Scientific Annals of Polish Society of Animal Production - Vol. 15 (2019), No 2, 33-45

Dependence of the frequency of sperm defects and dimensions on sperm motility in ejaculates of Polish Landrace boars

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An attempt was made to determine the dependence of the frequency of sperm defects and dimensions on sperm motility in ejaculates of Polish Landrace boars. The study was conducted on 393 ejaculates collected from 33 Polish Landrace boars. Ejaculates were grouped according to the percentage of sperm with progressive motility, distinguishing ejaculates in which the percentage of motile sperm was 70% and 80%. In each ejaculate, the frequency of morphological changes in the sperm was determined and morphometric measurements of the sperm were made. Ejaculates with a higher proportion of sperm with progressive motility were found to contain more sperm. The ejaculate volume and sperm concentration in the ejaculate were not found to be directly associated with sperm motility. The frequency of primary defects was linked to sperm motility. Ejaculates with higher sperm motility contained fewer sperm with primary defects. The frequency of minor morphological changes, however, shows no significant dependence on sperm motility in the ejaculate. The primary morphological sperm defects most often found in ejaculates are a proximal droplet and the Dag defect. Both of these morphological forms are more common in ejaculates with lower sperm motility. The most common secondary sperm defects include sperm with a simple bent tail, sperm with a free normal head, and sperm with a distal droplet. These defects were not found to depend on sperm motility in the ejaculate. Sperm cells in ejaculates with greater sperm motility had slightly larger dimensions than sperm in ejaculates with lower sperm motility. Ejaculates with higher sperm motility are preferable for use in practice, not only because more insemination portions can be prepared from them, but also due to the lower frequency of primary defects.

KEY WORDS: sperm motility/ morphological changes in sperm / sperm dimensions

Sperm motility indicates the ability of sperm to fertilize the ovum and is the basis for evaluation of sperm survivability. Sperm motility can be assessed by microscopic examination, by determining the percentage of sperm showing progressive linear movement, or by computer-assisted sperm analysis (CASA), immediately after collecting the ejaculate [22, 25]. The quality and fertilization capacity of sperm is associated with their morphology. The frequency of morphological changes as well as the dimensions and shape of sperm are important because they determine sperm motility and the ability to penetrate the ovum. Changes in the structure of the sperm cell membrane during capacitation and the acrosomal reaction are significant for the combining of the sperm with the oocyte. Therefore, sperm morphology is of key importance in determining male fertility [1]. The use of boars with reduced semen quality for breeding leads to reduced litter size [28]. Sperm dimensions and shape may affect the suitability of semen for insemination [10], as a link has been found between sperm dimensions and male fertility [6]. The characteristics of the ejaculate and the sperm contained in it depend on the breed [33, 40], on the age and intensity of use of the breeders [2, 21], on the season and semen collection conditions [35, 42, 43], and on the diet and libido of the male [19, 31, 44].

The aim of the study was to determine the dependence of the frequency of sperm defects and dimensions on sperm motility in ejaculates of Polish Landrace boars.

Material and methods

The study was conducted on 393 ejaculates collected from 33 Polish Landrace breeding boars, aged 1–2.5 years. The semen was collected manually twice a week. The following physical characteristics were determined in freshly collected ejaculates: ejaculate volume, sperm concentration, sperm motility, sperm count in the ejaculate, and the number of insemination doses obtained per ejaculate. The ejaculate volume was determined after filtering out the gelatinous fraction. The sperm concentration was determined by the photometric method using a spectrophotometer. The method involves measuring the intensity of light passed through a suspension of sperm in a sodium chloride solution isotonic for semen. Sperm motility was assessed by microscopic examination, based on the proportion of spermatozoa showing progressive movement. A light microscope with a heated stage was used. The percentage of sperm that showed normal movement in the total number of sperm visible in the field of view of the microscope was determined under 200 x magnification. Sperm motility in the ejaculate is determined as the percentage of sperm showing progressive rectilinear movement. Ejaculates with sperm motility of 60% or less (such ejaculates are not used for breeding), 70%, 80% and 90% or more (uncommon) are distinguished. In this

study, spermatozoa from ejaculates with sperm motility of 70% and 80% were examined. The sperm count in the ejaculate and the number of insemination doses obtained per ejaculate were calculated using SYSTEM SUL computer software.

