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Research Article

The Importance of Ejaculate Volume for the Physical Parameters of Ejaculates and Sperm Morphology of Hypor Boars

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Abstract

The study aimed at analysing the effects of ejaculate volume on the physical parameters of ejaculates and the sperm morphology of Hypor boars. The analyses involved 114 ejaculates collected from 12 Hypor insemination boars. The ejaculates were classified according to the criterion of ejaculate volume. Three groups were specified: ejaculates with a volume of 251 ml or lower (Group I), ejaculates with a volume of 252-310 ml (Group II), and ejaculates with a volume of 311 ml or higher (Group III). The ejaculates were assessed to identify the basic physical traits and determine the incidence of morphological abnormalities in the spermatozoa, specifying major and minor abnormalities. Furthermore, the morphological structure indices for the spermatozoa were also calculated. Rising ejaculate volume accompanied with a rise in the total number and motility of spermatozoa, and a simultaneous slight fall in sperm concentration in the ejaculates. The ejaculates with the highest volumes turned out to contain more morphologically well-formed spermatozoa. We also determined that rising ejaculate volume is accompanied with increasing sperm dimensions, especially those of the head. The increased parameters were the length and the width of sperm heads, as well as their perimeters and areas. Ejaculate volume has an impact on the shape of Hypor boar spermatozoa. As the ejaculate volume increases, the shape of sperm heads becomes increasingly more oval. Additionally, spermatozoa in ejaculates with greater volumes have larger heads in relation to flagellum length.

Keywords: *Boar, Ejaculate volume, Morphometric traits, Semen, Spermatozoa*

Hypor Domuzlarında Sperm Morfolojisi ve Ejakülatların Fiziksel Özellikleri Yönünden Ejakulat Hacminin Önemi

Özet

Çalışmada Hypor erkek domuzların sperm morfolojisi ve ejakülatın fiziksel özellikleri üzerine ejakülat hacminin etkilerini incelemek amaçlandı. Bu amaçla, 12 adet Hypor ırkı suni tohumlama domuzundan alınan 114 ejakülat incelendi. Ejakülatlar hacme göre gruplandırıldı: Numuneler, 251 ml ve altında (Grup 1), 251-310 ml (Grup 2) ve 310 ml ve üstünde (Grup 3) olarak ayrıldı. Sperma, major ve minor morfolojik sperm anomalilerinin sıklığı ve temel fiziksel özellikleri yönünden değerlendirildi. Ayrıca, spermatozoa'nın morfolojik yapısına ait değerler saptandı. Ejakülat hacmi artış ve ejakülasyondaki sperm konsantrasyonunda az miktarda düşüş ile, motilite ve toplam sperm sayısında bir artışla beraber bulundu. En yüksek hacimli ejakülatlarda düzgün yapıda morfolojiye sahip daha fazla sayıda spermatozoa saptandı. Ayrıca, daha yüksek ejakülat hacmiyle birlikte spermatozoonların özellikle baş kısmında olmak üzere ebatlarının büyüdüğü saptandı. Spermatozoonun baş uzunluğu ve genişliği ile çevre uzunluğu ve yüzey alanı büyüdü. Ejakülat hacmi Hypor domuz spermatozoonlarının şekli üzerinde bir etkiye sahipti. Ejakülat hacminin artmasıyla spermatozoa başı daha oval bir şekil aldı. Ek olarak, hacmin artmasıyla ejakülattaki spermatozoa kuyruk uzunluğu ile daha uzun başa sahip olma arasında bir ilişki saptandı.

Anahtar sözcükler: *Erkek domuz, Ejakülasyon hacmi, Morfolojik özellikler, Sperma, Sperm*

INTRODUCTION

The efficacy of insemination largely depends on the quality of spermatozoa inferred from analyses of sperm morphology [1,2]. Sperm quality can be also determined on the basis of an analysis of the damage to the sperm membrane [3], the state of chromatin structure, the anti-oxidative potential of the spermatozoa, apoptotic changes or on the basis of sperm ATP level assays [4,5].

Morphological abnormalities in spermatozoa reduce male fertility [6]. Particular individuals exhibit differences in the quality of spermatozoa they produce. This includes difference in the incidence of spermatozoa with morpho-

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logical abnormalities [7]. The shape and dimensions of spermatozoa are also important. Their dimensions and shapes determine their motility and capacity to penetrate the egg [8]. This issue has been dealt with by numerous researchers who concentrated on the variation in morphometric parameters of spermatozoa, depending on the physical traits of the ejaculates produced by the sires [9,10]. It is believed that the presence of spermatozoa with head abnormalities in the semen can be the reason for reduced embryo quality [11] and miscarriages in the initial period of pregnancy [12].

The size and shape of the sperm head is a species-related trait. However, differences have been identified between males representing different breeds of the same species, or even between particular individual animals [13]. Some scientists believe that motor parameters of spermatozoa depend on their sizes and shapes [14-16]. This has impact on the competitiveness of spermatozoa in the reproductive organs of the female. Faster and viable spermatozoa undergo spontaneous selection following capacitation. Spermatozoa negotiate a long way in the reproductive tract of the female, surmounting the immunological barrier, disadvantageous pH, complex oviduct topography and the untoward conditions prevalent there [17].

The volume of ejaculated semen varies among animals depending on nutrition, genetics, breed and management [18]. High-volume ejaculates are considered to be particularly useful for insemination because they make it possible to prepare numerous insemination doses containing the required numbers of spermatozoa. Ejaculate volume is also important for fertilization efficacy and embryo survival [19]. Semen volume affects the distribution of spermatozoa [20]. Increased ejaculate volume positively influences the transport of semen by stimulating the central layer of the uterine accelerate its contractions and inducing the pituitary body to release hormones that stimulate contractions of the smooth muscles of the matrix [19]. Ejaculate volume may also affect the quality dimensions and shape of sperms, as well as their motor parameters that determine their fertilizing ability. Currently, there are studies that indicate a connection between the sperm morphometric parameters and concentration in the ejaculates of boars [9].

The present study was aimed at analysing the relationship between ejaculate parameters and sperm morphology and the volume of ejaculates produced by Hypor boars, on the basis of physical traits of the ejaculates, morphometric measurements of the spermatozoa, and an evaluation of the incidence of morphological sperm abnormalities.

MATERIAL and METHODS

The study concerned 114 ejaculates collected from 12 Hypor boars used at three insemination centres. The boars (aged 8 to 18 months), were managed in accordance with the rules of animal welfare [21]. The individual pen area was 10 m^2/boar, and the pen had a concrete slatted floor. The boars were fed according to Swine Nutrition Requirements [22], with *ad libitum* access to water. Temperature, relative air humidity, and atmospheric pressure were measured during semen collections. Temperature was measured with a precision of one degree Celsius. Humidity, expressed as a percentage, was measured with a precision of one percentage point. Temperature and humidity was recorded using a thermo-hygrometer TERMIK PLUS (1000209, Termo-produkt, PL). Atmospheric pressure was measured using an ADLER barometer (Bar 003, Demus, PL) with a resolution one hPa. Relative humidity was close to 70%. The air temperature in the boar pens was 16ºC (average minimum 13ºC and maximum 21ºC). Air pressure inside the buildings averaged 1005 hPa (min. 987 hPa, max. 1016 hPa). Ejaculates were collected using the manual method [23] in one-month intervals over a period of nine months. A total of 114 ejaculates were collected from July 2013 to April 2014. Each boar provided at least 10 ejaculates for the analysis. The ejaculates were grouped according by volume as follows:

Group I : ejaculates with a volume below 251 ml - 32 ejaculates,

Group II : ejaculates with a volume between 251 ml and 310 ml - 38 ejaculates,

Group III : ejaculates with a volume above 310 ml - 44 ejaculates.

The following physical parameters were determined in the freshly collected ejaculates: ejaculate volume (ml), sperm concentration (x10^6/ml), sperm motility (%), total number of spermatozoa (x10^9), and number of insemination doses per ejaculate (n). Ejaculate volumes were determined by weight, without the gelatinous fraction, using electronic scales. Sperm concentration in the ejaculates was determined with a photometric method, using a spectrophotometer (IMV Technologies, France). Sperm motility was evaluated with a Nikon Eclipse 50i light microscope equipped with a heated stage. A sample of 5 µl of sperm suspension was placed on a pre-warmed slide and sealed with a coverslip at 37ºC. Under 200x magnification, the percentage of normally motile spermatozoa was determined in the overall number of sperms present in the field of vision of the microscope. The total number of motile spermatozoa and the number of insemination doses per ejaculate were calculated using SYSTEM SUL (v. 6.35; Gogosystem, Poland) software package.

Semen samples from the collections were used to prepare microscopic slides. The slides for morphological analyses were stained using eosin and gentian violet, according Kondracki et al.[24]. Microscopic analyses of the smears were performed under 100x magnification with immersion lenses, using the Nikon Eclipse 50i light microscope. The morphology of 500 spermatozoa was assessed per slide, identifying the number of well-formed and malformed spermatozoa and differentiating those

with primary and secondary changes, according to Blom's classification [25].

Also, sperm morphometric measurements were carried out on 15 randomly selected normal spermatozoa in each slide. The measurements were performed using a suite for computer image analysis (Screen Measurement v. 4.1), according to methodology proposed by Kondracki et al.[26]. The following sperm measurements were taken: head length (µm), head width (µm), head area (µm^2), head perimeter (µm), flagellum length (µm), and total length (µm). The following morphological indices were calculated on the basis of the measurements:

head width/head length,
head length/total length,
head length/flagellum length,
flagellum length/total length,
head perimeter/total length,
head area/total length,
head length x width/total length.

Experimental data were analysed using a program STATISTICA® 10 PL (StatSoft, Tulsa, USA) [27]. All results are expressed as mean (X) ± standard deviation (Sx). The obtained material was statistically analysed according to the following mathematical model:

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

Where: Y_{ij} is the value of the analysed parameter, µ is the population mean, a_i is the effect of ejaculate volume, e_{ij} is the error. The significance of the differences between the groups was assessed with the Tukey test at $P \leq 0.05$ and $P \leq 0.01$.

RESULTS

Table 1 contains data on the physical parameters of the Hypor boar ejaculates in relation to ejaculate volume. The data reveal that ejaculate physical parameters are correlated with ejaculate volume. An increase in ejaculate volume was accompanied by a decrease in sperm concentration and an increase in sperm motility. With an increase in volume, the total number of sperm and the number of insemination doses per ejaculate significantly increased too. Group I, which comprised the lowest-volume ejaculates, showed the highest sperm concentrations, which averaged 430.66 x 10^6/ml and were by more than 18 x 10^6/ml higher than in Group II, and by nearly 29 x 10^6/ml higher than in Group III, the one with the highest ejaculate volumes. These differences were not, however, statistically confirmed.

The data in *Table 1* show that spermatozoa in the higher-volume ejaculates have greater progressive motility. Group III, comprising ejaculates with the highest volume, was found to contain spermatozoa with the highest motility. The percentage of motile spermatozoa in the ejaculates from this group was more than 3% higher than in Group II ($P \leq 0.01$) and almost 5.5% higher than in Group I ($P \leq 0.01$). The highest sperm counts were found in Group III. The ejaculates in this group contained over 114 billion spermatozoa with progressive motility, over 26 billion more than those in Group II ($P \leq 0.01$) and approximately 40 billion more than those in Group I, with the lowest volumes ($P \leq 0.01$). The number of spermatozoa in the ejaculate also determines the number of insemination doses that can be prepared out of the ejaculate. The most numerous doses were prepared from the ejaculates in Group III, those with the highest volumes. Each ejaculate in this group provided more than 37 insemination doses, approximately 8.4 doses more than the ejaculates in Group II and over 13 doses more than the ejaculates in Group I ($P \leq 0.01$). *Table 2* contains the results of the analysis of morphological abnormalities in the spermatozoa.

The mean percentage of normally formed spermatozoa remained within the range from 94.72% to 96.90%. The fewest spermatozoa with normal morphology were found in the ejaculates in Group III. The data presented in *Table 2* show Hypor boar semen quality to be very high. The mean percentage of spermatozoa with major morphological abnormalities did not exceed 1.71%. The differences

Table 1. *Physical traits of ejaculates in relation to ejaculate volume*
Tablo 1. *Ejakülat hacmine ilişkin ejekülatların fiziksel özellikleri*

Specification		Groups & Ejaculate Volume (ml)		
		I (< 251)	II (251-310)	III (> 310)
Number of ejaculates (n)		32	38	44
Ejaculate volume (ml)	X±Sx	217.19±33.14^A	284.47±15.54^B	364.09±46.37^C
Spermatozoa concentration (x 10^6/ml)	X±Sx	430.66±131.65	412.50±129.98	401.86±103.20
Percentage of spermatozoa with progressive motility (%)	X±Sx	73.44±4.82^a	75.79±5.00^b	78.86±3.21^c
Total number of spermatozoa (x 10^9)	X±Sx	75.21±38.57^a	88.39±25.15^b	114.58±18.54^c
Number of insemination doses per ejaculate (n)	X±Sx	24.22±8.49^A	29.37±8.78^B	37.75±8.00^C

^{a,b} Differences between average values, represented by different letters in the same row, are important ($P \leq 0.05$); ^{A,B} Differences between average values, represented by different letters in the same row, are important ($P \leq 0.01$)

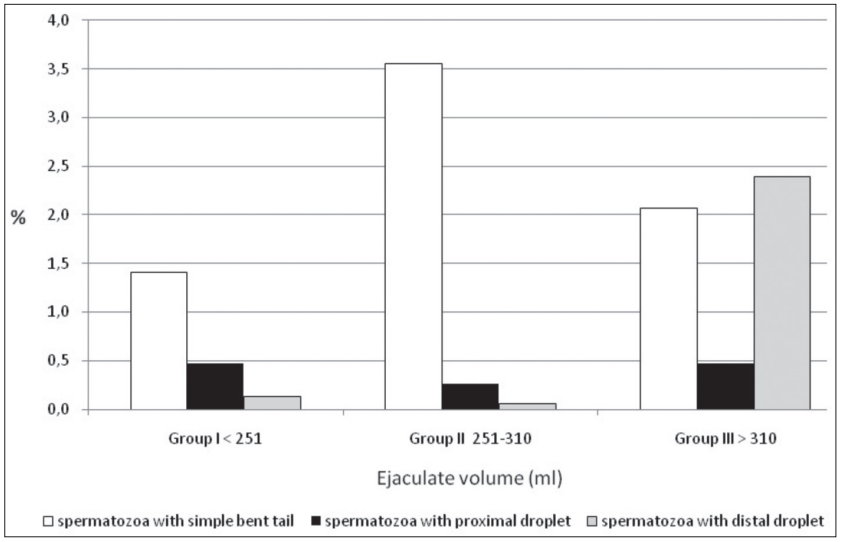

Fig 1. Frequency of occurrence of chosen anomaly of morphological spermatozoa depending on the ejaculate volume

Şekil 1. Ejakülat hacmine ilişkin seçilmiş morfolojik sperm anomalilerinin görülme sıklığı

Table 2. *Frequency of spermatozoa with morphological changes in relation to ejaculate volume*
Tablo 2. *Ejakülat hacmine ilişkin morfolojik değişikliklere sahip spermatozoa sıklığı*

Specification		**Groups & Ejaculate Volume (ml)**		
		I (< 251)	**II (251-310)**	**III (> 310)**
Number of ejaculates (n)		32	38	44
Ejaculate volume (ml)	X±Sx	217.19±33.14^{A}	284.47±15.54^{B}	364.09±46.37^{C}
Percentage of normal spermatozoa (%)	X±Sx	96.08±3.20^{B}	96.90±2.63^{B}	94.72±3.72^{A}
Spermatozoa with major abnormalities (%)	X±Sx	1.09±1.08	0.91±1.80	1.71±2.05
Spermatozoa with minor abnormalities (%)	X±Sx	2.88±2.69	2.18±1.81	3.60±2.93

^{A,B} Differences between average values, represented by different letters in the same row, are important (P≤0.01)

Table 3. *Morphometric traits of spermatozoa with regard to ejaculate volume*
Tablo 3. *Ejakülat hacmine ilişkin morfometrik spermatozoa özellikleri*

Specification		**Groups & Ejaculate Volume (ml)**		
		I (< 251)	**II (251-310)**	**III (> 310)**
Number of ejaculates (n)		32	38	44
Ejaculate volume (ml)	X±Sx	217.19±33.14^{A}	284.47±15.54^{B}	364.09±46.37^{C}
Head length (µm)	X±Sx	8.99±0.55	9.10±0.56	9.19±0.46
Head width (µm)	X±Sx	4.73±0.32	4.85±0.30	5.02±0.34
Head perimeter (µm)	X±Sx	23.24±1.02	23.59±1.14	23.75±1.32
Head area (µm^2)	X±Sx	37.88±4.39^{A}	39.16±4.57^{AB}	40.53±4.28^{B}
Flagellum length (µm)	X±Sx	43.19±1.12^{a}	43.84±1.16^{b}	43.59±1.37^{ab}
Total length (µm)	X±Sx	52.18±1.00^{A}	53.02±1.15^{B}	52.80±1.62^{AB}

^{a,b} Differences between average values, represented by different letters in the same row, are important (P≤0.05); ^{A,B} Differences between average values, represented by different letters in the same row, are important (P≤0.01)

between the groups were slight and statistically unconfirmed. The highest percentage of spermatozoa with major abnormalities was found in the semen of the boars with the highest ejaculate volumes. Among the major morphological abnormalities, cytoplasmic droplets in the proximal position in the spermatozoa were most frequent. The mean percentage of spermatozoa with this defect was low and did not exceed 0.5% *(Fig. 1)*. The highest numbers of sperm with minor morphological abnormalities were found in the semen of Group III (3.60%). The differences between the groups were, however, low and statistically non-significant.

The data in *Table 2* suggest that the volume of ejaculates collected from Hypor boars insignificantly affected the frequency of morphological abnormalities in the spermato-

Table 4. *Morphometric indices of spermatozoa in relation to ejaculate volume*
Tablo 4. *Ejakülat hacmine ilişkin morfometrik spermatozoa endeksleri*

Specification		Groups & Ejaculate Volume (ml)		
		I (< 251)	II (251-310)	III (> 310)
Number of ejaculates (n)		32	38	44
Ejaculate volume (ml)	X±Sx	217.19±33.14^{A}	284.47±15.54^{B}	364.09±46.37^{C}
Head width/head length	X±Sx	52.71±3.08^{A}	53.42±2.55^{AB}	54.61±2.88^{B}
Head length/total length	X±Sx	17.23±1.09	17.15±0.96	17.40±0.66
Head length/flagellum length	X±Sx	20.84±1.59	20.76±1.40	21.09±0.99
Head area/total length	X±Sx	82.76±1.09	82.69±1.31	82.54±0.79
Head length x width/total length	X±Sx	44.54±2.09	44.48±1.94	44.96±1.69
Perimeter of the head/total length	X±Sx	72.61±8.50^{a}	73.79±7.94^{ab}	76.66±6.82^{b}
Flagellum length/total length	X±Sx	81.79±9.54^{a}	83.46±8.94^{a}	87.37±7.88^{b}

^{a,b} Differences between average values, represented by different letters in the same row, are important (P≤0.05); ^{A,B} Differences between average values, represented by different letters in the same row, are important (P≤0.01)

zoa. The results of the morphometric measurements of the spermatozoa are presented in *Table 3*.

The data in *Table 3* show that the spermatozoa in the ejaculates with the highest volumes (Group III) have larger head dimensions than those in the ejaculates with intermediate and low volume (Group II and I). The head lengths and widths increase with ejaculate volume. The spermatozoa in the ejaculates with the highest volumes (Group III) had 0.17 µm wider heads than those in the ejaculates in Group II ($P \leq 0.05$) and 0.29 µm wider heads than the spermatozoa in Group I ($P \leq 0.01$). The spermatozoa in the ejaculates of the highest volumes were also characterized by the largest head areas. The head area exhibits a clear rising trend in line with the increase in ejaculate volume ($P \leq 0.01$).

Sperm flagella in the ejaculates in Group II were on average 0.65 µm longer than those in in Group I ($P \leq 0.05$), and 0.25 µm longer compared to Group III. The spermatozoa total length was also the greatest in the ejaculates in Group II, principally due to the longer flagella. *Table 4* contains data on the structural indices defining the sperm shape.

The data in *Table 4* suggest that the effect of ejaculate volume on the shape of Hypor boar spermatozoa is non-significant. Most of the structural sperm morphology indices assumed similar values in all groups, and the observed differences largely remained within the range of statistical error. It was recorded that the spermatozoa in the ejaculates with the lowest volume (Group I) had the most elongated heads, and as the ejaculate volume grew, the shape of the sperm heads turned increasingly more oval. This has been confirmed in the head width/head length index, the highest in the ejaculates in Group III - 1.90 times higher than in Group I ($P \leq 0.01$). The data in *Table 4* also show that as the ejaculate volume rises, the proportions between the spermatozoa head and the flagellum change as well. With an increase in ejaculate volume, the head area/total length and head length x width/total length ratios also increased. Both indices were higher in Group III, compared to those in Groups II and I ($P \leq 0.05$). This suggests that spermatozoa in ejaculates with higher volumes have larger heads in relation to the flagellum length.

DISCUSSION

The ejaculate volume has a physiological basis and is associated with the secretory function of the accessory sexual glands, which produce seminal plasma forming environment for development, and existence of sperm. Functionality of the accessory sexual glands depends on many factors including genetic and non-genetic factors. Important for the physiology of plasma secretion of semen components is sexual development of pig males. The sexual development of pig males is not over at 8-9 months of age, when boars start to be used for insemination, but proceeds until a much more advanced age. Some authors have reported that boar ejaculate volume and sperm count of the boars grows until the age of around 27-28 months [28,29]. The further development of sexual glands in sexually mature and active breeding boars is confirmed by testicular morphology analysis. It was shown that boar testes increase in size until the age of 20 months [30]. Oestrogens play a crucial role in the control of testicular development and functionality [31,32]. Dynamically rising weight of testes during pubescence as well as the number of reproductive and somatic cells within the parenchyma of testes may be determined by oestrogen levels.

An essential parameter in the qualitative assessment of boar semen is the percentage of sperm with superior motility. The reason is that motility is a symptom of viability and indirectly reveals the fertilization capability of spermatozoa. Acceptable, fertilisation-capable boar semen should contain at least 70% of spermatozoa with progressive rectilinear motion [33]. The data of the present study showed

the spermatozoa in all the analysed groups having a good motility, much above the values reported by Shipley [34]. It was essential to identify the positive effect of ejaculate volume on sperm motility. Raising ejaculate volume was accompanied with a significant increase in sperm motility. Numerous factors affecting sperm motility have been reported. Some authors have reported the negative impact of morphological defects on sperm motility [35], while others have pointed out the considerable impact of the hyperosmotic environment of spermatozoa on their motility [36]. Frequent causes of reduced sperm motility include spermatogenetic disorders, anomalies in the functioning of the epididymal epithelium and debilitated functioning of the additional sexual glands [7]. The correlation between sperm motility and the physical parameters of the ejaculate has not been clearly confirmed yet. Publications on the subject provide inconclusive observations. The study of Pietrain boars by Kondracki et al.[9] showed the highest motility in spermatozoa in ejaculates with the lowest volumes. The progressively motile spermatozoa identified in the previous study ranged from 75 to 79%, and was slightly higher in ejaculates with the lowest volumes, i.e. contrarily to the correlation observed in this study. The observed changes were, however, non-significant, and the percentage of spermatozoa with progressive motility was not too much. Studies of the importance of sperm concentration have revealed that sperm motility is not in significant correlation with sperm concentration in the ejaculate [37,38]. There was no correlation between sperm motility and the total number of spermatozoa in the ejaculate has been identified either [17]. The results of the present study showed that sperm concentration was slightly higher in ejaculates with lower volumes. This confirms the existence of an inversely proportional correlation between ejaculate volume and sperm concentration in boar ejaculates, as identified in previous studies [24,39].

The total number of sperm increased with ejaculate volume. The differences were significant and very pronounced. This is consistent with the expectations, since the content of motile spermatozoa in the ejaculate depends on ejaculate volume and sperm concentration. A directly proportional correlation between the number of spermatozoa and ejaculate volume has also been observed in other studies [9].

The results of the present study justify the conclusion that ejaculate volume affects sperm morphology. The spermatozoa in ejaculates with different volumes differ in their sizes and shapes, as well as in the incidence of morphological abnormalities. The spermatozoa in the ejaculates with the highest volumes (above 310 ml) were larger in size than those in the ejaculates in Group I - with the lowest volumes (below 251 ml). Sperm size affects the motility and fertilization capacity [14,30]. According to Noorafshan & Karbalay-Doust [16], sperm length is positively correlated with the speed of sperm motion. Spermatozoa with longer mid-pieces and flagella have stronger tails [40]. The correlation between flagellum length, and primarily mid-piece length, and sperm motility has also been revealed [14,15,41].

It is probable that, sperm mid-piece length can be associated with the level of energy originating in mitochondria [42]. Spermatozoa with longer flagella are more competitive since they might reach the egg faster. The present authors found that spermatozoa with the longest flagella were present in ejaculates with intermediate volumes (251-310 ml). A study by Marmor and Grob-Menendez [43] revealed that spermatozoa with low motility could have flagella that are shorter by as much as 50%. The results of the above study were confirmed by Noorafshan and Karbalay-Dust [16]. The data presented in this work also validate this correlation, because the lower flagellum length in ejaculates containing spermatozoa with low motility was statistically confirmed. The interrelation between flagellum length and ejaculate parameters has already been identified in several studies [44,45]. It has been revealed that ejaculates with a high sperm concentration contain spermatozoa with shorter flagella [37,46]. However, no clear-cut correlation was identified between flagellum length and the total number of spermatozoa in the ejaculate [17].

The rising ejaculate volume was accompanied with increasing sperm head dimensions, including the length, width, perimeter and area *(Table 3)*. The sperm head contains the cellular nucleus, which is the primary carrier of genetic information transferred during fertilization. The variation in the dimensions of sperm heads can stem from differences in chromatin structure [47]. Some reports informed that even slight modifications in the sperm head shape can be associated with changes in chromatin structure in the cellular nucleus [48], leading to reduced fertility [49]. A correlation has been found between sperm head dimensions and male fertility. It was observed that the spermatozoa of males with higher fertility had narrower and shorter heads [50,51]. The studies by Villalobos et al.[52] demonstrated a positive correlation between fertility and spermatozoa head morphometry in swine. It was concluded that males with high fertility showed the values of 8.9 µm in length and 4.5 µm in width. The data of the present work revealed that the spermatozoa in the ejaculates with the lowest volumes had the lowest head dimensions. Their heads were shorter and narrower than the heads of spermatozoa in the ejaculates with higher volumes. The association of sperm head dimensions with the physical parameters of ejaculates has also been identified in other studies [9]. Sperm head dimensions have been observed to be dependent on the sperm concentration in bull [24] and boar ejaculates [37,38].

The head shape can be significant in the context of sperm motility. Spermatozoa with an elongated head shape move faster than those with rounded heads [53]. The current data showed that the spermatozoa in the ejaculates

with the lowest volumes (Group I) had the most elongated heads, and as the ejaculate volume increases, the shape of the sperm heads turned increasingly more oval *(Table 4)*. Helfenstein et al.[54] have reported the existence of a correlation between the length of the head and flagellum and the speed of sperm motion. Spermatozoa with a lower ratio of head length to tail length move faster. Considering the results of the experiment, this refers to the spermatozoa from the ejaculates classified in Group II in terms of the volume.

Sperm head dimensions can be affected by the manner of storage and preservation of semen [55,56]. A study by Hidalgo et al.[56] revealed that buck sperm heads in refrigerated were smaller than those in fresh semen. This was explained with the damage to or loss of the acrosome, or a possible change in sperm chromatin structure as a result of refrigeration. The detection of abnormalities in sperm heads makes it possible to recognize fertile animals and those with reduced fertility [57]. Morphometric analyses of ram spermatozoa have revealed that sires with reduced fertility have larger sperm heads than fertile males [58]. The reason for the increase in head size can be disordered spermatogenesis, or changes in chromatin structure during the maturation and transport of spermatozoa. Sperm head defects often cause deterioration in the quality of embryos and lead to miscarriages in the first period of pregnancy [12,59].

The data presented in this work indicate a moderate correlation between the incidences of sperm morphological abnormalities and ejaculate volume. However, ejaculates with the highest volumes had the lowest proportions of spermatozoa with correct morphology. The presence of morphologically abnormal spermatozoa reduces male fertility and was an index of a reduced performance of the seminiferous epithelium. The incidence of morphological abnormalities in spermatozoa can result from the influence of seasonal factors [60], genetic conditions [12,61], and individual predispositions [62]. The incidences of morphologically abnormal spermatozoa also depend on feeding factors [63]. Large differences in the frequency of morphological abnormalities in spermatozoa have also been identified in relation to the age of sires [28,64-66].

Boars with normal fertility always have a certain percentage of morphologically abnormal spermatozoa [63]. A maximum of 15% spermatozoa with major and 10-15% with minor abnormalities is acceptable. The presence of spermatozoa with major abnormalities that have appeared during spermatogenesis is particularly disadvantageous. A high percentage of spermatozoa with major modifications, especially acrosome defects, substantially reduces the chances for insemination. The data presented in this study showed that the samples of spermatozoa with major abnormalities was low and did not exceed 1.71% in any of the groups. Among the major morphological abnormalities, the proximal cytoplasmic droplet in the spermatozoa was the most frequent defect. Such defects result from anomalies in sperm maturation. The reason for the appearance of the abnormalities can be a short a time of sperm maturation in the epididymal duct [67]. It should be noted that the incidence of the tail defects could be a consequence of an osmotic difference between the spermatozoa and the solution in which the sample is immersed [68]. According to Martin-Rillo et al.[69], a maximum of 20% spermatozoa with a proximal droplet is acceptable in collected semen. Any amount in excess of this level leads to a considerable reduction of male fertility [70].

Ejaculate parameters depend on the volume of produced ejaculates. The rise in ejaculate volume was accompanied with an increase in the total number and motility spermatozoa, as well as with a concomitant slight fall in sperm concentration. Ejaculates with the highest volume were highly usable for preparation of more insemination doses. Ejaculates with the highest volumes had a larger proportion of spermatozoa with normal morphology. However, ejaculate volume does not substantially affect the frequency of morphological sperm abnormalities in Hypor boar ejaculates. Ejaculate volume influences morphometric parameters of Hypor boar spermatozoa. The rise in ejaculate volume is accompanied with an increase in sperm dimensions, especially with regard to the sperm head. The increased parameters were the length and the width of sperm heads as well as their perimeters and areas. Ejaculate volume had an impact on the shape of Hypor boar spermatozoa. As the ejaculate volume increases, the shape of the sperm heads changes from elongated to increasingly more oval. Spermatozoa in ejaculates with higher volume had a larger heads in relation to the flagellum length. When using Hypor boars for insemination purposes, it is preferred to choose sires with a high ejaculatory efficacy and producing ejaculates of high volume. Such ejaculates allow not only for generating more insemination doses, but also doses including spermatozoa of higher mobility and quality.

REFERENCES

1. De Vos A, Van De Velde H, Joris H, Verheyen G, Devroey P, Van Steirteghem A: Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. *Fertil Steril*, 79, 42-48, 2003. DOI: 10.1016/S0015-0282(02)04571-5

2. Knecht D, Środoń S, Duziński K: The influence of boar breed and season on semen parameters. *S Afr J Anim Sci*, 44, 1-9, 2014. DOI: 10.4314/sajas.v44i1.1

3. Wysokińska A, Kondracki S: Assessment of changes in sperm cell membrane integrity occurring during the storage of semen from genetically different males using two diagnostic methods. *Can J Anim Sci*, 94, 601-606, 2014. DOI: 10.4141/cjas2013-095

4. Waberski D, Magnus F, Ardon F, Petrunkina AM, Weitzke KF, Töpfer-Petersen E: Binding of boar spermatozoa to oviductal epithelium in vitro in relation to sperm morphology and storage time. *Reproduction*, 131, 311-318, 2006. DOI: 10.1530/rep.1.00814

5. Wolf J: Genetic parameters for semen traits in AI boars estimated from data on individual ejaculates. *Reprod Domest Anim*, 44, 338-344, 2009. DOI: 10.1111/j.1439-0531.2008.01083.x

6. Çebi Şen Ç, Faundez R, Jurka P, Akçay E, Petrajtis-Golobow M,

Ambarcıoğlu P: Evaluation of the canine epididymal sperm morphology with two different staining methods, one fixative solution and motile sperm organelle morphology examination (MSOME). *Kafkas Univ Vet Fak Derg*, 22, 57-62, 2016. DOI: 10.9775/kvfd.2015.13887

7. Pinart E, Camps R, Briz MO, Bonet S, Egozcue J: Unilateral spontaneous abdominal cryptochidism: Structural and ultrastructural study of sperm morphology. *Anim Reprod Sci*, 49, 247-268, 1998. DOI: 10.1016/S0378-4320(97)00074-2

8. Suarez S: Interactions of spermatozoa with the female reproductive tract: Inspiration for assisted reproduction. *Reprod Fertil Dev*, 19, 103-110, 2007. DOI: 10.1071/RD06101

9. Kondracki S, Górski K, Wysokińska A, Jóźwik I: Correlation of ejaculate parameters and sperm morphology with the ejaculate volume of Pietrain boars. *Bulg J Agric Sci*, 20, 721-727, 2014.

10. Holt WV, Hernandez M, Warrell L, Satake N: The long and the short of sperm selection *in vitro* and *in vivo*: Swim-up techniques select for the longer and faster swimming mammalian sperm. *J Evol Biol*, 23, 598-608, 2010. DOI: 10.1111/j.1420-9101.2010.01935.x

11. De Jarnette JM, Saake RG, Barne J, Volger CJ: Accessory sperm: Their importance to fertility and embryo quality and attempts to alter their numbers in artificially inseminated cattle. *J Anim Sci*, 70, 484-491, 1992.

12. Chenoweth PJ: Genetic sperm defects. *Theriogenology*, 64, 457-468, 2005. DOI: 10.1016/j.theriogenology.2005.05.005

13. García-Vázquez FA, Hernández-Caravaca I, Yánez-Quintana W, Matás C, Soriano-Úbeda C, Izquierdo-Rico MJ: Morphometry of boar sperm head and flagellum in semen backflow after insemination. *Theriogenology*, 84, 566-574, 2015. DOI: 10.1016/j.theriogenology.2015.04.011

14. Gil MC, García-Herreros M, Barón FJ, Aparicio IM, Santos AJ, García-Marín LJ: Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. *Theriogenology*, 71, 254-263, 2009. DOI: 10.1016/j.theriogenology.2008.07.007

15. Ciftci HB, Zülkadir U: The correlation between bull sperm head dimensions and mitochondria helix length. *J Anim Vet Adv*, 9, 1169-1172, 2010. DOI: 10.3923/javaa.2010.1169.1172

16. Noorafshan A, Karbalay-Doust S: A simple method for unbiased estimating of ejaculated sperm tail length in subject with normal and abnormal sperm motility. *Micron*, 41, 95-99, 2010. DOI: 10.1016/j.micron.2009.09.002

17. Wysokińska A, Kondracki S, Banaszewska D: Morphometrical characteristics of spermatozoa in Polish Landrace boars with regard to the number of spermatozoa in an ejaculate. *Reprod Biol*, 9, 271-282, 2009. DOI: 10.1016/S1642-431X(12)60031-X

18. Alonso R, Cama JM, Rodriguez J: El cerdo. Editorial Félix Varela, Vedado Ciudad de La Habana, Cuba, 2004.

19. Stratman FW, Self HL: Effect of semen volume and number of sperm on fertility and embryo survival in artificially inseminated gilts. *J Anim Sci*, 19, 1081-1088, 1960.

20. Kaeoket K, Persson E, Dalin AM: The influence of pre- and post-ovulatory insemination on sperm distribution in the oviduct, accessory sperm to the zona pellucida, fertilization rate and embryo development in sows. *Anim Reprod Sci*, 71, 239-248, 2002. DOI: 10.1016/S0378-4320(02)00230-0

21. Ordinance of the Minister of Agriculture and Rural Development: Journal of Laws, No. 56, item 344, 15 February 2010. www.isap.sejm.gov.pl, *Accessed:* 08.04.2010.

22. Swine Nutrition Requirements: The Kielanowski Institute Animal Physiology and Nutrition, Polish Academy of Sciences. Omnitech-Press, Warsaw, Poland (in Polish), 1993.

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

24. Kondracki S, Iwanina M, Wysokińska A, Huszno M: Comparative analysis of Duroc and Pietrain boar sperm morphology. *Acta Vet Brno*, 81, 195-199, 2012. DOI: 10.2754/avb201281020195

25. Blom E: The morphological estimation of the spermatozoa defects of bull. II. The proposal of new classification of spermatozoa defects (in Polish). *Med Weter*, 37, 239-242, 1981.

26. Kondracki S, Banaszewska D, Mielnicka C: The effect of age on the morphometric sperm traits of domestic pigs. *Cell Mol Biol Lett*, 1, 3-13, 2005.

27. STATISTICA®: Data Analysis, Software System. Version 10 StatSoft Inc, 2012.

28. Jankeviciute N, Zilinskas H: Influence of some factors on semen quality of different breeds of boars. *Vet Med Zoot*, 19, 15-19, 2002.

29. Banaszewska D, Kondracki S: An assessment of the breeding maturity of insemination boars based on ejaculate quality changes. *Folia Biol*, 60, 151-162, 2012. DOI: 10.3409/fb60_34.151162

30. Clark SG, Schaeffer DJ, Althouse GC: B-mode ultrasonographic of paired testicular diameter of mature boars in relation to average total sperm numbers. *Theriogenology*, 60, 1011-1023, 2003. DOI: 10.1016/S0093-691X(03)00127-4

31. Kula K, Walczak-Jędrzejowska R, Słowikowska-Hilczer J, Oszukowska E: Estradiol enhances the stimulatory effect of FSH on testicular maturation and contributes to precocious initiation of spermatogenesis. *Mol Cell Endocrinol*, 178, 89-97, 2001. DOI: 10.1016/S0303-7207(01)00415-4

32. Oliveira CA, Carnes K, Franca LR, Hess RA: Infertility and testicular atrophy in the antiestrogen-treated adult male rat. *Biol Reprod*, 72, 214-220, 2001. DOI: 10.1095/biolreprod65.3.913

33. Kuster CE, Althouse GC: The fecundity of porcine semen stored for 2 to 6 days in Androhep® and X-cell™ extenders. *Theriogenology*, 52, 365-376, 1999. DOI: 10.1016/S0093-691x(99)00135-1

34. Shipley C: Breeding soundness examination of the boar. *J Swine Health Prod*, 7, 117-120, 1999.

35. Šerniene L, Riškeviciene V, Banys A, Žilinskas H: Effects of age and season on sperm qualitative parameters in Lithuanian White and Pietrain boars. *Vet Med Zoot*, 17, 1-5, 2002.

36. Rutllant J, Pommer AC, Meyers SA: Osmotic tolerance limits and properties of rhesus monkey *(Macaca mulatta*) spermatozoa. *J Androl*, 24, 534-541, 2003. DOI: 10.1002/j.1939-4640.2003.tb02705.x

37. Kondracki S, Wysokińska A, Iwanina M, Banaszewska D, Sitarz D: Effect of sperm concentration in an ejaculate on morphometric traits of spermatozoa in Duroc boars. *Pol J Vet Sci*, 14, 35-40, 2011. DOI: 10.2478/v10181-011-0005-z

38. Kondracki S, Banaszewska D, Bajena M, Komorowska K, Kowalewski D: Correlation of frequency of spermatozoa morphological alterations with sperm concentration in ejaculates of Polish Landrace boars. *Acta Vet Beograd*, 63, 513-524, 2013. DOI: 10.2298/AVB1306513K

39. Smital J: Effects influencing boar semen. *Anim Reprod Sci*, 110, 335-346, 2009. DOI: 10.1016/j.anireprosci.2008.01.024

40. Katz DF, Drobnis EZ: Analysis and interpretation of the forces generated by spermatozoa. **In,** Bavister BD, Cummins J, Roldan ERS, Norwell MA (Eds): Fertilization in Mammals. 125-137, Serono Symposia, 1990.

41. Lüpold S, Calhim S, Immler S: Sperm morphology and sperm velocity in passerine birds. *Proc Biol Sci*, 276, 1175-1181, 2009. DOI: 10.1098/rspb.2008.1645

42. Bierła JB, Giżejewski Z, Leigh CM, Ekwall H, Söderquist L, Rodriguez-Martinez H, Zalewski K, Breed WG: Sperm morphology of the Eurasian beaver Castor fiber: an example of a species of rodent with highly derived and pleiomorphic sperm populations. *J Morphol*, 268, 683-689, 2007. DOI: 10.1002/jmor.10544

43. Marmor D, Grob-Menendez F: Male infertility due to asthenozoospermia and flagellar anomaly: detection in routine semen analysis. *Int J Androl*, 14, 108-116, 1991. DOI: 10.1111/j.1365-2605.1991.tb01072.x

44. Levitan DR: Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegates. Proc R Soc Lond*, 267, 531-534, 2000. DOI: 10.1098/rspb.2000.1032

45. Burness G, Casselman SJ, Schulte-Hostedde AJ, Moyes CD, Montgomerie R: Sperm swimming speed and energetics vary with sperm competition risk in bluegill *(Lepomis macrochirus)*. *Behav Ecol Sociobiol*, 56, 65-70, 2004. DOI: 10.1007/s00265-003-0752-7

46. Rijsselaere T, Soom A, Hoflack G, Meas D, Kruif A: Automated sperm morphometry and morphology analysis of canine semen by the

Hamilton-Thorne analyser. *Theriogenology*, 62, 1292-1306, 2004. DOI: 10.1016/j.theriogenology.2004.01.005

47. Sailer BL, Jost LK, Evenson DP: Bull sperm head morphometry related to abnormal chromatin structure and fertility. *Cytometry*, 24, 167-173, 1996. DOI: 10.1002/(SICI)1097-0320(19960601)24:2<167::AID-CYTO9>3.0.CO;2-G

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

49. Evenson DP, Wixon R: Clinical aspects of sperm DNA fragmentation detection and male infertility. *Theriogenology*, 65, 979-991, 2006. DOI: 10.1016/j.theriogenology.2005.09.011

50. Casey PJ, Gravance CG, Davis RO, Chabot DD, Liu IKM: Morphometric differences in sperm head dimensions of fertile and subfertile stallions. *Theriogenology*, 47, 575-582, 1997. DOI: 10.1016/S0093-691X(97)00015-0

51. Hirai M, Boersma A, Hoeflich A, Wolf E, Föll J, Aumüller R, Braun AJ: 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, 2001.

