EFFECT OF SPERM CONCENTRATION ON EJACULATE FOR MORPHOMETRIC TRAITS OF SPERMATOZOAS OF THE PIETRAIN BREED BOARS

WPŁYW KONCENTRACJI PLEMNIKÓW W EJAKULACIE NA CECHY MORFOMETRYCZNE PLEMNIKÓW KNURÓW RASY PIETRAIN

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ABSTRACT

An attempt to evaluate the effect of spermatozoa concentration in one ejaculate on their measurements, shape, frequency of occurrence of morphological abnormalities in spermatozoa and physical traits of boar ejaculates in Pietrain breed was made.

It was concluded that there was a slight dependence between the content of spermatozoa in one ejaculate and morphometrical traits of spermatozoa. In semen with lower content of spermatozoa (I and II group), the spermatozoa had slightly longer heads (by about 0.18 μm) than in semen with large spermatozoa concentration (III group). Spermatozoa in ejaculates with the lowest spermatozoa concentration were characterized by the longest flagellum and the largest total length. The total length of spermatozoa was decreasing in groups of larger concentration, which was caused by both lower length of heads and flagella. Some differences in spermatozoa shape in relation to their concentration in one ejaculate were found. Spermatozoa in ejaculates, which were classified into II group, seemed to have less prolate shape than spermatozoa in ejaculates of I and III groups. It was stated that the content of spermatozoa in one ejaculate affected the frequency of spermatozoa with morphological changes. Semen assigned to II group was distinguished by the best quality.

KEY WORDS: sperm, morphometry, boar, sperm concentration

STRESZCZENIE

Podjęto próbę oceny wpływu koncentracji plemników w ejakulacie na wymiary, kształt i częstość występowania anomalii morfologicznych plemników oraz cechy fizyczne ejakulatu knurów rasy Pietrain. Wykazano, że jest nieduża zależność pomiędzy koncentracją plemników w ejakulacie a cechami morfometrycznymi plemników. W nasieniu o mniejszej koncentracji (grupa I i II) plemniki miały nieco dłuższe główki (o około 0,18 μm) niż w nasieniu o dużej koncentracji plemników (grupa III). Najdłuższymi witkami i największą łączną długością charakteryzowały się plemniki z ejakulatów o najmniejszej koncentracji plemników. W grupach o większej koncentracji łączna długość plemnika malała, co wynikało z mniejszej długości zarówno główki jak i witki plemnika. Stwierdzono pewne różnice w kształcie plemników w zależności od ich koncentracji w ejakulacie. Plemniki z ejakulatów zakwalifikowanych do II grupy zdają się mieć kształt mniej wydłużony niż plemniki w ejakulatach z grupy I i III. Stwierdzono, że koncentracja plemników w ejakulacie wywiera wpływ na frekwencję plemników wykazujących zmiany morfologiczne. Najlepszą jakością charakteryzowało się nasienie zakwalifikowane do II grupy.

SŁOWA KLUCZOWE: plemnik, morfometria, knur, koncentracja plemników w ejakulacie

STRESZCZENIE SZCZEGÓŁOWE

Podjęto próbę oceny wpływu koncentracji plemników w ejakulacie na wymiary, kształt i częstość występowania anomalii morfologicznych plemników oraz cechy fizyczne ejakulatu knurów rasy Pietrain.

Badaniami objęto 63 ejakulaty pobrane od 11 knurów rasy Pietrain użytkowanych w dwóch stacjach należących do Mazowieckiego Centrum Hodowli i Rozrodu Zwierząt w Łowiczu. Od każdego knura raz w miesiącu pobierano próbkę ejakulatu do badań morfologii plemników. Z pobranych próbek wykonano preparaty mikroskopowe. W każdym preparacie wykonano pomiary morfometryczne piętnastu losowo wybranych plemników według metodyki opracowanej przez Kondrackiego i in. [38]. Obliczono też wskaźniki budowy morfologicznej plemników i przeprowadzono ocenę częstości występowania zmian morfologicznych wyszczególniając plemniki wykazujące zmiany główne i podrzędne zgodnie z klasyfikacją Bloma. Przeprowadzono również standardową ocenę badanych ejakulatów według metod stosowanych w polskich stacjach unasieniania loch. Zebrany materiał podzielono na podgrupy według kryterium koncentracji plemników w ejakulacie wyodrębniając: ejakulaty o koncentracji plemników mniejszej niż $400x10^3 \cdot mm^{-3}$ (grupa I), ejakulaty o koncentracji plemników $400\text{-}500x10^3 \cdot mm^{-3}$ (grupa II), ejakulaty o koncentracji plemników większej niż $500x10^3 \cdot mm^{-3}$ (grupa III).

Wykazano, że jest nieduża zależność pomiędzy koncentracją plemników w ejakulacie a cechami morfometrycznymi plemników. W nasieniu o mniejszej koncentracji (grupa I i II) plemniki miały nieco dłuższe główki (o około 0,18 μm) niż w nasieniu o dużej koncentracji plemników (grupa III). Najdłuższymi witkami i największą łączną długością charakteryzowały się plemniki z ejakulatów o najmniejszej koncentracji plemników. W grupach o większej koncentracji łączna długość plemnika malała, co wynikało z mniejszej długości zarówno główki jak i witki plemnika. Stwierdzono pewne różnice w kształcie plemników w zależności od ich koncentracji w ejakulacie. Plemniki z ejakulatów zakwalifikowanych do II grupy zdają się mieć kształt mniej wydłużony niż plemniki w ejakulatach z grupy I i III. Stwierdzono, że koncentracja plemników w ejakulacie wywiera wpływ na frekwencję plemników wykazujących zmiany morfologiczne. Najlepszą jakością charakteryzowało się nasienie zakwalifikowane do II grupy. W nasieniu tym niemal 96% plemników charakteryzowało się prawidłową budową tj. o około 3,8% więcej niż w nasieniu o największej koncentracji plemników ($P \leq 0,05$). Najwięcej plemników wykazujących podrzędne zmiany morfologiczne stwierdzono w nasieniu knurów o największej koncentracji plemników, o 3,8% więcej niż w nasieniu knurów z grupy II ($P \leq 0,01$) i o 2,09% więcej niż w nasieniu z grupy I.

INTRODUCTION

Fertilizing ability of spermatozoa depends on the status of their ultrastructure which conditions the course of the acrosomal reaction and sperm penetration of the ovum. However, even the spermatozoa characterized by appropriate morphology may differ in shape, which can influence the velocity of sperm's reaching the egg cell and their getting through the strata surrounding the egg cell. Sperm head size and shape result from the size and shape of the nucleus and acrosome. It is, first of all, a species characteristic but there occur some within-species differences and even differences between individuals of the same breed [3, 11, 28, 37, 39, 43]. It has been shown that the level of sperm competitiveness within the reproductive tract of insect females is connected with sperm dimensions [21]. Spermatozoa have to cover a long way in the female reproductive tract, overcome the immunological barrier, unfavourable pH, complex oviduct topography and its unfavourable conditions. Some studies have indicated an existence of an association between the dimensions and shape of spermatozoa and male fertility [48, 50]. What is also important is semen density expressed as the concentration of spermatozoa in an ejaculate. It is also possible that sperm concentration in an ejaculate influences sperm shape and dimensions and, as a result, motility characteristics and fertilizing ability. What also matters is the frequency of an incidence of sperm morphological defects which decreases fertility of not only boars [32, 54] but also stallions [31], bulls [14], bucks and rams [13, 44], and even humans [6, 15, 42]. A spermiogram, which is a result of examinations of kinds of sperm morphological changes and frequency of their occurrence, provides useful information on the physiological status and function of spermatogenic epithelium and epididymis. Among all the morphological anomalies of spermatozoa, head defects are most difficult to classify as even the heads with apparently appropriate shape may be characterized by a disturbed chromatin structure in the nucleus or changes in the acrosome [33]. It has been found that the presence in semen of spermatozoa with head defects may result in early deaths of embryos, reduced embryo quality [19] and decreased fertilizing ability of such spermatozoa [41].

In the present work has made attempted to assess sperm morphometric characteristics of Pietrain boars according to the concentration of spermatozoa in an ejaculate.

MATERIAL AND METHODS

Studies included 11 boars of the Pietrain breed utilized at two saw insemination stations owned by the Mazovian Centre of Animal Breeding and Reproduction in Łowicz. There were assessed ejaculates collected from each boar at monthly intervals. At least 5 ejaculates from each boar were collected and assessed. The ejaculates were taken by means of the gloved-hand technique as about 6 a.m. [36]. Immediately after collection, the semen was filtered through of sterile gauze into a prewarmed beaker to remove gel particles. The whole period of experience took about one and a half years. Altogether the study was carried out on 63 ejaculates (tab. 1).

The ejaculates were classified into the following three groups according to the criterion of sperm concentration in an ejaculate:

- ejaculates with sperm concentration of less than $400x10^{3}$ mm^{-3} (group I),
- ejaculates with sperm concentration of 400 - $500x10^{3}$ mm^{-3} (group II),
- ejaculates with sperm concentration of more than $500x10^{3}$ mm^{-3} (group III).

In each ejaculate there were taken morphometric measurements of spermatozoa, and determined the frequency of sperm morphological changes. Additionally, there was carried out an evaluation of physical properties of the ejaculates.

Sperm morphometric measurements and semen morphology assessment for each boar were made on the basis of results of microscopic examination of slides prepared using fresh ejaculates. Microscope slides were prepared from ejaculate samples. The slides were stained by the Bydgoska method previously described in the work by Kondracki et al. [40].

Microscopic examination of the preparations was carried out using immersion objectives at 100x objective magnification and a light microscope Nikon Eclipse 50i (Japan). Morphometric tests were carried out employing Screen Measurement v. 4. 1 (Laboratory Imaging S.r.o. LIM Czech Republic, Praha) for computer analysis of a picture.

Morphometric measurements of 15 randomly selected spermatozoa which were well visible within the microscope vision field were taken from each slide according to the methodology by Kondracki et al. [38]. Altogether there were taken 675 measurements of spermatozoa.

The following sperm morphometric measurements were taken:

- ✓ sperm head length – the distance between the point of head junction with the mid-piece and the furthest point in the front part of the sperm head,
- ✓ sperm head width – the distance between the furthest points located on the sperm head perimeter, measured perpendicularly to the long axis of the sperm head,
- ✓ tail length – the distance measured along the long axis of the tail and limited by the point where the head is connected with the mid-piece and the point of tail's end,
- ✓ sperm head perimeter – length of the boundary limiting the microscopic image of a sperm head,
- ✓ head area – area of a figure limited by a curve running along the perimeter of the sperm head microscopic image,
- ✓ total sperm length – the distance from the furthest point on the front part of the head to the point of tail's end.

On the basis of morphometric measurements there were calculated the following parameters of sperm morphology:

1. width-to-length ratio of sperm head (%),
2. ratio of head length to total sperm length (%),
3. ratio of head length to sperm tail length (%),
4. ratio of tail length to total sperm length (%),
5. ratio of sperm head perimeter to total sperm length (%),
6. ratio of sperm had area to total sperm length (%),
7. ratio of a product of sperm head length and width to total sperm length (%).

Morphological structure of 500 spermatozoa was evaluated

Table 1. Number of ejaculates depending on the sperm concentration
Tabela 1. Liczba ejakulatów objętych oceną w zależności od koncentracji plemników w ejakulacie

Specification Wyszczególnienie	Sperm concentration ($x10^{3}$ mm^{-3}) Koncentracja plemników w ejakulacie ($x10^{3}$ mm^{-3})			Including Łącznie
	Group I Grupa I <400	Group II Grupa II 400 - 500	Group III Grupa III >500	
Number of ejaculates Liczba ejakulatów	20	25	18	63

in each slide, indicating the number of spermatozoa with appropriate morphology and morphologically changed ones which were further divided into forms with major and minor changes according to the Blom classification [8].

Moreover, there was carried out an assessment of physical properties of an ejaculate. The following physical properties were considered in freshly collected ejaculates:

- ejaculate volume (in ml),
- sperm concentration ($x10^{3} \cdot mm^{-3}$),
- sperm progressive motility (%),
- total number of spermatozoa ($x10^{9}$),
- number of insemination doses per ejaculate.

Ejaculate volume was determined after filtering of the gel fraction on the basis of measurements of ejaculate weight by means of electronic scales. Sperm concentration in an ejaculate was determined by the colorimetric method using a spectrophotometer (Casou). Sperm motility was determined by means of microscopic examination (Nikon Eclipse 50i, Japan). There was used 200x magnification to determine percentage share of spermatozoa with appropriate motility at 37˚C in the total number of spermatozoa visible under microscope. The total sperm count in an ejaculate and the number of insemination doses obtained from one ejaculate were calculated using the computer software SYSTEM SUL (calculated using the computer program SYSTEM SUL used in Polish Centres of Animal Breeding and Reproduction).

The analysis of variability of spermatozoa morphology traits was carried out according to the following mathematical model:

$Y_{ij}=\mu+a_i+ e_{ij}$

where:

Y_{ij} – traits value,

μ - population mean,

a_i – effect sperm concentration,

e_{ij} – error.

Differences beetwen means were evaluated based upon Tukey's test.

RESULTS AND DISCUSSION

Table 2 presents results of sperm morphometric measurements according to sperm concentration in an ejaculate. The data reveal some differences in sperm dimensions. The semen with the smallest sperm concentration (group I and II) was characterized by spermatozoa with slightly longer heads (by about 0.18 µm) than the semen with high sperm concentration (group III). Similar associations were observed in the case of sperm head perimeter (fig. 1) and area. An increase in sperm concentration in an ejaculate was followed by decreasing perimeter and area of sperm heads. The longest tails and the greatest total lengths were found in the case of spermatozoa from ejaculates with the lowest sperm concentration (fig. 2). In ejaculates characterized by a higher sperm concentration, the total sperm length decreases, which is a result of both smaller sperm head and tail lengths.

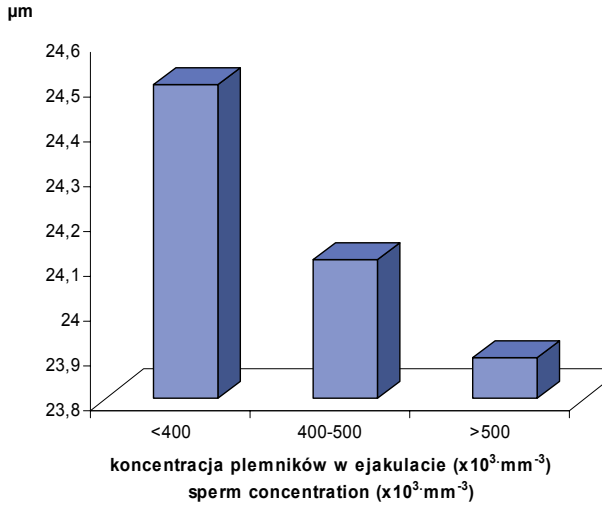

Figure 1. Sperm head perimeter depending on the sperm concentration

Rysunek 1. Obwód główki plemnika w zależności od koncentracji plemników w ejakulacie

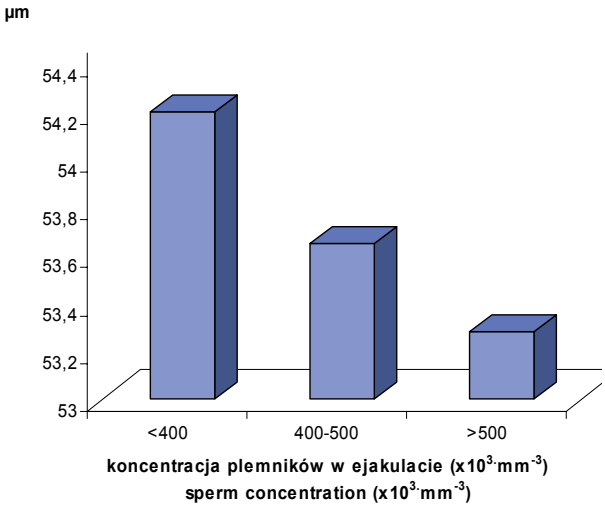

Figure 2. Total sperm length depending on the sperm concentration

Rysunek 2. Łączna długość plemnika w zależności od koncentracji plemników w ejakulacie

Table 2. Morphometric traits of sperms depending on the sperm concentration
Tabela 2. Cechy morfometryczne plemników w zależności od koncentracji plemników w ejakulacie

Specification Wyszczególnienie		Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)			$LSD_{0.05}$ $NIR_{0,05}$	$LSD_{0.01}$ $NIR_{0.01}$
		Group I Grupa I <400	Group II Grupa II 400-500	Group III Grupa III >500		
Number of ejaculates Liczba ejakulatów		20	25	18		
Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)	$\bar{x}$	331.50	446.00	567.22	25.408	31.597
	Sd	53.14	35.24	40.56		
Sperm head length (µm) Długość główki plemnika (µm)	$\bar{x}$	9.25	9.25	9.07	0.230	0.285
	Sd	0.47	0.29	0.40		
Sperm head width (µm) Szerokość główki plemnik (µm)	$\bar{x}$	4.69	4.62	4.67	0.153	0.190
	Sd	0.26	0.18	0.48		
Tail length (µm) Długość witki plemnika (µm)	$\bar{x}$	44.95	44.40	44.20	1.014	1.262
	Sd	2.22	1.43	1.43		
Sperm head perimeter (µm) Obwód główki plemnika (µm)	$\bar{x}$	24.50	24.11	23.89	0.595	0.740
	Sd	1.11	1.02	0.87		
Head area (μm^2) Pole powierzchni główki (μm^2)	$\bar{x}$	41.01	40.67	40.49	1.627	2.024
	Sd	2.78	2.48	3.09		
Total sperm length (µm) Łączna długość plemnika (µm)	$\bar{x}$	54.20	53.65	53.28	1.047	1.302
	Sd	2.32	1.36	1.58		

Legend: $\bar{x}$ - mean, Sd – standard deviation, LSD - NIR

III). Ejaculates with the lowest sperm concentration yielded the most insemination doses (over 31). The number of insemination doses produced from an ejaculate decreased as the sperm concentration in the ejaculate increased. Group I ejaculates yielded by 3 insemination doses more than group III ejaculates.

The data presented indicate that there existed some association between sperm morphometric characteristics and sperm concentrations in an ejaculate because there were found differences for sperm dimensions and size as well as frequency of incidence of sperm morphological changes in individual groups of sperm concentration. Typical characteristics of spermatozoa of individual animal species are characterized by certain specificity. Sperm shape first of all depends on the shape and size of its nucleus and acrosome, as well as the kind of connection of the tail with the head and the amount and size of spirally located mitochondria on the mid-piece [7]. Some works point to an existence of a relationship between tail length, the mid-piece in particular, and sperm motility. It is possible that sperm mid-piece length can be associated with the amount of energy produced in the mitochondria [7]. According to Katz and Drobins [34] spermatozoa with a longer mid-piece and tail are characterized by the stronger tail. There also exist works indicating differences in the intensiveness and forms of sperm motility according to head shape [52]. Hingst et al. [29] suggest that inappropriate sperm head shape and larger dimensions are positively associated with incompletely condensated chromatin, variability in sperm head dimensions being associated with an incorrect chromatin structure and DNA condensation disorders [47, 48]. An appropriate chromatin structure is formed during sperm passing through the epididymis duct. It has been shown that the percentage of spermatozoa with an appropriate chromatin structure, collected from the head of epididymis, increased form 68 to 92% of spermatozoa collected from the tail of epididymis [29]. Some studies indicate that spermatozoa with inappropriate morphology, especially those with an enlarged head, may have a disturbed chromosome structure. Examinations by Saravia et al. [49] have revealed that spermatozoa of Duroc boars have got larger heads of a more elliptic shape than boars of other breeds, which may be directly associated with a higher frequency of major changes in spermatozoa found in this breed [4]. It can be assumed that they are less motile, which reduces their changes of reaching an egg cell. Perhaps a more round shape of the head makes spermatozoa to be eliminated quicker than the remaining ones taking part in the journey to the oocyte. Studies of human sperm containing spermatozoa with incorrect chromosome number indicate that this characteristic may be conditioned by genetic factors and can be passed on to

There were found some differences in sperm shape according to their concentration in an ejaculate (tab. 3). Spermatozoa from ejaculates classified into group II ($400\text{-}500 \times 10^3 \cdot mm^{-3}$) seem to have been less elongated in shape than spermatozoa in ejaculates from group I and III, which was indicated by a greater ratio of sperm head length to total sperm length and to sperm tail length, and from a greater ratio of sperm head perimeter to total sperm length.

Table 4 displays data on the frequency of incidence of sperm morphological abnormalities according to sperm concentration in an ejaculate. The data indicate that sperm concentration in an ejaculate influenced the frequency of spermatozoa affected by morphological changes. The best quality was found for the semen classified into group II. In the semen as much as almost 96% of spermatozoa had appropriate morphology (fig. 3), that is by about 3.8% more than in the semen with the highest sperm concentration ($P \leq 0.05$). The percentage of spermatozoa with major morphological changes was also the smallest in the semen representing group II and amounted to 1.00%, which was by 0.16-0.55% less than in the ejaculates classified into group I and III ($P \leq 0.05$). However, the most spermatozoa with changes within the head were found in group III (fig. 4). The largest percentage of spermatozoa, that is 6.8%, displaying minor morphological changes was determined in the semen of boars with the highest sperm concentration (group III), which was by 3.8 ($P \leq 0.01$) and 2.09% more than in the semen of group II and group I boars, respectively (fig. 5). In the highest-concentration group of ejaculates there were also determined much more spermatozoa with a protoplasmic drop in the further (distal) location than in the remaining groups (fig. 6).

Table 5 lists data on ejaculate physical properties according to sperm concentration in the ejaculate. The data indicate that sperm concentration was inversely proportional to the ejaculate volume. As sperm concentration increased, the ejaculate volume decreased. Group I ejaculates characterized by the lowest sperm concentration had the highest volume which amounted to over 365 ml, that is, it was by almost 111 ml higher than group II ejaculates and by almost 160 ml higher than group III ejaculates characterized by the largest sperm concentration ($P \leq 0.01$). No differences for sperm motility according to sperm concentration in an ejaculate were found. In group I with the smallest sperm concentration the percentage of spermatozoa displaying progressive motility was by about 1% smaller than in the remaining groups. Ejaculates classified into group I and II had by about 6×10^9 greater total number of spermatozoa than ejaculates with the largest sperm concentration (group

Table 3. Indices sperm morphology
Tabela 3. Wskaźniki budowy morfologicznej plemników

Specification Wyszczególnienie		Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)			$LSD_{0.05}$ $NIR_{0.05}$	$LSD_{0.01}$ $NIR_{0.01}$
		Group I Grupa I <400	Group II Grupa II 400-500	Group III Grupa III >500		
Number of ejaculates Liczba ejakulatów		20	25	18		
Sperm concentration ($x10^3 \cdot mm^{-3}$)	$\bar{x}$	331.50	446.00	567.22	25.408	31.597
Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)	Sd	53.14	35.24	40.56		
Width-to-length ratio of sperm head (%)	$\bar{x}$	50.76	50.05	51.19	1.932	2.403
Stosunek szerokości główki plemnika do długości główki plemnika (%)	Sd	3.46	2.54	3.93		
Ratio of head length to total sperm length (%)	$\bar{x}$	17.09	17.25	17.04	0.471	0.585
Stosunek długości główki plemnika do łącznej długości plemnika (%)	Sd	0.95	0.74	0.68		
Ratio of head length to sperm tail length (%)	$\bar{x}$	20.63	20.86	20.54	0.688	0.855
Stosunek długości główki plemnika do długości witki plemnika (%)	Sd	1.39	1.09	1.98		
Ratio of tail length to total sperm length (%)	$\bar{x}$	82.91	82.75	82.94	0.471	0.585
Stosunek długości witki plemnika do łącznej długości plemnika (%)	Sd	0.95	0.74	0.68		
Ratio of sperm head perimeter to total sperm length (%)	$\bar{x}$	45.24	44.95	44.85	1.081	1.345
Stosunek obwodu główki plemnika do łącznej długości plemnika (%)	Sd	2.15	1.82	1.43		
Ratio of sperm had area to total sperm length (%)	$\bar{x}$	75.74	75.83	75.96	2.854	3.550
Stosunek pola powierzchni główki plemnika do łącznej długości plemnika (%)	Sd	5.45	4.49	4.60		
Ratio of a product of sperm head length and width to total sperm length (%)	$\bar{x}$	80.03	79.74	79.10	3.169	3.941
Stosunek iloczynu długości i szerokości główki plemnika do łącznej długości plemnika (%)	Sd	5.13	3.90	7.15		

Legend: $\bar{x}$ - mean, Sd – standard deviation, LSD - NIR

Table 4. Frequency of occurrence of spermatozoas morphologically changed depending on the sperm concentration
Tabela 4. Częstość występowania anomalii morfologicznych plemników w zależności od koncentracji plemników w ejakulacie

Specification Wyszczególnienie			Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)			$LSD_{0.05}$ $NIR_{0,05}$	$LSD_{0.01}$ $NIR_{0.01}$
			Group I Grupa I <400	Group II Grupa II 400-500	Group III Grupa III >500		
Number of ejaculates Liczba ejakulatów			20	25	18		
Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)		$\bar{x}$	331.50	446.00	567.22	25.408	31.597
		Sd	53.14	35.24	40.56		
Normal spermatozoa Plemniki o prawidłowej budowie morfologicznej	szt. No.	$\bar{x}$	468.15	479.60	459.61	16.137	20.068
		Sd	18.73	15.03	43.96		
	%	$\bar{x}$	93.70	95.90	92.03	3.241	4.031
		Sd	3.81	2.98	8.82		
Sperm with major abnormalities Plemniki ze zmianami głównymi	szt. No.	$\bar{x}$	7.75	5.00	5.78	5.772	7.177
		Sd	11.04	5.88	12.38		
	%	$\bar{x}$	1.55	1.00	1.16	1.154	1.435
		Sd	2.21	1.17	2.48		
Sperm with minor abnormalities Plemniki ze zmianami podrzędnymi	szt. No.	$\bar{x}$	23.60	14.76	34.06	12.914	16.060
		Sd	17.39	11.98	33.96		
	%	$\bar{x}$	4.72	2.95	6.81	2.583	3.212
		Sd	3.48	2.40	6.79		

Legend: $\bar{x}$ - mean, Sd – standard deviation, LSD - NIR

the offspring [16], although it is possible that the egg cell's zona pellucida may be able to select against spermatozoa with disturbed karyotype, incorrect chromosome number in particular [53]. Sperm genetic abnormalities may be displayed as late as after fertilization [51], that is why the nucleus chromatin structure is so important.

In the present work there were detected differences in sperm dimensions and shape according to sperm concentration in an ejaculate. There was observed a certain relationship between sperm head dimensions and increasing concentration. In lower-concentration ejaculates spermatozoa had slightly larger, longer heads with greater perimeter and area than in ejaculates with high sperm concentrations. There are not many works on an association between sperm concentration in an ejaculate and sperm morphometric characteristics. Studies by Rijsselaere et al. [45] on dog semen have shown that sperm concentration is associated with their dimensions. In ejaculates with lower sperm concentration ($50x10^3 \cdot mm^{-3}$) the spermatozoa had shorter and larger heads whose area and perimeter were smaller and tails were shorter than in ejaculates with higher sperm concentration ($200x10^3 \cdot mm^{-3}$). The largest heads and the longest tails were characteristic of spermatozoa in ejaculates with an average sperm concentration ($100x10^3 \cdot mm^{-3}$). Similar findings pertain to sperm head shape expressed as a head width-to-length ratio [45]. An impact of sperm concentration in an ejaculate on their morphometric characteristics has also been reported in stallions. Stallions which produced ejaculates with high sperm concentration ($628x10^3 \cdot mm^{-3}$) had spermatozoa characterized by smaller heads which were less elongated than spermatozoa from low-concentration ejaculates. The largest and the most elongated heads were found for spermatozoa obtained from ejaculates which low sperm concentration ($40x10^3 \cdot mm^{-3}$), whereas the longest heads were found in the case of spermatozoa from ejaculates whose sperm concentration was $150x10^3 \cdot mm^{-3}$ [18].

Many authors have studied the shape and dimensions of spermatozoa of different animal species, however,

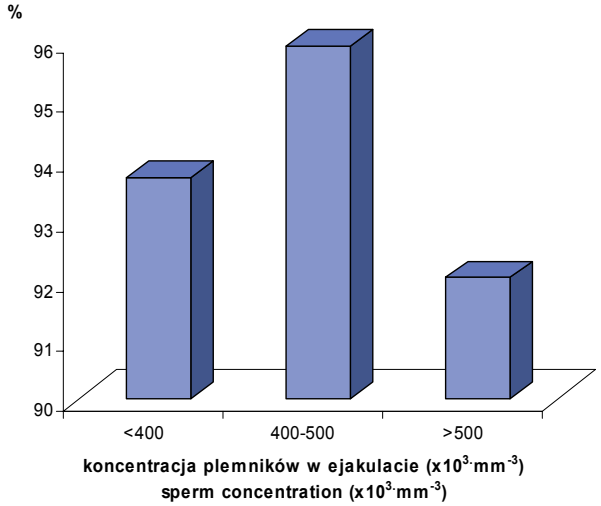

Figure 3. Frequency of normal spermatozoas depending on the sperm concentration

Rysunek 3. Frekwencja plemników o prawidłowej budowie morfologicznej w zależności od koncentracji plemników w ejakulacie

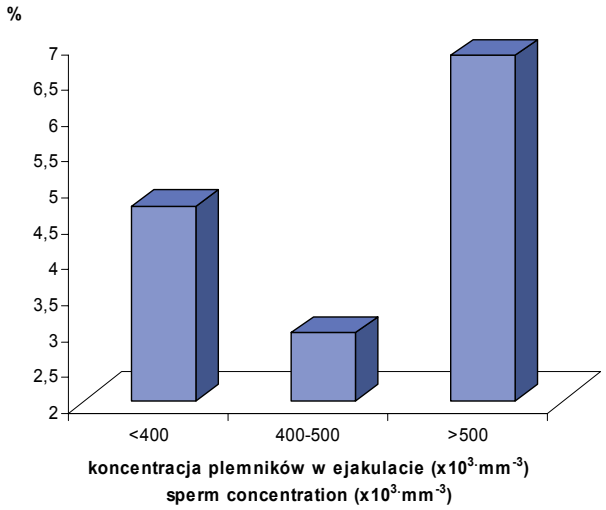

Figure 5. Frequency of occurrence of sperms with minor abnormalities depending on the sperm concentration

Rysunek 5. Frekwencja występowania plemników z podrzędnymi zmianami morfologicznymi w zależności od koncentracji plemników w ejakulacie

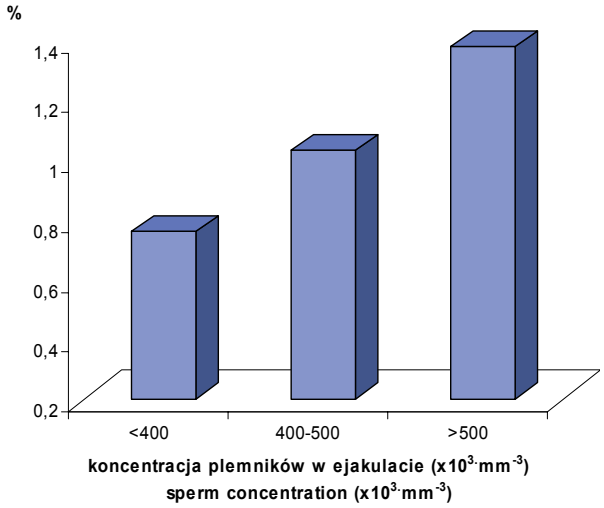

Figure 4. The percentage of sperms showing the change of head (the head reduced at basis pear-shaped head, head about obliterated contour, small incorrect head and the loose incorrect head) depending on the sperm concentration

Rysunek 4. Odsetek plemników wykazujących zmiany główki (główka gruszkowata, główka zwężona u podstawy, główka o zatartym konturze, główka mała nieprawidłowa i główka luźna nieprawidłowa) w zależności od koncentracji plemników w ejakulacie

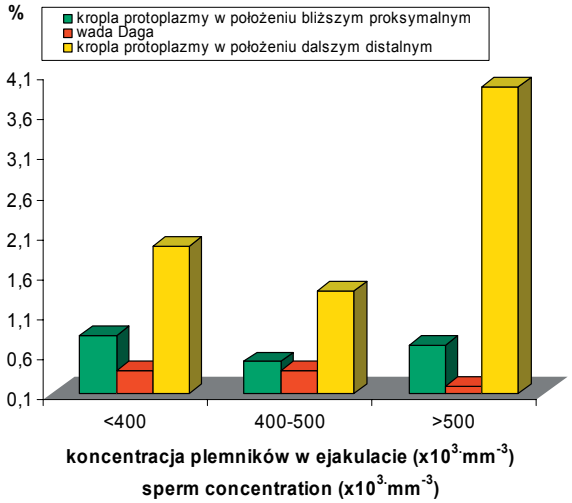

Figure 6. Frequency of occurrence of chosen of anomaly of morphological sperms depending on the sperm concentration

Rysunek 6. Częstość występowania wybranych anomalii morfologicznych plemników w zależności od koncentracji plemników w ejakulacie

Table 5. Physical properties of ejaculates of Pietrain boars depending on the sperm concentration
Tabela 5. Cechy fizyczne ejakulatu knurów rasy Pietrain w zależności od koncentracji plemników w ejakulacie

Specification Wyszczególnienie		Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)			$LSD_{0.05}$ $NIR_{0,05}$	$LSD_{0.01}$ $NIR_{0.01}$
		Group I Grupa I <400	Group II Grupa II 400-500	Group III Grupa III >500		
Number of ejaculates Liczba ejakulatów		20	25	18		
Sperm concentration ($x10^3 \cdot mm^{-3}$) Koncentracja plemników w ejakulacie ($x10^3 \cdot mm^{-3}$)	$\bar{x}$	331.50	446.00	567.22	25.408	31.597
	Sd	53.14	35.24	40.56		
Ejaculate volume (in ml) Objętość ejakulatu (ml)	$\bar{x}$	365.50	254.80	206.11	48.427	60.224
	Sd	91.97	85.20	64.09		
Sperm progressive motility (%) Odsetek plemników wykazujących ruch postępowy (%)	$\bar{x}$	78.50	79.60	79.44	1.607	1.998
	Sd	3.66	2.00	2.36		
Total number of spermatozoa ($x10^9$) Ogólna liczba plemników w ejakulacie ($x10^9$)	$\bar{x}$	92.58	92.44	86.58	17.256	21.459
	Sd	23.00	31.11	32.64		
Number of insemination doses per ejaculate Liczba dawek inseminacyjnych z jednego ejakulatu (szt.)	$\bar{x}$	31.45	29.76	28.44	5.346	6.648
	Sd	7.59	10.14	8.98		

an effect of these parameters on animal fertility is not unambiguous. Some researchers have seed a connection between sperm head dimensions and male fertility. Sperm head dimensions of humans [2, 35] and stallions [10, 23] characterized by reduced fertility differ from spermatozoa of individuals characterized by high fertilization effectiveness. According to Casey et al., [12] sperm of high-fertility stallions have got smaller and shorter heads as well as smaller head area and perimeter than spermatozoa of low-fertility stallions. Similar observations are presented in works by Hirai et al., [30], who have assessed morphometric characteristics of boar semen. According to the above authors sperm of boars characterized by lower fertilization effectiveness had larger and more elongated heads than the semen of high-fertility boars. Larger sperm heads found in the semen of males characterized by reduced fertility may indicate that there have occurred disorders in spermatogenesis and, first of all, changes in chromatin structure taking place during maturation and transportation of spermatozoa in the epididymis duct [12].

Some authors associate sperm head dimensions with the seasonal character of reproduction. Giżejewski [22] observed an increase in sperm head dimensions in stags during the rutting season. Also, the process of semen storage and preservation may influence sperm dimensions, but the results of different studies are not uniform. Gravance et al. [25] have reported that buck sperm in thawed ejaculates have got larger heads than in fresh ejaculates, which is probably connected with changes in the nucleus taking place during the process of cryopreservation. In turn, Arrudy et al. [1] showed that stallion sperm heads in fresh semen were statistically larger than in the semen after thawing. Similar conclusions have been presented in other works [27] which report that decreased sperm head dimensions after the process of cryopreservation may result from cell's dehydration as a result of chilling and freezing [20], as well as acrosome damage [26, 46]. Similar associations have been observed in the case of bull semen. In fresh ejaculates bull spermatozoa were characterized by smaller heads than

in diluted ejaculates [24]. Thurston et al. [52] distinguished groups of boar spermatozoa with different head shapes. They observed that the percentage of spermatozoa with rectangle-like heads was inversely proportional to the percentage of spermatozoa with undamaged acrosomes after thawing. Additionally, the percentage of narrowly-ended heads was positively correlated with the percentage of spermatozoa with undamaged plazmalemma after thawing. There were found differences in the sperm plazmalemma tolerance of the freezing process between individuals of the same species. Moreover, morphological and morphometric characteristics of spermatozoa may follow from predisposition of the individual [9, 17]. The data of the current work indicate that the most spermatozoa with morphological changes were found in ejaculates with the highest sperm concentration, the percentage of spermatozoa with changes into the head being almost twice as high in ejaculates characterized by the concentration of over $500x10^3 \cdot mm^{-3}$ as in ejaculates with the concentration of less than $400x10^3 \cdot mm^{-3}$. Worsening of the semen morphological image may be connected with many factors. An increased incidence of major changes and polymorphism of sperm heads may be related to a low testosterone level [5], how closely related the animals are [7], temperature in the testes, genetic factors [14] as well as seasonal and environmental factors. Studies by Rijsselaere [45] of dog semen have revealed that ejaculates with lower sperm concentrations contained less sperm with morphological changes. As the concentration increased, the percentage of morphological abnormalities increased. Higher sperm concentration in an ejaculate usually indicates their higher concentration in epididymises where they are stored and mature before ejaculation. It may create less favourable conditions of the environment of sperm development. The studies by Banaszewska [4] indicate that a Duroc boars' distinctive feature is a markedly higher frequency of sperm morphological changes compared with the males of other breeds. The percentage of sperm morphological abnormalities in the semen of Duroc boars was first of all determined by an incidence of many spermatozoa with minor changes. Ejaculates of the boars of this breed are characterized by a very high sperm concentration. A higher frequency of sperm morphological defects in the sperm of Duroc boars may be associated with high sperm concentration in their ejaculates.

CONCLUSION

An analysis of the data presented reveals that sperm concentration in ejaculates of Pietrain boars may be of significance as far as morphometric characteristics of spermatozoa are concerned. The differences shown between particular sperm dimensions indicate the occurrence of gametes differing in shape depending on the sperm concentration. In ejaculates with lower sperm concentration spermatozoa are slightly longer and their heads are more round in shape than spermatozoa of ejaculates characterized by higher concentration. In ejaculates with concentration of over $500x10^3 \cdot mm^{-3}$ there were found the least spermatozoa characterized by appropriate morphology, which was first of all determined by a high frequency of minor changes.

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