^{A} ±0.93
Flagellum length/total length	82.45^{B} ±0.52	82.48^{B} ±0.65	82.81^{A} ±0.64
Perimeter of the head/total length	44.62^{b} ±1.25	44.38^{b} ±1.72	43.96^{a} ±1.66
Head area/total length	75.52^{b} ±2.82	75.51^{b} ±3.02	73.90^{a} ±5.11
Head length x width/total length	83.26^{b} ±6.59	82.71^{b} ±5.34	80.11^{a} ±7.85

*Different superscripts mean significant differences among means within particular rows; lower-case letters: $P \leq 0.05$; upper-case letters: $P \leq 0.01$

An association was found in sperm cell shape ratios in relation to ejaculate volume. Spermatozoa from largest-volume ejaculates (group III) exhibited the lowest ratios of head dimensions to flagella length or total sperm length, as compared with sperm of groups I and II. This is evidenced by significantly lower ($P \leq 0.01$) ratios: head length/total sperm length, head length/flagellum length, and flagellum length/total sperm length. It was demonstrated that spermatozoa of the largest-volume ejaculates had significantly lower ($P \leq 0.05$) ratios: head area/total sperm length and head length x head width/total sperm length, as compared with sperm cells of ejaculate volume groups I and II. On the other hand, the largest-volume ejaculate had a larger ($P \leq 0.01$) flagellum length/total length ratio.

Discussion

The total number of sperm increased with ejaculate volume. The differences were significant. A directly proportional correlation ($r = 0.63$) between the number of spermatozoa and ejaculate volume has been previously demonstrated (26). The results of the present study showed that sperm concentration was higher in ejaculates with lower volumes. Sperm concentration is usually reversely correlated with ejaculate volume, as identified in previous studies (30). The data presented in this work indicate a low correlation between the incidences of sperm morphological abnormalities and ejaculate volume. Boars with normal fertility always have a certain percentage of morphologically abnormal spermatozoa. A high percentage of spermatozoa with major modifications reduces the chances for insemination. Sperm cell abnormalities belonging to the major abnormalities group are formed most often in the process of spermatogenesis. High percentage of these abnormalities diminishes male's fertility (5). According to Noorafshan and Karbalay-Doust (20), sperm length is positively correlated with the speed of sperm motion. Spermatozoa with longer flagella are more competitive since they might reach the ovum faster. We found that spermatozoa with the longest flagella were present in ejaculates with high volumes (>201 mL).

The data generated by this study revealed that spermatozoa from the largest-volume ejaculate were more elongated in shape. They had a relatively long flagellum and a relatively smaller head. Such an elongated shape may be preferable in terms of ovum fertilising ability. Namely, it has been found that sperm cells vary in relation to their ability to penetrate the female reproductive cells, depending on their shape. Higher chances of fertilisation belong to longer sperm cells attaining higher progressive speeds (3). In the semen of low sperm motility, sperm cells have shorter tails compared to semen comprising a higher fraction of progressively motile spermatozoa (20). Sperm of the largest-volume ejaculates (group III) had also slightly longer heads as illustrated by the head width/head length ratio, which was the lowest in the sperm of the largest-volume group of ejaculates. Increasing length of sperm cells (including head and flagellum) with an increase in ejaculate volume was found in previous studies on Pietrain boars (13). It is possible, therefore, that this relationship is a feature of these two breeds or, perhaps, it occurs regardless of boar breed.

Another study demonstrated a relationship between sperm head dimensions and semen sperm concentration. In porcine ejaculates with higher sperm concentrations, sperm cells had smaller heads (14, 15). Differences in head dimensions may represent a useful marker in discriminating between fertile males and those of limited fertility (24). Humphries *et al.* (8) and Helfenstein *et al.* (6) point out that there is a relationship between the head and flagellum length and the forward progression velocity of the sperm. The authors claim that sperm cells exhibiting a lower head length/flagellum length ratio move faster. Head shape is important for the hydrodynamics of the sperm cell (4). The sperm cells of boars that exhibit poorer fertilising efficiency, as compared to boars of high fertility, are characterised by larger heads (7). Sperm cells with more oblong shape of head are faster than those with more round-shaped heads (18). The shape of sperm head may be related to the breed of the boar. Saravia *et al.* (23) demonstrated that Duroc boars had larger sperm cell heads which are more elliptical in shape than the sperm of boars of other breeds.

In conclusion, the volume of ejaculate has little impact on the occurrence of morphological abnormalities and the size of sperm cells. Ejaculate volume is, however, important for the shape of the sperm cells. In ejaculates of large volumes, spermatozoa are more elongated in shape. The heads of sperm cells are also more elongated in ejaculates of the

largest volume. With an increase in the ejaculate volume, sperm concentration in the ejaculate decreases; however, the total number of sperm in the ejaculate increases, and the number of insemination doses obtained from a single ejaculate is higher.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: This study was financed by statutory research and development activity funds from the Polish Ministry of Science and Higher Education assigned to the Faculty of Natural Sciences, Siedlce University of Natural Sciences and Humanities.

Animal Rights Statement: The boars were maintained in accordance with the principles of animal welfare. Approval for this study was given by the Ethical Committee of UPH, Faculty of Natural Sciences, and the Decision of the District Veterinary Officer.

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