52. Villalobos DG, Quintero-Moreno A, López-Brea JJG, Esteso MC, Fernández-Santos MR, Rubio-Guillén J, Silva WM, Marval YG, Atencio GL, Bohórquez CL: Caracterización morfométrica de la cabeza del espermatozoide porcinomediante análisis computarizado (Resultados Preliminares). *Revta Cient FCV-LUZ*, 18, 570-577, 2008.

53. Malo AF, Gomendio M, Garde J, Lang-Lenton B, Soler AJ, Roldan ERS: Sperm design and function. *Biol Lett*, 22, 246-249, 2006. DOI: 10.1098/rsbl.2006.0449

54. Helfenstein F, Podevin M, Richner H: Sperm morphology, swimming velocity, and longevity in the house sparrow *Passer domesticus. Behav Ecol Sociobiol*, 64, 557-565, 2010. DOI: 10.1007/s00265-009-0871-x

55. Arruda RP, Ball BA, Gravance CG, Garcia AR, Liu IKM: Effects of extenders and cryoprotectants on stallion sperm head morphometry. *Theriogenology*, 58, 253-256, 2002. DOI: 10.1016/S0093-691X(02)00858-0

56. Hidalgo M, Rodriguez I, Dorado JM: The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Anim Reprod Sci*, 100, 61-72, 2007. DOI: 10.1016/j.anireprosci.2006.07.003

57. Gravance CG, Liu IKM, Davis RO, Hughs JP, Casey PJ: Quantification of normal stallion sperm-head morphometry. *J Reprod Fertil*, 108, 41-46, 1996. DOI: 10.1530/jrf.0.1080041

58. De Paz P, Mata-Campuzano M, Tizado EJ, Álvarez M, Álvarez-Rodríguez M, Herraez P, Anel L: The relationship between ram sperm head morphometry and fertility depends on the procedures of acquisition and analysis used. *Theriogenology*, 76, 1313-1325, 2011. DOI: 10.1016/j.theriogenology.2011.05.038

59. Kot MC, Handel MA: Binding of morphologically abnormal sperm to mouse egg *zonae pellucidae in vitro*. *Gamete Res*, 18, 57-66, 1987. DOI: 10.1002/mrd.1120180107

60. Purwantara B, Arifiantini RI, Riyadhi M: Sperm morphological assessments of Friesian Holstein bull semen collected from Tyree artificial insemination center in Indonesia. *J Indonesian Trop Anim Agric*, 35, 89-94, 2010.

61. Wysokińska A, Kondracki S: Assessment of sexual activity levels and their association with ejaculate parameters in two-breed hybrids and purebred Duroc and Pietrain boars. *Ann Anim Sci*, 14, 559-571, 2014. DOI: 10.2478/aoas-2014-0030

62. Boersma AA, Braun J, Stolla R: Influence of random factors and two different staining procedures on computer-assisted sperm head morphometry in bulls. *Reprod Domest Anim*, 34, 77-82, 1999. DOI: 10.1111/j.1439-0531.1999.tb01387.x

63. Bonet S: Immature and aberrant spermatozoa in the ejaculate of *Sus domesticus*. *Anim Reprod Sci*, 22, 67-80, 1990. DOI: 10.1016/0378-4320(90)90039-I

64. Hallap T, Nagy S, Haard M, Jaakma U, Johannisson A, Rodriguez-Martinez H: Sperm chromatin stability in frozen-thawed semen is maintained over age in AI bulls. *Theriogenology*, 63, 1752-1763, 2005. DOI: 10.1016/j.theriogenology.2004.08.001

65. Makhzoomi A, Lundeheim N, Haard M, Rodriguez-Martinez H: Sperm morphology and fertility of progeny-tested AI Swedish dairy bulls. *Theriogenology,* 70, 682-691, 2008. DOI: 10.1016/j.theriogenology.2008.04.049

66. Sarder MJU: Effects of age, body weight, body condition and scrotal circumference on sperm abnormalities of bulls used for artificial insemination (AI) programme in Bangladesh. *Univ J Zool Rajshahi Univ*, 27, 73-78, 2008. DOI: 10.3329/ujzru.v26i0.706

67. Pruneda A, Pinart E, Briz DM, Sancho S, Garcia-Gil N, Badia E, Kádár E, Bassols J, Bussalleu E, Yeste M, Bonet S: Effects of a high semen-collection frequency on the quality of sperm from ejaculates and from six epididymal regions in boars. *Theriogenology*, 63, 2219-2232, 2005. DOI: 10.1016/j.theriogenology.2004.01.005

68. Herman H, Mitchell JR, Doak GA: Evaluation of semen-morphology. **In,** Herman HA, Mitchell JR, Doak GA (Eds): The Artificial Insemination and Embryo Transfer of Dairy and Beef Cattle. 8th ed., 87-92, Interstate Publishers, Dauville, 1994.

69. Martin-Rillo S, Martinez E, Garcia C, Artiga C, De Alba C: Boar semen evaluation in practice. *Reprod Domest Anim*, 31, 519-526, 1996.

70. Soderquist L, Janson L, Larsson K, Einarsson S: Sperm morphology and fertility in A. I. bulls. *J Vet Med A*, 38, 534-543. 1991. DOI: 10.1111/j.1439-0442.1991.tb01045.x