Ejaculates were grouped according to the percentage of sperm showing progressive movement, as follows:

– ejaculates in which the percentage of motile sperm was 70% (group I)

– ejaculates in which the percentage of motile sperm was 80% (group II)

In each ejaculate with sperm motility of 70% or 80%, the frequency of morphological changes in the sperm was determined and morphometric measurements were performed. Microscope slides were prepared from samples taken from all ejaculates examined. The slides were stained with eosin and gentian dye according to Kondracki et al. [20]. The morphological structure of 500 sperm cells was assessed in each slide, indicating the number of sperm with normal structure and of sperm with morphological abnormalities, distinguishing forms with primary defects and minor anomalies according to Blom's classification [4]. The frequency of morphological defects was determined on the basis of microscopic examinations of sperm morphology, carried out with a Nikon Eclipse-50i light microscope using immersion objectives at 100x magnification.

Morphometric sperm measurements were performed on microscopic slides prepared in the same way as for examination of morphological changes. For each ejaculate, measurements were made of 10 randomly selected morphologically normal sperm that were well visible in the field of view of the microscope. In total, 3930 sperm cells were measured. The measurements were carried out manually with a computer image analysis system (Screen Measure v. 4.1), according to Kondracki et al. [17]. Head circumference, head length, head width, head area, tail length, and total sperm length were measured (Fig.).

The data were subjected to statistical analysis and means and standard deviations were calculated for the groups. Significance of differences between group means was verified by Student's t-test.

Results and discussion

Table 1 summarizes the data illustrating the physical characteristics of the ejaculates depending on sperm motility in the ejaculate. The data show that the physical characteristics of the ejaculate depend to a small extent on sperm motility. However, in the group of ejaculates with higher motility (group II), the average number of sperm suitable for insemination (showing rectilinear movement) was higher by nearly 10 billion than in the group of ejaculates with lower motility, which was statistically confirmed

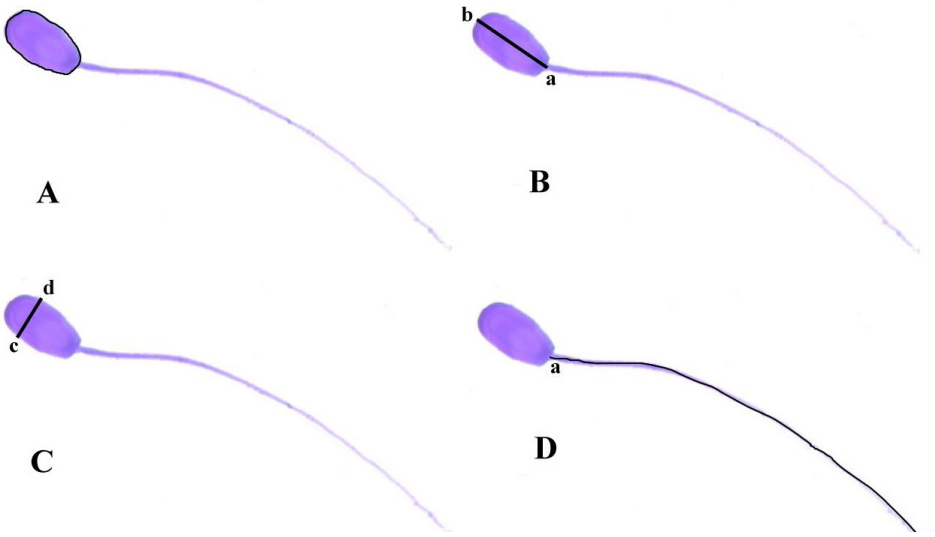

Fig. Means of determining sperm dimensions: A – head area, B – head length, C – head width, D – tail width [17]

Table 1
Physical characteristics of ejaculates depending on the motility of spermatozoa in the ejaculate

Ejaculate traits	Percentage of spermatozoa with progressive motility		
	group I 70%	group II 80%	total
Number of ejaculates (n)	94	299	393
Percentage of spermatozoa with progressive motility (%)	70.00 ±0.00	80.00 ±0.00	77.61 ±4.27
Ejaculate volume (ml)	272.23 ±86.29	262.85 ±91.05	265.09 ±89.92
Sperm concentration (x10^6/ml)	448.51 ±100.95	458.97 ±97.56	456.47 ±98.35
Number of sperm in ejaculate (x 10^9)	84.89^a ±33.97	94.51^b ±33.49	92.21 ±33.82
Number of insemination doses (n)	29.90 ±11.30	31.75 ±11.09	31.31 ±11.16

a, b – $P \leq 0.05$

at $P \leq 0.05$. More insemination doses were prepared from these ejaculates. Ejaculate volume and sperm concentration were not shown to be dependent on sperm motility in the ejaculate.

Table 2 presents the frequency of morphological abnormalities in sperm depending on their motility. Sperm with primary defects were more frequent in ejaculates with lower sperm motility. In ejaculates from group I, in which 70% of spermatozoa showed progressive movement, the frequency of sperm with major morphological changes was more than twice as high as in group II, in which sperm motility was 80% ($P \leq 0.05$). However, the frequency of sperm with primary defects was very low, irrespective of motility. The low frequency of major abnormalities in the semen of breeding boars has been shown in other studies as well [18, 45]. No intergroup differences in the frequency of sperm with minor abnormalities were demonstrated.

Table 2
Incidence of morphologically normal and abnormal sperm depending on sperm motility in the ejaculate (%)

Parameter	Percentage of spermatozoa with progressive motility		
	group I 70%	group II 80%	total
Normal spermatozoa (%)	95.41 ±4.90	96.25 ±4.62	96.05 ±4.69
Sperm with major abnormalities (%)	1.02[a] ±2.54	0.46[b] ±0.65	0.60 ±1.38
Sperm with minor abnormalities (%)	3.57 ±3.92	3.28 ±4.46	3.35 ±4.33

a, b – $P \leq 0.05$

Table 3 presents the occurrence of specific major defects of sperm in ejaculates with higher and lower sperm motility. The most common were spermatozoa with a proximal droplet or the Dag defect. Both of these defects were more common in ejaculates with lower sperm motility ($P \leq 0.05$). The frequency of other major defects was negligible and was not dependent on the motility of the sperm in the ejaculate.

Table 4 presents the occurrence of specific minor morphological anomalies of sperm in ejaculates with higher and lower sperm motility. The most common were sperm with a simple bent tail (about 2% of sperm), with a free normal head (about 0.63%), and with a distal droplet (about 0.55%). Sperm with a giant head and abaxial implantation

Table 3
Frequency of individual major defects depending on sperm motility in the ejaculate (%)

Major sperm defects	Percentage of spermatozoa with progressive motility		
	group I 70%	group II 80%	total
Underdeveloped sperm	0.04 ±0.10	0.03 ±0.15	0.03 ±0.14
Double forms	0.04 ±0.15	0.02 ±0.08	0.03 ±0.10
Pear-shaped head	0.04 ±0.10	0.03 ±0.12	0.03 ±0.11
Narrow head at base	0.02 ±0.07	0.01 ±0.07	0.02 ±0.08
Abnormal head contour	0.01 ±0.07	0.01 ±0.06	0.01 ±0.06
Proximal droplet	0.31^{a} ±1.32	0.13^{b} ±0.40	0.18 ±0.74
Pseudodroplet	0.06 ±0.14	0.04 ±0.23	0.04 ±0.21
Strongly coiled or folded tail (Dag defect)	0.26^{a} ±0.38	0.17^{b} ±0.33	0.19 ±0.35

a, b – P≤0.05

were relatively common, with a frequency of about 0.42%. Such anomalies were more common in Group I ejaculates, with lower sperm motility (P≤0.05). The rate of other minor abnormalities was very low (less than 0.2% of sperm).

Table 5 presents the results of morphometric measurements of spermatozoa in ejaculates differing in sperm motility. They show that the sperm dimensions depend to a small extent on sperm motility in the ejaculate. Certain tendencies can be seen in the width of the sperm head. Sperm with greater motility (group II) had slightly wider heads than sperm ejaculates with lower sperm motility (group I). Sperm in ejaculates with greater motility (group II) also had a larger head area (by 1.07 μm), which was confirmed statistically (P≤0.05).

The data presented in this paper indicate that ejaculates with greater sperm motility contain more sperm. A similar relationship has also been found by Górski et al. [13]. The results of our study indicate that the frequency of morphological changes in sperm depends on sperm motility. The data show that in ejaculates with a higher per-

Table 4
Frequency of sperm with the most common minor defects depending on sperm motility in the ejaculate (%)

Minor sperm defects	Percentage of spermatozoa with progressive motility		
	group I 70%	group II 80%	total
Narrow heads	0.012[a] ±0.06	0.004[b] ±0.03	0.006 ±0.04
Small normal heads	0.021 ±0.08	0.007 ±0.05	0.009 ±0.06
Giant and short broad heads	0.071[a] ±0.16	0.031[b] ±0.09	0.042 ±0.12
Free normal heads	0.490 ±0.94	0.673 ±3.19	0.633 ±2.82
Detached acrosome membrane	0.006 ±0.06	0.007 ±0.07	0.007 ±0.07
Abaxial implantation	0.073[a] ±0.16	0.032[b] ±0.10	0.042 ±0.12
Distal droplet	0.611 ±1.32	0.511 ±1.09	0.542 ±1.15
Simple bent tail	2.094 ±2.97	1.983 ±2.68	2.011 ±2.75
Terminally coiled tail	0.201[a] ±0.99	0.034[b] ±0.12	0.074 ±0.50

a, b – $P \leq 0.05$

centage of sperm showing progressive movement, the frequency of sperm with major abnormalities is lower. Mažeika et al. [26] have shown that the sperm motility in boar ejaculates decreases as the percentage of morphologically abnormal sperm increases. Primary defects are usually due to abnormalities during spermiogenesis and during sperm maturation, as they pass through successive segments of the epididymis [36]. Genetic and environmental disturbances during spermatogenesis or maturation of sperm in the epididymis result in the production of sperm with abnormal morphology. Damage to DNA or chromatin structures is often found in deformed spermatozoa [7]. Increased frequency of morphological defects in sperm may result from chromatin instability and the occurrence of chromosomal abnormalities [5, 23, 37]. The presence of morphologically altered sperm reduces male fertility and is indicative of reduced function of the seminiferous epithelium. A high percentage of sperm with primary defects, especially defects of the acrosome, significantly reduces the chance of fertilization. The data presented in this study indicate

Table 5
Sperm dimensions depending on sperm motility in the ejaculate

Sperm dimensions	Percentage of spermatozoa with progressive motility		
	group I 70%)	group II 80%	total
Head length (μm)	9.21 ±0.35	9.17 ±0.33	9.18 ±0.33
Head width (μm)	4.67^{a} ±0.27	4.78^{b} ±0.29	4.75 ±0.29
Head perimeter (μm)	23.38 ±0.77	23.53 ±0.95	23.50 ±0.91
Head area (μm^2)	39.81^{a} ±2.34	40.88^{b} ±2.39	40.62 ±2.42
Tail length (μm)	45.03 ±1.69	45.35 ±1.33	45.27 ±1.43
Total length (μm)	54.24±1.90	54.52±1.49	54.45±1.60

a, b – P≤0.05

that the percentage of sperm with major defects was not high. It was far from the threshold values for semen of fertile boars [4, 30], but in the group I ejaculates, with lower sperm motility, the frequency of sperm with major morphological abnormalities was more than twice that in the group II ejaculates ($P \leq 0.05$). This indicates that the frequency of major morphological defects is linked to sperm motility. The most common defects in the sperm of Polish Landrace boars in the present study were a proximal droplet and the Dag defect (Table 3). Both sperm with a proximal droplet and sperm with the Dag defect were more common in ejaculates with lower sperm motility ($P \leq 0.05$). A proximal protoplasmic droplet is a consequence of disturbed sperm maturation in the initial stage of spermatogenesis. Sperm maturation disorders in the final stage of spermatogenesis lead to the formation of a distal droplet, which is considered a minor defect. Proximal and distal droplets are the most common morphological defects in sperm, which was also demonstrated in this study. Some data show that spermatozoa with a cytoplasmic droplet differ in head dimensions and shape from morphologically normal sperm [11].

The results of this study indicate that the morphometric characteristics of sperm are associated with sperm motility, as differences were demonstrated in the dimensions of sperm heads from ejaculates differing in sperm motility. In ejaculates with higher

sperm motility, the spermatozoa had somewhat wider heads and a larger surface area than sperm from ejaculates with lower motility ($P \leq 0.05$). The shape of the sperm head is determined by the organization of DNA [34]. It is thought that even small deviations in the shape of the sperm head may be caused by changes in chromatin structure in the nucleus [29], which may result in decreased fertility [8]. Sperm with a cytoplasmic droplet have a greater share of decondensed chromatin than sperm with normal morphology [11]. Therefore, finding differences in sperm head dimensions may be helpful in the diagnosis of fertile individuals and individuals with reduced fertility [14, 32].

Some researchers [15, 16] draw attention to the dependence of the speed of sperm movement on the length of the sperm head and tail. Sperm with elongated heads are faster than those with rounded heads [24]. The form of the sperm's movement also depends on the shape of the head [39]. Ejaculates with a low percentage of motile sperm contain sperm with shorter tails. The sperm tails of fertile males are straight, while males with a reduced fertility rate are bent at the site of the cytoplasmic droplet [27]. The length of the sperm midpiece may be linked to the level of energy derived from the mitochondria [3] and affects the movements of the tail, and thus the strength and speed of movement of the sperm [27]. Sperm with longer tails are more competitive, because they can move and reach the egg faster [9, 12]. Sperm motility affects litter size and the number of live piglets born [38, 41]. Thus, an accurate assessment of sperm motility in the ejaculate can be used to predict boar fertility [41].

Summing up, ejaculates of Polish Landrace boars with a higher percentage of sperm showing progressive movement also contain more sperm. Ejaculate volume and the sperm concentration in the ejaculate are not directly associated with sperm motility. Ejaculates with higher sperm motility contain fewer sperm cells with major morphological defects. The frequency of minor abnormalities, however, does not show a significant dependence on sperm motility in the ejaculate. The most common primary sperm defects in the ejaculates of the Polish Landrace boars are a proximal droplet and the Dag defect. Both of these forms are more common in ejaculates with lower sperm motility. The most common minor sperm anomalies are sperm with a simple bent tail, sperm with a free normal head, and sperm with a distal droplet. These changes were not shown to depend on sperm motility in the ejaculate. In ejaculates with higher sperm motility, sperm are slightly larger than sperm from ejaculates with a lower motility rate. Ejaculates with higher sperm motility are more suitable for insemination, not only because more insemination doses can be prepared from them, but also due to the lower frequency of primary defects.

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