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INTER- AND INTRA-BREED VARIATION IN EJACULATE CHARACTERISTICS AND IN THE MORPHOLOGY, DIMENSIONS AND SHAPE OF SPERMATOZOA OF BOARS USED FOR ARTIFICIAL INSEMINATION

MIĘDZYRASOWA I WEWNĄTRZRASOWA ZMIENNOŚĆ CECH EJAKULATU ORAZ MORFOLOGII, WYMIARÓW I KSZTAŁTU PLEMNIKÓW KNURÓW INSEMINACYJNYCH

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Streszczenie. W niniejszej pracy podjęto próbę oceny międzyrasowej i wewnątrzrasowej zmienności ejakulatów oraz morfologii, wymiarów i kształtu plemników knurów inseminacyjnych. Podstawowe cechy ejakulatów cechuje relatywnie duża zmienność wewnątrzrasowa i międzyrasowa. Ejakulaty knurów ras Landrace i Large White mają wyraźnie większą objętość niż ejakulaty knurów rasy Duroc oraz mieszańców Duroc × Pietrain, a ejakulaty knurów rasy Landrace zawierają więcej plemników niż ejakulaty knurów innych ras. Plemniki knurów mieszańców wykazują mniejszą ruchliwość niż plemniki knurów innych ras. W ejakulatach knurów wszystkich ras stwierdzono bardzo małą frekwencję plemników ze zmianami głównymi i podrzędnymi. Frekwencję zmian morfologicznych plemników cechuje bardzo duża zmienność wewnątrzrasowa, przy stosunkowo małej zmienności międzyrasowej. Wymiary morfometryczne plemników cechuje mała zmienność wewnątrzrasowa, przy relatywnie dużej zmienności międzyrasowej. Plemniki knurów rasy Landrace mają wyraźnie dłuższe witki oraz większe pole powierzchni główki niż plemniki knurów innych ras.

Key words: boar, traits of ejaculate, sperm morphology, variation, breed.
Słowa kluczowe: knur, cechy ejakulatu, morfologia plemników, zmienność, rasa.

INTRODUCTION

Artificial insemination plays a key role in animal reproduction. It enables far more efficient use of boar semen than in the case of natural mating (Oh et al. 2006; McCann et al. 2008; Lewis and Bunter 2011). Boars of various breeds chosen for commercial use are evaluated in terms of fattening performance and carcass traits (Oh et al. 2006). In addition to these traits, insemination stations must also consider characteristics predisposing boars for breeding, and thus the libido level and the quantity and quality of semen produced (Robinson and Buhr 2005; Flowers 2008; Safranski 2008; Kondracki et al. 2013b). The sperm production process varies between individual boars (Stockley and Purvis 1993; Miller et al. 2015). Ejaculates of boars used for insemination are highly varied. The sources of this variation

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include the genotype of the boar (Smital et al. 2005; Wolf 2008; Schulze et al. 2014), environmental factors (Kunavongkrit et al. 2005; Fuerst-Waltl et al. 2006; David et al. 2007; Buranawit and Imboonta 2016; Kowalewski et al. 2016) and organizational factors (Bajena et al. 2016). Individual variation is also significant, some individuals are more predisposed for use for artificial insemination than others.

Elements of ejaculate variation include effects resulting from the breed of boar or crossbreeding variant (Bertani et al. 2002; Wysokińska and Kondracki 2013). The ejaculates of boars of different breeds differ in volume, sperm concentration, total sperm count in the ejaculate, and thus the number of insemination doses obtained (Kondracki et al. 2014; Górski et al. 2017). Significant differences are also noted in sperm motility and fertilization capacity (Petrunkina et al. 2007; Sonderman and Luebbe 2008). The basic criterion for evaluating a boar's fertility is sperm morphology. The results of examination of sperm morphology can be used to draw conclusions about the course of the spermatogenesis process and to predict the boar's suitability for breeding (Banaszewska et al. 2015). Individual boars exhibit substantial variation in the frequency of morphological sperm anomalies, which have a significant impact on the fertilization capacity of the semen (Walters et al. 2005; Saacke 2008). A morphologically normal spermatozoon successfully reaches the ovum and initiates activation of the oocyte (Menkveld et al. 2011). The morphological characteristics of spermatozoa may be influenced by the rate of sperm production in the testicular tissue and thus the number of sperm stored in the epididymis and released in the ejaculate (Hossain et al. 1997; Silva et al. 2012). The dimensions and shape of sperm have been shown to be correlated with ejaculate characteristics and male fertility (Rijsselaere et al. 2004; Ramm et al. 2014; Górski et al. 2016). Even morphologically normal sperm cells differ in shape, which may affect the speed with which they reach the ovum (Banaszewska et al. 2009). Competition between spermatozoa in the female reproductive organs and their ability to penetrate the ovum may depend on the dimensions and shape of the sperm (Gage and Cook 1994; Parisi et al. 2014).

In the present study an attempt was made to evaluate inter-breed and intra-breed variation in ejaculates and the morphology, dimensions and shape of spermatozoa of boars used for artificial insemination.

MATERIAL AND METHODS

The evaluation was performed on 1047 ejaculates collected from 86 boars used for artificial insemination, including 29 Large White boars, 30 Landrace boars, 11 Duroc boars and 16 Duroc × Pietrain crosses, which were 8–9 months old at the start of the study. All the boars from which the semen was collected had been managed under standarized conditions, conforming with the current animals welfare regulations (Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 15 lutego 2010 r.). Feed intake was individualized for each boar according to nutrition requirements (Normy Żywienia Świń 1993), with *ad libitum* access to water. The animals were free from contagious and reproduction-related diseases, covered by a routine disease prevention program and received permanent veterinary care. The ejaculates were collected by the manual method (King and Macpherson 1973) in the early morning. The ejaculates were collected every four days. Ten ejaculates from each boar collected at one-month intervals were evaluated.

Immediately after collection the ejaculates were examined to determine the following physical characteristics: ejaculate volume, sperm concentration, and the percentage of sperm displaying progressive motility in the ejaculate. Ejaculate volume was determined following removal of the gel fraction. Sperm concentration in the ejaculate was determined by the photometric method with a Cassou spectrophotometer. Sperm motility was determined by microscopic examination. The results were used to calculate the total number of sperm with progressive motility. The total ejaculate sperm count was calculated using SYSTEM SUL software.

Morphometric measurements and evaluation of the morphology of the spermatozoa of each boar were based on microscopic examination of slides prepared from fresh ejaculates. Microscopic analysis of the slides was carried out using immersion lenses at ×100 magnification, with a Nikon-E 50i light microscope. In each slide morphometric measurements were performed on 15 randomly selected morphologically normal sperm cells that were clearly visible in the field of view of the microscope. The measurements were made using image analysis software (Screen Measurement v. 4.1), according to a method developed by Kondracki et al. (2005). A total of 15705 spermatozoa were measured. The following morphometric measurements were made of each sperm cell: the perimeter, area, length, and width of the head; tail length; and total sperm length. In addition, the morphological structure of 500 spermatozoa was evaluated in each ejaculate; the numbers of morphologically normal and morphologically abnormal cells was determined, distinguishing forms with major and minor defects according to Blom's classification (1981).

Experimental data were analysed using Statistica 10 PL (StatSoft, USA). Intra-breed variation was characterized by calculating the arithmetic mean, standard error and coefficient of variation for each ejaculate trait and morphological sperm parameter within each genetic group. Inter- breed variation of the traits examined was analysed according to the following mathematical model:

$$Y_{ijk} = \mu + a_i + b_{jk} + e_{ijk}$$

where:

Y_{ijk} – value of trait,
μ – mean for population,
a_i – effect of breed,
b_{jk} – effect of boar within breed,
e_{ijk} – error.

Significance of differences between groups was determined by Tukey's test.

RESULTS

Table 1 presents data pertaining to the physical characteristics of the ejaculate of boars of each breed.

The volume of the ejaculates of the Landrace and Large White boars was over 249 ml and was significantly greater than the volume obtained in the Duroc breed and in the Duroc × × Pietrain crossbreds ($P \leq 0.01$). The ejaculates of the Duroc boars had the smallest volume. It averaged 200.66 ml and was at least 48 ml smaller ($P \leq 0.01$) than in the boars of the

Landrace and Large White breeds. The Duroc crossbreds also had a low ejaculate volume; it was significantly lower than in the Landrace and Large White breeds (P ≤ 0.01). The ejaculate volume of the Duroc boars was characterized by relatively low intra-breed variation (V% = 26.59). Relatively low variation in ejaculate volume was also noted in the group of Duroc × Pietrain crossbreds (V% = 29.09), while relatively high variation in ejaculate volume was observed in the Landrace and Large White boars (V% > 35).

Table 1. Variability evaluation of boar semen characteristics according to breed
Tabela 1. Zmienność cech ejakulatów pobieranych od knurów poszczególnych ras

Variable Parametr		Breed – Rasa			
		Landrace	Large White	Duroc	Duroc × Pietrain
Number of ejaculates Liczba ejakulatów		394	360	133	160
Ejaculate volume Objętość ejakulatu [ml]	$\bar{x}$ ± SEM	264.87 ± 4.53^{B}	249.01 ± 5.10^{B}	200.66 ± 4.63^{A}	221.12 ± 5.10^{A}
	V%	35.94	38.78	26.59	29.09
Sperm concentration Koncentracja plemników [×10^{6}cells/ml]	$\bar{x}$ ± SEM	456.60 ± 4.95^{AB}	433.98 ± 5.88^{A}	521.33 ± 9.44^{C}	475.01 ± 12.83^{B}
	V%	21.52	20.90	20.89	34.18
Percentage of spermatozoa with progressive motility Procent plemników o ruchu postępowym [%]	$\bar{x}$ ± SEM	77.51 ± 0.23^{b}	77.47 ± 0.43^{b}	78.79 ± 0.28^{b}	75.12±0.39^{a}
	V%	6.01	10.70	4.14	6.67
Total number of spermatozoa Ogólna liczba plemników [×10^{9}]	$\bar{x}$ ± SEM	91.97 ± 1.72^{B}	81.17 ± 1.64^{A}	85.65 ± 1.94^{AB}	76.32 ± 2.10^{A}
	V%	37.06	38.80	27.03	34.84

Different superscripts designate significant differences between means within rows; lower-case letters: P ≤ 0.05, upper-case letters: P ≤ 0.01 – Dużymi literami oznaczono istotne różnice przy P ≤ 0,01; małymi literami oznaczono istotne różnice przy P ≤ 0,05.
$\bar{x}$ – arithmetic mean – średnia arytmetyczna.
SEM – standard error – standardowy błąd pomiaru.
V% – coefficient of variation – współczynnik zmienności.

The highest sperm concentration was noted in the ejaculates of the Duroc boars (on average 521.33 × 10^{6} cells/ml). It was significantly higher than in the ejaculates of the boars of the other groups (P ≤ 0.01). The lowest sperm concentration (433.98 × 10^{6} cells/ml) was noted in the ejaculates of the Large White boars. A high sperm concentration was observed in the ejaculates of the Duroc × Pietrain crossbreds. However, it was markedly lower than in the ejaculates of the Duroc boars (P ≤ 0.01). The sperm concentration in the ejaculates of the crossbreds showed relatively high variation (V% = 34.18); it was much higher than in the ejaculates of the purebred boars (V% ≈ 21).

Variation in sperm motility within breed groups was relatively low (V% ≈ 4.14 – 10.70%). Inter-breed variation in sperm motility was greater, and the differences between breed groups were confirmed statistically. In the ejaculates of the purebred boars the percentage of spermatozoa with progressive motility was higher than in the ejaculates of the crossbreds. The differences ranged from about 2.3% to over 3.6% and were significant (P ≤ 0.05).

The data in Table 1 indicate high variation in the total sperm count in the ejaculates of the boars. Coefficient of variation within breed groups (V%) ranged from 27.03 to 38.80. The lowest sperm count (about 76 × 10^9 cells per ejaculate) was noted in the ejaculates of the Duroc × Pietrain crossbreds, and the highest in the ejaculates of the Landrace boars (on average about 92 × 10^9 cells per ejaculate) – P ≤ 0.01.

Table 2 presents data on the frequency of major and minor morphological sperm defects in the ejaculates of boars of different breeds.

Table 2. Variability evaluation of boar spermatozoa morphology according to breed
Tabela 2. Frekwencja zmian morfologicznych plemników w ejakulatach knurów w zależności od rasy

Item Wyszczególnienie		Breed – Rasa			
		Landrace	Large White	Duroc	Duroc × Pietrain
Number of ejaculates Liczba ejakulatów		394	360	133	160
Percentage of normal spermatozoa	x̄ ± SEM	96.05 ± 0.24^{BC}	95.14 ± 0.30^{AB}	94.26 ± 6.98^{A}	96.77 ± 0.21^{C}
Odsetek plemników prawidłowych [%]	V%	4.93	6.02	0.57	2.78
Sperm with major abnormalities	x̄ ± SEM	0.58 ± 0.07^{A}	1.20 ± 0.10^{B}	1.52 ± 0.22^{B}	0.48 ± 0.06^{A}
Odsetek plemników ze zmianami głównymi [%]	V%	236.20	164.16	165.13	164.58
Sperm with minor abnormalities	x̄ ± SEM	3.36 ± 0.22^{ab}	3.65 ± 0.25^{ab}	4.20 ± 0.53^{b}	2.74 ± 0.20^{a}
Odsetek plemników ze zmianami podrzędnymi [%]	V%	130.06	129.58	144.18	90.51

Explanations see Table 1 – Objaśnienia zob. tab. 1.

The data in Table 2 indicate that the ejaculates were characterized by very good semen quality. This can be seen in the low frequency of morphological sperm defects. The mean percentage of sperm with major or minor abnormalities did not exceed 5% in any of the breeds studied. The most abnormalities in morphological sperm structure were noted in the ejaculates of the Duroc boars, while the least in the ejaculates of the Duroc × Pietrain crossbreds. The fewest spermatozoa with major or minor defects were also noted in the ejaculates of the Duroc × Pietrain crossbreds. The semen of the Large White boars contained relatively few morphologically normal sperm (on average 95.14%), with a relatively high percentage of sperm displaying major or minor defects. In the semen of the Landrace boars, 0.62% fewer sperm with major defects were noted (P ≤ 0.01) than in the semen of the

Large White breeders. The results obtained in the study indicate that crossbreeding of the Duroc and Pietrain breeds to obtain two-breed crossbreds yields positive effects in terms of improving the reproductive characteristics of males. The crossbred boars produce ejaculates with intermediate levels of the main quantitative characteristics as compared to purebred breeders, but the level of qualitative characteristics is very high (a small percentage of sperm with major or minor morphological defects). The data in Table 2 indicate high variation in the occurrence of morphological sperm defects within breed groups. The coefficient of variation (V%) for the frequency of sperm with major defects ranged from 164.16% in the ejaculates of the Large White boars to 236.20% in the ejaculates of the Landrace boars, and for the frequency of minor defects, from 90.51% in the Duroc × Pietrain crossbreds to 144.18% in the Duroc breed. The frequency of morphological sperm abnormalities is thus distinguished by high intra-breed variation.

Table 3 presents the results of morphometric measurements of sperm from the ejaculates of boars of each breed.

Table 3. Variability evaluation of morphometric traits of sperm according to the breed of boar
Tabela 3. Wyniki pomiarów morfometrycznych plemników z ejakulatów knurów badanych ras

Parameter Parametr		Breed – Rasa			
		Landrace	Large White	Duroc	Duroc × Pietrain
Number of ejaculates Liczba ejakulatów		394	360	133	160
Head length Długość główki [µm]	$\bar{x}$ ± SEM	9.17 ± 0.02^{AB}	9.09 ± 0.02^{A}	9.36 ± 0.03^{C}	9.26 ± 0.03^{BC}
	V%	3.59	3.96	3.31	4.42
Head width Szerokość główki [µm]	$\bar{x}$ ± SEM	4.75 ± 0.01	4.77 ± 0.05	4.72 ± 0.02	4.79 ± 0.02
	V%	6.10	20.75	6.14	6.05
Head perimeter Obwód główki [µm]	$\bar{x}$ ± SEM	23.49 ± 0.04	23.35 ± 0.06	24.19 ± 0.14	23.41 ± 0.13
	V%	3.87	5.09	6.98	6.87
Head area Pole główki [µm^2]	$\bar{x}$ ± SEM	40.62 ± 0.12^{D}	39.21 ± 0.16^{A}	40.33 ± 0.20^{C}	39.82 ± 0.32^{B}
	V%	5.96	7.60	5.65	10.27
Flagellum length Długość witki [µm]	V%	45.27 ± 0.07^{D}	43.50 ± 0.13^{A}	44.51 ± 0.15^{C}	44.06 ± 0.11^{B}
		3.15	5.63	3.84	3.29
Total length Długość plemnika [µm]	$\bar{x}$ ± SEM	54.45 ± 0.08^{C}	52.59 ± 0.13^{A}	53.88 ± 0.16^{BC}	53.32 ± 0.13^{AB}
	V%	2.94	4.73	3.39	3.00

Explanations see Table 1 – Objaśnienia zob. tab. 1.

The sperm of Landrace boars had the largest head area (on average 40.62 µm^2). The head area of the sperm of these boars was larger than in the case of the Large White, Duroc × Pietrain and Duroc boars by 1.41, 0.8 and 0.29 µm^2, respectively ($P \leq 0.01$). The spermatozoa of the Large White boars had the shortest heads and their tails were significantly shorter than those of the sperm of other breeds ($P \leq 0.01$). No differences were demonstrated between breeds in the perimeter and width of the sperm heads. Sperm

dimensions and shape are therefore characterized by distinct inter-breed variation. Intra-breed variation, on the other hand, was very low and did not exceed a few percent for most of the morphometric sperm characteristics.

DISCUSSION

The data presented in the study indicate pronounced differences in ejaculate volume between the analysed boar breeds. The ejaculates of the Landrace and Large White boars had the greatest volume (over 249 ml). Wolf and Smital (2009), in a study conducted on Landrace and Large White boars, noted a somewhat higher ejaculate volume for these breeds (about 275 ml). Duroc breeders produced ejaculates of smaller volume, over 64 ml less than in the case of Landrace boars, but with higher sperm concentration by over 64 $\times 10^6$ cells/ml. Duroc boars are distinguished by high sperm concentration in a small volume of ejaculate (Park and Yi 2002; Smital 2009; Kondracki et al. 2011). This also applies to crossbreds with this breed, due to paternal heterosis (Wysokińska and Kondracki 2013; Knecht et al. 2014; Carvajal et al. 2004). The highest variation in the total ejaculate sperm count was noted in the Landrace and Large White breeds (V% > 37). Variation in the total number of sperm with progressive motility in the ejaculate is determined by breed, individual variation between boars, and individual variation in ejaculates from the same boar (Chukwumeka et al. 2005). A significant problem at insemination stations is the high individual variation of boars of a given breed, and even variation between individual ejaculates from the same boar. That is why there is a need for greater attention to individual variation and to the suitability of individual animals for insemination.

The results of the study indicate a small dependency of the frequency of morphological sperm abnormalities on the breed of boar. The percentage of morphologically normal sperm in the semen of the boars analysed was over 94%. The increased frequency of morphological sperm abnormalities in the semen of the Duroc boars may be secondarily generated in the tail of the epididymis and in the vas deferens as a result of less comfortable conditions for the development of spermatozoa after they leave the seminiferous tubules, due to their high concentration (Banaszewska and Kondracki 2012). The dependency of the frequency of morphological sperm defects on genetic factors is confirmed in the literature. Some studies have shown pronounced differences in the frequency of morphological sperm anomalies in the semen of boars of different breeds (Park and Yi 2002). Semen obtained from breed crosses of Duroc, Hampshire and Pietrain boars is of high quality (Wysokińska and Kondracki 2013). High-quality ejaculates obtained from Landrace boars have been reported in previous studies (Wysokińska et al. 2009). According to Blom (1981), semen should contain no more than 15% sperm cells with major defects and 10–15% with minor defects. Bonet (1990) posits that if the percentage of morphological abnormalities in boar sperm exceeds 20%, the semen is disqualified for insemination. According to Pena et al. (2006), boars used for insemination should produce ejaculates in which at least 90% of sperm have normal head structure. The breed factor has a certain influence on the frequency of morphological sperm abnormalities. The present study, however, indicates that factors other than breed have a much greater influence on the frequency of morphological sperm defects, as intra-breed variation was very high: V% = 164.16–236.20 for the frequency of major morphological defects and V% = 90.51–144.18 for the frequency of minor morphological defects.

The results of the present study document the occurrence of inter-breed variation in the dimensions and shape of spermatozoa. The sperm of the Landrace boars had longer tails and greater total length than the sperm of the other breeds, which may beneficially affect their motility. Sperm motility is influenced by the length of the midpiece and tail (Simpson et al. 2013). In semen with a reduced percentage of sperm exhibiting progressive motility, the sperm usually have shorter tails than the sperm in semen in which this percentage is high (Noorafshan and Karbalay-Doust 2010). Sperm with longer tails are likely to be more competitive than other sperm and able to reach the ovum faster (Helfenstein et al. 2010; Garcia-Vazquez et al. 2015). Radwan (1996) suggests that the total length of the spermatozoon influences the percentage of sperm competing to reach the ovum. The shape of sperm may also depend on the number of sperm in the ejaculate (Wysokińska et al. 2009), and their dimensions depend on the sperm concentration in the ejaculate (Pinart et al. 1998; Kondracki et al. 2013a).

A study conducted on dogs showed that in ejaculates with a high sperm concentration the sperm have shorter tails than sperm from ejaculates with a lower sperm concentration (Rijsselaere et al. 2004). The data obtained in the present study indicate that the sperm of the Large White boars had a smaller head area than the sperm of the other breeds. The size and shape of the sperm head may play a significant role in the fertilization process (Hirai et al. 2001; Yaniz et al. 2015). Variation in sperm head dimensions may result from differences in chromatin structure (Sailer et al. 1996). According to some authors, even small changes in the shape of the sperm head may be linked to changes in chromatin structure in the nucleus (Ostermeier et al. 2001), and this may reduce fertility in the male (Evenson and Wixon 2006) and affect sperm sensitivity (Wysokińska et al. 2015). The shape and size of sperm heads may influence sperm motility (Malo et al. 2006). The sperm of highly fertile boars have smaller and shorter heads than the sperm of boars with a lower fertilization rate (Hirai et al. 2001). Saravia et al. (2007) suggest that detection of subtle differences in the morphometry of the sperm head may lead to new possibilities for precise evaluation of the boar spermiogram. The data obtained in the present study document the occurrence of marked differences in sperm dimensions and shape in boars of different breeds. Inter-breed variation is thus pronounced, whereas intra-breed variation was very low, not exceeding a few percent for most of the morphometric sperm traits.

RECAPITULATION

In conclusion, there is relatively high intra- and inter-breed variation in the basic characteristics of ejaculates. Characteristics with high variation include ejaculate volume and ejaculate sperm count. The Duroc breed is distinguished by relatively low variation in these traits. There is also pronounced inter-breed variation for these characteristics. The ejaculates of the Landrace and Large White boars have markedly greater volume than those of the Duroc boars or the Duroc × Pietrain crossbreds, while the ejaculates of the Landrace boars contain more sperm cells than those of boars of other breeds. The spermatozoa of the crossbred boars were exhibited lower motility than the sperm of other breeds. In the ejaculates of all breeds a very low frequency of sperm with major and minor defects was observed. The frequency of morphological sperm abnormalities is highly varied within breeds, but shows

relatively little variation between breeds. The morphometric dimensions of sperm cells are characterized by low intra-breed variation and relatively high inter-breed variation. The spermatozoa of the Landrace boars have markedly longer tails and a larger head area than the sperm of the other breeds.

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Abstract. The aim of this study was to evaluate inter-bred and intra-breed variation in ejaculates and the morphology, dimensions and shape of spermatozoa of boars used for artificial insemination. The results obtained from this study suggested that there is relatively high intra- and inter-breed variation in the basic characteristics of ejaculates. The ejaculates of the Landrace and Large White boars have markedly greater volume than those of the Duroc boars or the Duroc × Pietrain crossbreds, while the ejaculates of the Landrace boars contain more sperm cells than those of boars of other breeds. The spermatozoa of the crossbred boars exhibit lower motility than the sperm of other breeds. Results revealed that factors other than breed have a much greater influence on the frequency of morphological sperm defects. The morphometric dimensions of sperm cells are characterized by low intra-breed variation and relatively high inter-breed variation. The results showed that the sperm of Landrace boars had the largest head area. The spermatozoa of the Large White boars had the shortest heads and their tails were significantly shorter than those of the sperm of other breeds.