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CORRELATION OF EJACULATE PARAMETERS AND SPERM MORPHOLOGY WITH THE EJACULATE VOLUME OF PIETRAIN BOARS

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Abstract

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The objective was to analyze the connection between physical ejaculate parameters and sperm morphology and the volume of Pietrain boar ejaculates. The study covered 87 ejaculates collected from 10 Polish Pietrain boars. Ejaculate samples were collected from each of the boars once a month for sperm morphology analyses. The samples were used for preparing microscopic slides. Fifteen randomly selected spermatozoa were morphometrically measured. Their morphologic structure indices were additionally calculated. The frequency of morphological abnormalities in the spermatozoa was also determined. Spermatozoa with major and minor abnormalities were specified according to Blom's classification. Also, conventional evaluation of ejaculates was made following methods used at Polish sow insemination centers. A relationship was identified between ejaculate volume and morphometric parameters of spermatozoa. A rise in ejaculate volume was accompanied with increasing head and tail length, with the longest sperms identified in the most voluminous ejaculates. It was found that a rise in ejaculate volume was accompanied with a decrease in sperm concentration and an increase in the total sperm count.

Key words: boar, ejaculate volume, morphometric characteristics, spermatozoa

Introduction

One of the elementary factors that determine the profitability of animal production is male and female fertility. This has specific importance in the case of the pig which is a multi-birth species. Sizeable and even litters as well as the number of raised piglets significantly influence the efficiency of pig livestock production (Gadea, 2005). The utilization of the sow fertility potential largely depends on the boar (Tsakmakidis et al., 2010). Boars employed in reproduction should have high levels of sexual activity parameters, as well as producing large amounts of high-quality semen (Frydrychová et al., 2011).

The generation of spermatozoa of substantial biological value is conditioned by numerous factors. They include: the age of the male, season of the year, bioclimatic conditions, type of farming, diet, size of testes, intensity of animal use and individual features of the animal (Pruneda et al., 2005). