Polish Journal of Veterinary Sciences Vol. 26, No. 4 (2023), 695–704

DOI 10.24425/pjvs.2023.148289

Original article

Association between sperm morphology and sperm count of boar semen

S. Kondracki[1], K. Górski[1], M. Iwanina[1], W. Kordan[2], M. Lecewicz[2]

[1] University of Siedlce, Faculty of Agricultural Sciences, Prusa 14, 08-110 Siedlce, Poland
[2] University of Warmia and Mazury in Olsztyn,
Department of Animal Biochemistry and Biotechnology, Oczapowskiego 13, 10-719 Olsztyn, Poland

Abstract

The number of spermatozoa in the ejaculate is important for its quality and that of the sperm contained in it. The number of ejaculated spermatozoa is also associated with sperm dimensions. The aim of this study was to assess the morphological structure of sperm and the frequency of morphological abnormalities in sperm on the ejaculation performance of boars, measured as the total number sperm per ejaculate. The study was conducted using 648 ejaculates collected from 31 Large White boars and 30 Landrace boars. All ejaculates were analysed for basic physical characteristics and the frequency of sperm with morphological abnormalities. In addition, morphometric measurements of the sperm were made and used to calculate their shape indexes. As a result of our study it was noted that sperm from ejaculates with the most spermatozoa have shorter heads with a smaller area than sperm from ejaculates with a small or intermediate number of spermatozoa. Landrace boars produce semen of better quality, with a smaller percentage of sperm with major abnormalities, and the differences between the breeds increase with the number of spermatozoa in the ejaculate. The sperm from Landrace boars have larger heads and longer flagella than the sperm from Large White boars. The differences in sperm dimensions between breeds decrease as the total number of spermatozoa in the ejaculate increases. The number of spermatozoa in the ejaculate was shown to influence the dimensions of the sperm. The effect of the number of ejaculated sperm on ejaculate characteristics and sperm morphology depends on the breed of the male.

Keywords: boar, sperm count, sperm dimension, sperm morphology

Introduction

Predicting fertility is of great importance for optimizing and maximizing the reproductive capacity of males (Rahman et al. 2017). Boars used for artificial insemination should produce a large number of sperm with high fertilization capacity (Henning et al. 2022). Fertility is the result of a combination of factors associated with both the external environment and genetic determinants (Lopez Rodriguez et al. 2017). The ejaculation performance of males and the quality of the sperm produced depend on factors such as age and breed

Correspondence to: K. Górski, e-mail: krzysztof.gorski@uph.edu.pl

(Wysokińska and Kondracki 2019, Madrigal-Valverde et al. 2020). Ejaculate characteristics and sperm quality are also influenced by the time of year and the intensity of use for breeding (Kondracki et al. 2013, Górski et al. 2017). Analysis of sperm morphology plays an important role in semen assessment. Sperm morphology and morphometric measurements are important parameters in predicting male fertility (Lasiene et al. 2013). The frequency of sperm with morphological abnormalities reflects the degree of disturbance of the spermatogenesis process (Górski et al. 2018). Normal sperm morphology is a condition for fusion with the oocyte and enables genetic information to be transmitted during fertilization (Nagy et al. 2018). Changes taking place in the structure of the sperm cell membrane during capacitation and the acrosome reaction are important for the fusion of the spermatozoon with the oocyte (Barquero et al. 2021). The zona pellucida of the oocyte is a significant barrier for sperm with morphological abnormalities (Moros-Nicolás et al. 2021). The suitability of semen for artificial insemination may be influenced by the morphological characteristics of the sperm (Gaggini et al. 2017), and the size of the sperm head may be a factor determining fertilization capacity (Stasiak et al. 2021). The presence of sperm with head defects in the semen has been shown to be a cause of abortion in early pregnancy (Cao et al. 2017). Differences in the head size of the sperm of fertile males and those with reduced fertility have been noted in boars (Banaszewska and Andraszek 2021). Morphological characteristics may depend on the intensity of sperm production in the testicular tissue and the associated number of sperm stored in the epididymis and released in the ejaculate.

In the present study, an attempt was made to assess the dependency of ejaculate characteristics, the morphological structure of sperm, and the frequency of morphological abnormalities in sperm on the ejaculation performance of boars of the Large White and Landrace breeds, measured as the total number of sperm per ejaculate.

Materials and Methods

The study comprises accepted methods and standard operation procedures for boar semen processing. The centres in this research complied with the European Council Directive, 2008/120/EC outlining minimum standards for the protection of pigs, as well as with 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes. The study was approved by the Polish Laboratory Animal Science Association (Number 3401/2015).

Animals and ejaculate collections

The study was conducted using 648 ejaculates collected from 31 boars of the Large White breed and 30 boars of the Landrace breed used for artificial insemination. Young boars (7-9 months of age) at the start of their use for breeding were selected for the study. These boars were housed individually in pens, and were used regularly for AI (artificial insemination) purposes. Food intake was individualized for each boar according to nutrition requirements, with ad libitum access to water. Boars received 2.5-3.0 kg of full-ration feed per day with a content of 12 MJ/kg and 15.5% of total protein. Ejaculates were collected by the manual method (King and Macpherson 1973), by the gloved-hand technique discarding the gelatinous fraction, at one-month intervals. To eliminate the effect of the time of year, the ejaculates were collected regularly throughout the year. Each male provided at least 10 ejaculates for examination.

Experimental design

Ejaculates from Large White and Landrace boars were assigned to three groups according to the total number of spermatozoa in the ejaculate. Group I comprised ejaculates in which the number of sperm was lower than average for the breed by at least half the standard deviation ($<x \pm ½SD$). Group II consisted of ejaculates in which the number of sperm was within the range of ± half of the standard deviation from the mean for the breed ($x \pm ½SD$). Group III comprised ejaculates in which the number of sperm was higher than the mean for the breed by at least half the standard deviation ($>x \pm ½SD$).

Semen evaluation

The following physical characteristics were determined in the freshly collected ejaculates: ejaculate volume, sperm concentration, sperm motility, total number of spermatozoa in the ejaculate, and number of insemination doses obtained from one ejaculate. The ejaculate volume was identified by isolating the gelatinous fraction based on the weight of the ejaculate measured on an electronic scale. This was calculated using a density of 1.0 g/ml. Sperm concentration was measured using a spectrophotometer (IMV Technologies, France). The sperm was diluted in Biosolwens Plus (Biochefa, Poland). A blank tube was filled with 2.4 ml of 0.9% NaCl. A Nikon Eclipse 50i light microscope equipped with a heated stage was used to examine the percentage of sperm showing progressive movement thereby assessing the sperm motility. The number of spermatozoa in the ejaculate and the number

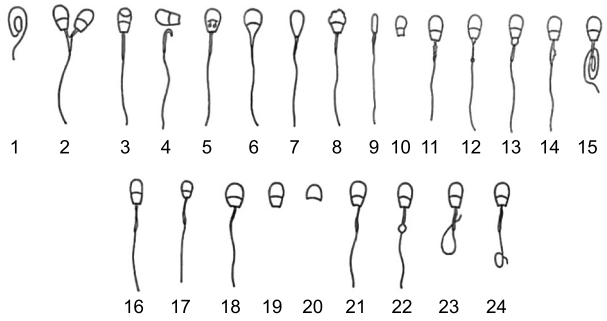

Fig. 1. Morphological sperm defects (Blom 1981).
Major sperm defects: 1. Underdeveloped, 2. Double forms, 3. Acrosome defect (knobbed acrosome), 4. Decapitated sperm defect (active tails), 5. Diadem defect, 6. Pear-shaped defect, 7. Narrow at base, 8. Abnormal contour, 9. Small abnormal heads, 10. Free pathological heads, 11. Corkscrew defect, 12. Tail stump, 13. Proximal droplet, 14. Pseudodroplet, 15. Strongly coiled or folded tail ("Dag" defect).
Minor sperm defects: 16. Narrow heads, 17. Small normal heads, 18. Giant and short broad heads, 19. Free normal heads, 20. Detached acrosome membranes, 21. Abaxial implantation, 22. Distal droplet, 23. Simple bent tail, 24. Terminally coiled tail.

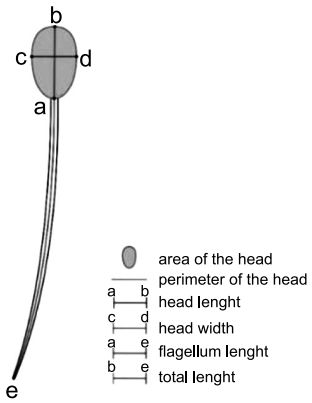

Fig. 2. Method of performing morphometric sperm measurements.
Head length – segment a-b (the blue line), head width – segment c-d (the black line), head area – the area of the region limited by the green line, head perimeter – the length of the green line, tail length – the length of the curve a-e (signed with the red colour).

of insemination doses obtained per ejaculate were calculated using WINSUL computer software.

Sperm morphology

Samples of all ejaculates were taken for the preparation of microscope slides. The slides were prepared and stained according to the methods described by Kondracki et al. (2017). The eosin-gentian staining method was used. Sperm morphology was examined using a Nikon Eclipse 50i light microscope with immersion lenses at x 100 magnification. The morphology of 500 spermatozoa on each slide was examined. The numbers of normal and abnormal sperm were determined, distinguishing forms with major and minor abnormalities according to the method described by Blom (1981). The morphological forms of the sperm are shown in Fig. 1.

Sperm morphometry

Morphometric measurements were made in 15 randomly selected normal spermatozoa in each ejaculate. A total of 9720 spermatozoa were measured. The measurements were made manually using image analysis software (Screen Measurement v. 4.1), according to the method described by Kondracki et al. (2005). The following measurements were performed: head length – established as the length of a-b line where a is the point where the head and the connecting piece (the tail neck) adjoin, and b is the farthest point a at the tip of the head; head width – the length of c-d line where c and d are the farthest sticking points from each other on the edge of sperm cell head, and the line was drawn perpendicular to the long axis of the sperm head at the height of ½ an acrosome; head area – measuring the area limited by a curve extending along the perimeter of the sperm head; head perimeter – length of the boundary limiting the sperm head; tail length – the length of a-e curve drawn along the long axis of the tail, where a is defined as above, and e is the tail tip and total sperm cell length – the length tail and head (Fig. 2).

Based on the measurements, the following indices of the morphological structure of the sperm were calculated:

– Head width/head length × 100
– Head length/total length × 100

Table 1. Basic characteristics of ejaculates and frequency of occurrence of normal and abnormal spermatozoa in relation to the total number of spermatozoa of Large White and Landrace boars (mean±SD).

Parameter	Total number of spermatozoa		
	Group I	Group II	Group III
Number of ejaculates (n)	232	244	172
Total number of spermatozoa (× 10^9)	56.59±11.08[a]	84.54±10.05[b]	127.24±23.85[c]
Ejaculate volume (mL)	198.29±64.14[a]	249.91±63.04[b]	347.34±95.28[c]
Sperm concentration (×10^6/mL)	405.13±104.26[a]	447.78±86.62[b]	474.35±102.96[c]
Percentage of spermatozoa with normal motility	76.03±4.90[a]	78.16±3.89[b]	78.90±3.32[b]
Number of insemination doses per ejaculate	20.50±4.37[a]	28.88±4.58[b]	41.59±9.17[c]
Percentage of normal spermatozoa	96.10±3.74	95.31±6.18	95.11±6.10
Percentage of major sperm abnormalities	0.89±1.63	0.86±1.84	0.92±1.43
Percentage of minor sperm abnormalities	3.01±3.09	3.83±5.48	3.98±5.17

[(a,b,c)] Values within a row are significantly different ($p \leq 0.05$).

– Perimeter of the head/total length × 100
– Head area/total length × 100
– Head length × head width/total length × 100
– Flagellum length/total length × 100
– Head length/flagellum length × 100

The slides were prepared and assessed microscopically by the same person.

Statistical analysis

Experimental data were analyzed using the Statistica 13.1 PL program (StatSoft, Tulsa, OK, USA). The material obtained was statistically analysed according to the following mathematical model:

$$Y_{ijk} = \mu + a_i + b_j + ab_{ij} + e_{ijk}$$

where: Y_{ijk} - value of the parameter analysed, μ - populational mean, a_i – the effect of sperm count, b_j – the effect of boar breed, ab_{ij} – the effect of interaction between factors, e_{ijk} – error. Two-way analysis of variance (ANOVA) was performed. The significance of the differences between the groups was assessed using the Tukey test at $p \leq 0.05$.

Results

Table 1 presents the physical characteristics of the ejaculate and the frequency of sperm abnormalities in relation to the total number of spermatozoa in the ejaculate.

The data indicate that an increase in the total number of spermatozoa is associated with an increase in ejaculate volume and sperm concentration and, to a lesser extent, with an increase in sperm motility ($p \leq 0.05$). In the group of ejaculates with the fewest sperm (group I) the average ejaculate volume was 198.29 ml, which was 51.62 ml lower than in group II and 149.05 ml lower than in group III ($p \leq 0.05$). The data in Table 1 also show that ejaculates containing more sperm have a higher sperm concentration, which was confirmed statistically ($p \leq 0.05$). In group III the average sperm concentration in the ejaculate was 474,350/mm^3, which was more than 26,000/mm^3 higher than in group II ($p \leq 0.05$) and more than 69,000/mm^3 higher than in group I ($p \leq 0.05$). The differences in sperm motility were much less pronounced. However, the lowest percentage of sperm with normal motility was noted in the ejaculates with the fewest sperm (group I). In those ejaculates, the percentage of sperm with normal motility was more than 2% lower than in groups II and III ($p \leq 0.05$). More insemination doses can be prepared from ejaculates containing more sperm. On average 12.71 more insemination doses were prepared from the ejaculates from group III than from the group II ejaculates, and 21.09 more than from the group I ejaculates I ($p \leq 0.05$).

Table 1 also presents the frequency of morphological abnormalities in the sperm of ejaculates with a low, medium and high total number of spermatozoa. The average percentage of normal sperm ranged from 95.11% to 96.10% and showed no significant association with the total number of spermatozoa in the ejaculate. The average percentage of sperm with major abnormalities was very small, not exceeding 0.92%, and the differences between groups were small and not confirmed statistically. The highest percentage of sperm with minor abnormalities was noted in the ejaculates assigned to group III (on average 3.98%). However, the differences between groups were not confirmed statistically.

Table 2 presents data enabling assessment of the relationship between the physical characteristics of the ejaculate and the total number of spermatozoa in the ejaculate, taking into account the breed of boar.

Table 2. Basic characteristics of spermatozoa and frequency of occurrence of normal and abnormal spermatozoa in relation to the total number of spermatozoa of Large White and Landrace boars (mean±SD).

Parameter	Breed	Total number of spermatozoa		
		Group I	Group II	Group III
Number of ejaculates (n)	LW	112	119	95
	L	120	125	77
Sperm concentration ($\times 10^6$/mL)	LW	382.79±108.54$^{a\,d}$	452.83±86.06^{b}	462.29±106.05^{b}
	L	425.99±95.93$^{a\,e}$	442.97±87.23^{a}	489.22±97.65^{b}
Ejaculate volume (mL)	LW	200.82±74.16^{a}	230.40±57.11$^{b\,d}$	336.55±104.53$^{b\,d}$
	L	195.93±53.33^{a}	268.48±63.02$^{b\,e}$	360.65±81.15$^{c\,e}$
Total number of spermatozoa ($\times 10^9$)	LW	52.05±10.93$^{a\,d}$	79.72±9.01$^{b\,d}$	120.01±19.51$^{c\,d}$
	L	60.83±9.44$^{a\,e}$	89.14±8.79$^{b\,e}$	136.16±25.75$^{c\,e}$
Percentage of spermatozoa with normal motility	LW	75.09±5.02$^{a\,d}$	78.15±3.90^{b}	79.47±2.68^{c}
	L	76.92±4.64$^{a\,e}$	78.16±3.89^{b}	78.18±3.88^{b}
Number of insemination doses per ejaculate	LW	19.51±4.65$^{a\,d}$	26.85±4.28$^{b\,d}$	38.66±7.77$^{c\,d}$
	L	21.42±3.88$^{a\,e}$	30.82±3.99$^{b\,e}$	45.21±9.51$^{c\,e}$
Percentage of normal spermatozoa	LW	96.15±3.67^{a}	95.07±6.26^{ab}	94.28±7.02$^{b\,d}$
	L	96.06±3.82	95.54±6.12	96.14±4.47^{e}
Percentage of major sperm abnormalities	LW	0.86±1.06^{a}	1.17±2.29$^{ab\,d}$	1.44±1.73$^{b\,d}$
	L	0.92±2.03^{a}	0.57±1.22$^{ab\,e}$	0.28±0.38$^{b\,e}$
Percentage of minor sperm abnormalities	LW	2.99±3.11^{a}	3.77±5.25^{ab}	4.29±5.74^{b}
	L	3.03±3.08	3.88±5.71	3.59±4.36

$^{(a,b,c)}$ Values within a row are significantly different ($p \leq 0.05$).
$^{(d,e)}$ Values within a column are significantly different ($p \leq 0.05$).
LW – Large White, L – Landrace.

The data in Table 2 show that the characteristics of the ejaculates of both the Large White and Landrace breeds are associated with the total number of spermatozoa in the ejaculate. An increase in the number of sperm was associated with significant increases in sperm concentration, ejaculate volume, the percentage of sperm with normal motility, and the number of insemination doses obtained per ejaculate ($p \leq 0.05$), irrespective of the breed of boar. Ejaculates from the two breeds differed significantly in sperm concentration and the percentage of sperm with normal motility only in group I. In the remaining groups, the differences between breeds were negligible and were not confirmed statistically. However, the data in Table 2 show marked differences between breeds in favour of the ejaculates of Landrace boars, as these contained many more sperm, and more insemination doses could be prepared from them. The differences between breeds were confirmed statistically for every group of ejaculates ($p \leq 0.05$). It is worth noting, however, that the differences between breeds were relatively small in ejaculates with a small number of spermatozoa (group I) but increased in ejaculates with an intermediate (group II) and large (group III) number of sperm (Figs. 3 and 4).

The data in Table 2 show that sperm quality was high in the ejaculates of boars of both breeds. The average percentage of sperm with major abnormalities did not exceed 1.44% in either breed, and the percentage of sperm with minor abnormalities did not exceed 4.29%. It is notable, however, that Landrace boars produced semen with better morphology than Large White boars, and the differences between breeds increase together with the total number of spermatozoa in the ejaculates (Figs. 5 and 6).

In the group of ejaculates with the highest number of sperm (group III), the percentage of sperm with major abnormalities was 1.16% lower in Landrace boars than in Large White boars ($p \leq 0.05$). In group II the difference was smaller but significant, amounting to 0.60% ($p \leq 0.05$), whereas in group I the differences between breeds were negligible. It is also worth noting that in the ejaculates of Landrace boars the percentage of sperm with major abnormalities decreased as the total number of spermatozoa in the ejaculate increased, whereas in the ejaculates of Large White boars it increased (Table 2).

Table 3 presents the results of morphometric measurements of sperm in relation to the total number of spermatozoa in the ejaculate.

The data in Table 3 show that some sperm dimensions depend on the total number of spermatozoa in the ejaculate. Certain trends can be observed for sperm

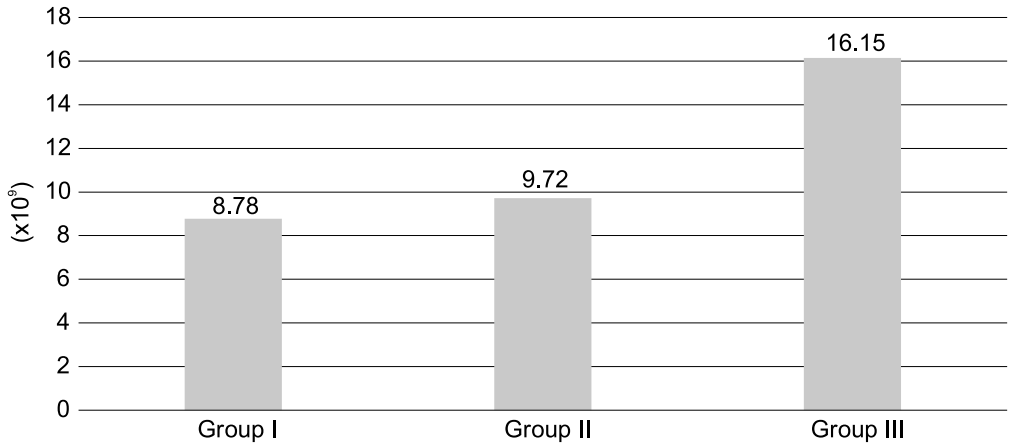

Fig. 3. Differences between breeds (L-LW) in the total number of spermatozoa in the ejaculates of groups I, II and III.

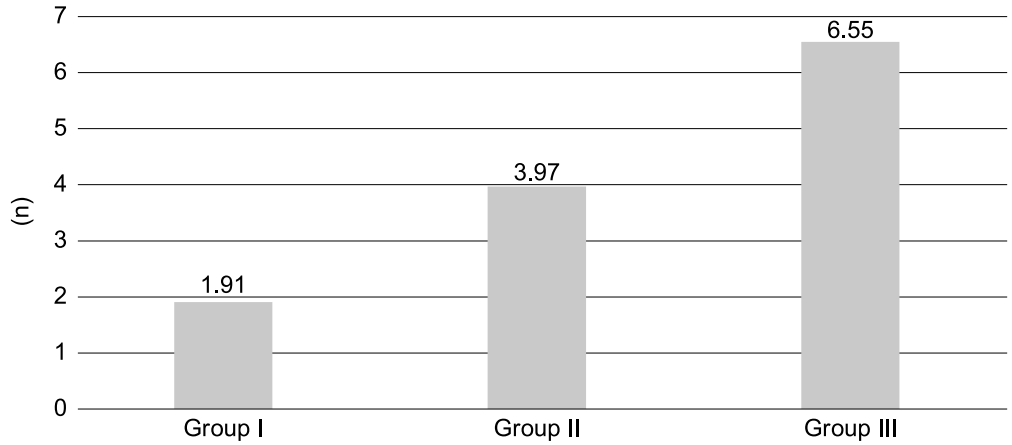

Fig. 4. Differences between breeds (L-LW) in the number of insemination doses in the ejaculates of groups I, II and III.

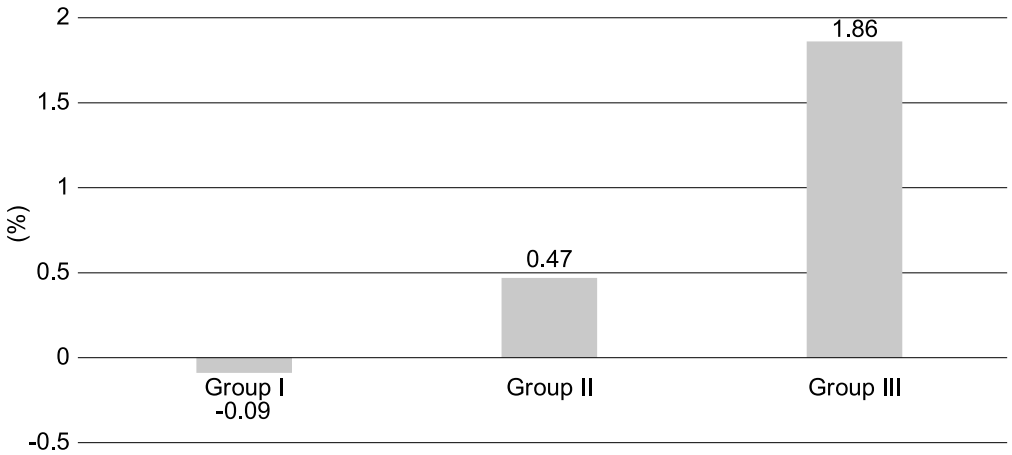

Fig. 5. Differences between breeds (L-LW) in the percentage of normal spermatozoa in the ejaculates of groups I, II and III.

head length. Sperm from ejaculates assigned to group III had significantly shorter heads ($p \leq 0.05$) than sperm from ejaculates with an intermediate or small number of sperm (groups I and II). Sperm heads in ejaculates from group III also had a smaller area than the heads of sperm from ejaculates from groups I and II ($p \leq 0.05$). Sperm from the ejaculates with the smallest number of sperm (group III) had the smallest total length. Sperm from these ejaculates were 0.50 µm shorter than sperm from the group I ejaculates ($p \leq 0.05$).

Table 3 also presents indexes enabling assessment of the differences in the shape of sperm released in ejaculates differing in the number of sperm. The data show that the total number of spermatozoa in the ejacu-

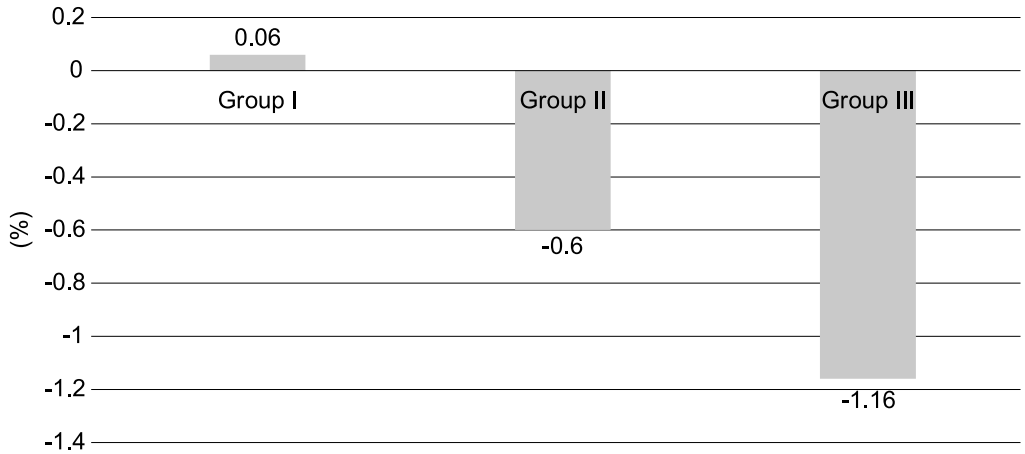

Fig. 6. Differences between breeds (L-LW) in the percentage of sperm with major abnormalities in the ejaculates of groups I, II and III.

Table 3. Morphometric characteristics and indexes of spermatozoa in relation to the total number of spermatozoa (mean±SD).

Parameter	Total number of spermatozoa		
	Group I	Group II	Group III
Number of ejaculates (n)	232	244	172
Head length (µm)	9.17±0.38[a]	9.19±0.33[a]	9.09±0.33[b]
Head width (µm)	4.73±0.30	4.85±1.19	4.72±0.28
Perimeter of the head (µm)	23.39±0.97	23.55±1.33	23.34±0.78
Head area (µm^2)	40.00±2.76[a]	40.19±2.76[a]	39.29±2.67[b]
Flagellum length (µm)	44.66±1.75	44.23±3.05	44.25±1.51
Total length (µm)	53.84±1.94[a]	53.42±3.13[ab]	53.34±1.55[b]
Head width/head length	51.56±3.10	52.91±4.13	51.90±3.09
Head length/total length	17.04±0.64	17.37±3.22	17.06±0.70
Perimeter of the head/total length	43.48±1.73	44.93±6.85	43.77±1.58
Head area/total length	74.30±4.76	76.11±8.18	73.67±4.81
Head length × head width/total length	80.57±6.30	87.98±1.93	80.48±6.13
Flagellum length/total length	82.96±0.64	82.63±3.22	82.94±0.70
Head length/flagellum length	20.51±0.95	20.57±0.88	21.56±1.84

[(a,b)] Values within a row are significantly different ($p \leq 0.05$).

late has a minor effect on the shape of boar sperm. The values of individual sperm shape indexes (Table 3) in the groups were similar; the differences between groups were small and not confirmed statistically.

Table 3 presents data enabling assessment of the dependency of sperm dimensions and shape on the total number of spermatozoa in the ejaculate and on the breed of boar.

The data reveal differences in the dimensions of sperm from Large White and Landrace boars. The sperm of Landrace boars had larger dimensions than the sperm from Large White boars. This was most evident in the case of flagellum length, which was about 2 µm longer in Landrace boars than in Large White boars. The total sperm length was also greater in the Landrace breed than in Large White boars. The differences between breeds in flagellum length and total sperm length were confirmed statistically for all groups of ejaculates ($p \leq 0.05$).

The sperm of Landrace boars also had larger heads than the sperm of Large White boars. This was most evident in the case of head area. The average head area of the sperm from Landrace boars was 1.04-2.40 µm^2 greater than in Large White boars ($p \leq 0.05$), with the greatest difference noted in the case of the ejaculates with the smallest number of spermatozoa (group I). This difference was smaller in groups II and III, as the total number of spermatozoa in the ejaculate increased (Fig. 7).

The larger head size of the sperm of Landrace boars can also be seen in the head length and perimeter, but this was demonstrated only for the ejaculates with the smallest number of spermatozoa (group I).

The data in Table 3 show that the differences in the

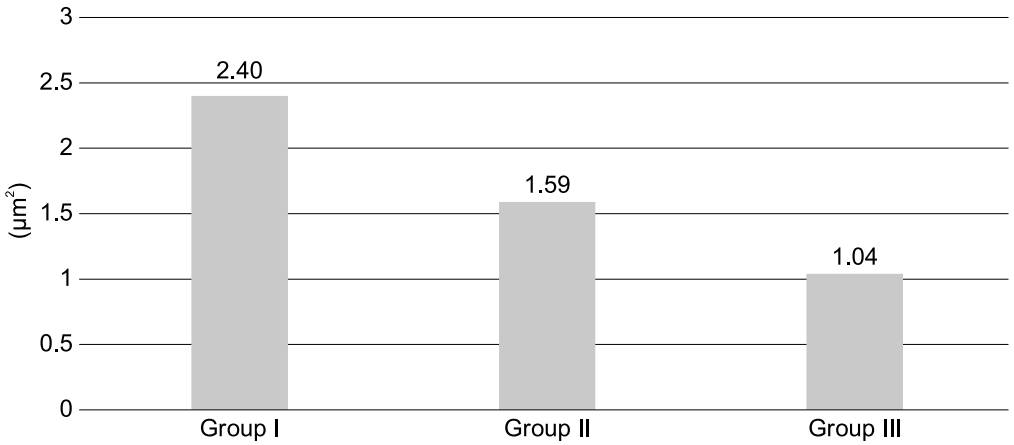

Fig. 7. Differences between breeds (L-LW) in the head area of sperm in the ejaculates of groups I, II and III.

morphometric indexes of spermatozoa, characterizing their shape, were generally minor and did not show a clear direction. Only the sperm of Large White boars from ejaculates assigned to group III (with the highest number of spermatozoa) were shown to be highly elongated. This is indicated by the head length/flagellum length ratio, which was 22.77 − about 2.0 times greater than in ejaculates from groups I and II and more than 2.4 times greater than in ejaculates from Landrace boars ($p \leq 0.05$).

Discussion

The results of the present study demonstrate a relationship between the number of spermatozoa in the ejaculate and other ejaculate characteristics. A study by Kondracki et al. (2006) in Large White boars demonstrated that sperm concentration has a marked effect on the number of sperm in the ejaculate. As the sperm concentration increased, there was an increase in the number of sperm but a decrease in the ejaculate volume. However, a study by Wysokińska et al. (2009) found that sperm motility was not dependent on the number of sperm in the ejaculate.

The ejaculates of Large White boars contained more sperm with major abnormalities than the ejaculates of Landrace boars, which was demonstrated for ejaculates with a medium and high content of sperm (groups II and III). This suggests that Large White boars produce semen of somewhat lower quality. The poorer quality of the sperm of this breed was also demonstrated in a recent study by Dotché et al. (2021) which showed that the semen of Large White boars contained more sperm with morphological defects than the semen of local breeds used in Benin. Determination of the normality of sperm head structure is currently taking on particular importance. The size and shape of the sperm head have become an important criterion for classifying morphologically normal sperm or characterizing abnormalities in their structure. Gaggini et al. (2017) showed that the heads of boar sperm with a cytoplasmic droplet (proximal or distal) are longer but narrower than those of normal sperm. Sperm with major abnormalities may have damaged chromatin structure or a high level of DNA damage (Enciso et al. 2011). Significant sperm abnormalities arise in the last stage of spermatogenesis. The percentage of sperm with major abnormalities determines the fertilization capacity of semen and the fertility of the male (Lee et al. 2014). Acrosome defects in particular reduce the chance of fertilization (Chenoweth 2005).

The results of the present study demonstrate that the number of sperm influences the head dimensions and flagellum length of sperm. Head length, perimeter and area as well as flagellum length and total sperm length in Landrace boars were inversely proportional to the total number of spermatozoa in the ejaculate. As the number of sperm increased, the head dimensions, flagellum length, and total sperm length decreased. This indicates that the association between sperm dimensions and the total number of spermatozoa in the ejaculate depends on the breed of boar. The dimensions and shape of sperm can be of significance for male fertility (Barquero et al. 2021). Larger sperm head dimensions may be evidence of disturbances in the spermatogenesis process or may result from changes in chromatin structure during the maturation and transport of sperm. A study using Nelore bulls showed that sperm with chromatin instability have heads with a larger area than sperm without chromatin anomalies (Kipper et al. 2017). Some researchers claim that males that produce sperm with larger heads, especially wider heads, are less fertile. Waheed et al. (2015) demonstrated that highly fertile stallions produce sperm whose heads have a smaller width, area and perimeter than stallions with low fertility. According to Hirai et al. (2001) the sperm of highly fertile boars have smaller,

shorter heads. The sperm head affects the hydrodynamics of the spermatozoon. According to Malo et al. (2006) sperm with elongated heads move faster than sperm with round heads. Sperm with greater ellipticity values (head length/head width) are less capable of straight progressive movement (Gil et al. 2009).

A study by Wysokińska et al. (2009) showed that the total number of spermatozoa in the ejaculate can influence the shape of sperm heads and the proportions of the dimensions of individual organelles. The morphometric characteristics of sperm were also shown to depend on ejaculate volume and sperm concentration in the ejaculate (Kondracki et al. 2014, Kondracki et al. 2020, Górski et al. 2021). The results of some studies indicate that variation in sperm head dimensions is associated with DNA organization (Jakubik-Uljasz et al. 2020). Even minor deviations from the typical sperm head shape may be due to changes in chromatin structure in the cell nucleus. In consequence, the capacity of the sperm to fertilize the oocyte is linked to the shape of the sperm head (Ostermeier et al. 2001). An abnormal head shape is often associated with disturbances in chromatin condensation, which results in elongation and narrowing of the sperm head. Assessment of the normality of sperm heads is particularly important because the size and shape of the head are an important criterion for qualifying the sperm as morphologically normal and for identifying abnormalities, in order to establish the fertilization capacity of the sperm (Maree et al. 2010). The structure and arrangement of microfibres in the sperm head may have a role in determining its dimensions. The cytoskeleton of the sperm head consists of nuclear proteins and a nuclear envelope, which are partially responsible for the formation of the nucleus (Dvořaková et al. 2005).

It is not only the size of the sperm head, however, that affects fertilization capacity. The dimensions and functioning of the flagellum and midpiece are important as well. According to Noorafshan and Karbalay-Doust (2009), sperm length is positively correlated with the speed of its movement. Sperm with longer flagella have greater flagellum strength, which allows them to move and reach the oocyte faster (Pesch and Bergmann 2006). In the light of these reports, the sperm from the Landrace boars seem to have an advantage, as they had much longer flagella and greater total length than the sperm from Large White boars.

Conclusion

The study showed that an increase in the number of spermatozoa in the ejaculates of boars is associated with an increase in ejaculate volume and sperm concentration, and to a lesser degree with the percentage of sperm with normal motility as well. Sperm from ejaculates with the highest number of sperm have shorter heads with a smaller area than sperm from ejaculates with a small or intermediate number of sperm. Their total length is shorter as well. The total number of spermatozoa in the ejaculate does not have a major influence on the shape indexes of the sperm. Landrace boars produce ejaculates with more favourable characteristics than those of Large White boars. Their ejaculates contain more sperm, and more insemination doses can be obtained from them. Landrace boars also produce semen of better quality, with a smaller percentage of sperm with major abnormalities. The differences between breeds increase together with the total number of spermatozoa in the ejaculate. The sperm of Landrace boars have larger heads and longer flagella than those of Large White boars. The differences in sperm dimensions between breeds decrease as the number of sperm in the ejaculate increases.

Acknowledgements

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland.

References

Banaszewska D, Andraszek K (**2021**) Assessment of the morphometry of heads of normal sperm and sperm with the Dag defect in the semen of Duroc boars. J Vet Res 65: 239-244.

Barquero V, Roldan ERS, Soler C, Yaniz JL, Camacho M, Valverde A (**2021**) Predictive capacity of boar sperm morphometry and morphometric sub-populations on reproductive success after artificial insemination. Animals 11: 920.

Blom E (**1981**) Morphological estimation of the spermatozoa defects of bull II. Proposal of new classification of spermatozoa defects. Med Weter 37: 239-242.

Cao X, Cui Y, Zhang X, Lou J, Zhou J, Wei R (**2017**) The correlation of sperm morphology with unexplained recurrent spontaneous abortion: a systematic review and meta-analysis. Oncotarget 8: 55646-55656.

Chenoweth PJ (**2005**) Genetic sperm defects. Theriogenology 64: 457-468.

Dotché IO, Gakou A, Bankolé CB, Dahouda M, Houaga I, Antoine-Moussiaux N, Dehoux JR, Thilmant P, Koutinhouin BG, Karim A (**2021**) Semen characteristics of the three genetic types of boars reared in Benin. Asian Pac J Reprod 10: 82-89.

Dvořaková K, Moore HD, Šebková N, Palecek J (**2005**) Cytoskeleton localization in the sperm head prior to fertilization. Reproduction 130: 61-69.

Enciso M, Cisale H, Johnston SD, Sarasa J, Fernández JL, Gosálvez J (**2011**) Major morphological sperm abnormalities in the bull are related to sperm DNA damage. Theriogenology 76: 23-32.

Gaggini TS, Rocha LO, Souza ET, de Rezende FM, Antunes RC, Beletti ME (**2017**) Head morphometry and chromatin instability in normal boar spermatozoa and in spermatozoa with cytoplasmic droplets. Anim Reprod 14 (Suppl 1): 1253-1258.

Gil MC, García-Herreros M, Barón FJ, Aparicio IM, Santos AJ, Garcia-Marin LJ (**2009**) Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. Theriogenology 71: 254-263.

Górski K, Kondracki S, Iwanina M, Kordan W, Fraser L (**2021**) Effects of breed and ejaculate volume on sperm morphology and semen parameters of boars. Anim Sci J 92: e13629.

Górski K, Kondracki S, Wysokińska A (**2017**) Effects of season on semen parameters and relationships between selected semen characteristics in Hypor boars. Turk J Vet Anim Sci 41: 563-569.

Górski K, Kondracki S, Wysokińska A, Iwanina M (**2018**) Dependence of sperm morphology and ejaculate characteristics on sperm concentration in the ejaculates of Hypor boars. J Vet Res 62: 353-357.

Henning H, Luther AM, Höfner-Schmiing L, Waberski D (**2022**) Compensability of an enhanced incidence of spermatozoa with cytoplasmic droplets in boar semen for use in artificial insemination: a single cell approach. Sci Rep 12: 21833.

Hirai M, Boersma A, Hoeflich A, Wolf E, Foll J, Aumüller TR, Braun J (**2001**) Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): relation to fertility and seminal plasma growth factors. J Androl 22: 104-110.

Jakubik-Uljasz J, Gill K, Rosiak-Gill A, Piasecka M (**2020**) Relationship between sperm morphology and sperm DNA dispersion. Transl Androl Urol 9: 405-415.

King GJ, Macpherson JW (**1973**) A comparison of two methods for boar semen collection. J Anim Sci 36: 563-565.

Kipper BH, Trevizan JT, Carreira JT, Carvalho IR, Mingoti GZ, Beletti ME, Perri SH, Franciscato DA, Pierucci JC, Koivisto MB (**2017**) Sperm morphometry and chromatin condensation in Nelore bulls of different ages and their effects on IVF. Theriogenology 87: 154-160.

Kondracki S, Banaszewska D, Mielnicka C (**2005**) The effect of age on the morphometric sperm traits of domestic pigs (*Sus scrofa domestica*). Cell Mol Biol Lett 10: 3-13.

Kondracki S, Banaszewska D, Wysokińska A, Sadowska A (**2006**) Ejaculate traits and spermatozoa morphology as related to spermatozoa concentration in ejaculates of Polish Large White boars. Anim Sci Pap Rep 24: 111-119.

Kondracki S, Górski K, Iwanina M (**2020**) Impact of sperm concentration on sperm morphology of large white and landrace boars. Livest Sci 241: 104214.

Kondracki S, Górski K, Wysokińska A, Jóźwik I (**2014**) Correlation of ejaculate parameters and sperm morphology with the ejaculate volume of pietrain boars. Bulg J Agric Sci 20: 703-709.

Kondracki S, Iwanina M, Wysokińska A, Górski K (**2013**) The use of sexual activity measurements to assess ejaculatory performance of boars. Arch Tierz 56: 1052-1059.

Kondracki S, Wysokińska A, Kania M, Górski K (**2017**) Application of two staining methods for sperm morphometric evaluation in domestic pigs. J Vet Res 61: 345-349.

Lasiene K, Gedrimas V, Vitkus A, Glinskyte S, Lasys V, Valanciute A, Sienkiewicz W (**2013**) Evaluation of morphological criteria of sperm quality before in vitro fertilization and intracytoplasmic sperm injection. Pol J Vet Sci 16: 773-785.

Lee WY, Lee R, Kim HC, Lee K.H, Cui XS, Kim NH, Kim SH, Lee IJ, Uhm SJ, Yoon MJ, Song H (**2014**) Pig spermatozoa defect in acrosome formation caused poor motion parameters and fertilization failure through artificial insemination and *in vitro* fertilization. Asian-Australas J Anim Sci 27: 1417-1425.

Lopez Rodriguez A, Van Soom A, Arsenakis I, Maes D (**2017**) Boar management and semen handling factors affect the quality of boar extended semen. Porc Health Manag 3: 15.

Madrigal-Valverde M, Bittencourt RF, Brito LS, Lents MP, Santos ES, Valverde-Abarca A (**2020**) Analysis of testicular variables, semen motility and kinematics-derived indexes in boar using a CASA-Mot system. Reprod Dom Anim 55: 309-317.

Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ, Roldan ER (**2006**) Sperm design and sperm function. Biol Lett 2: 246-249.

Maree L, Du Plessis SS, Menkveld R, Van der Horst G (**2010**) Morphometric dimensions of the human sperm head depend on the staining method used. Hum Reprod 25: 1369-1382.

Moros-Nicolás C, Chevret P, Jiménez-Movilla M, Algarra B, Cots-Rodriguez P, Gonzalez-Brusi L, Aviles M, Izquierdo-Rico MJ (**2021**) New insights into the mammalian egg *zona pellucida*. Int J Mol Sci 22: 3276.

Nagy S, Tamminen T, Andersson M, Rodriguez-Martinez H (**2018**) Ejaculated boar spermatozoa displaying a rare multivesicular defect. Acta Vet Scand 60: 21.

Noorafshan A, Karbalay-Doust S (**2010**) A simple method for unbiased estimating of ejaculated sperm tail length in subject with normal and abnormal sperm motility. Micron 41: 96-99.

Ostermeier GC, Sargeant GA, Yandell BS, Evenson DP, Parrish JJ (**2001**) Relationship of bull fertility to sperm nuclear shape. J Androl 22: 595-603.

Pesch S, Bergmann M (**2006**) Structure of mammalian spermatozoa in respect to viability, fertility and cryopreservation. Micron 37: 597-612.

Rahman MS, Kwon WS, Pang MG (**2017**) Prediction of male fertility using capacitation-associated proteins in spermatozoa. Mol Reprod Dev 84: 749-759.

Stasiak K, Cygan-Szczegielniak D, Bogucka J (**2021**) Spermatozoon head size – the main differentiating feature between spermatozoa of blue and white Arctic fox (*Vulpes lagopus*). Anim Reprod 18: e20210015.

Waheed MM, Ghoneim IM, Abdou MSS (**2015**) Morphometric characteristics of spermatozoa in the Arabian horse with regard to season, age, sperm concentration, and fertility. J Equine Vet Sci 35: 244-249.

Wysokińska A, Kondracki S (**2019**) Heterosis for morphometric characteristics of sperm cells from Duroc x Pietrain crossbred boars. Anim Reprod Sci 211: 106217.

Wysokińska A, Kondracki S, Banaszewska D (**2009**) Morphometrical characteristics of spermatozoa in Polish Landrace boars with regard to the number of spermatozoa in an ejaculate. Reprod Biol 9: 271-282.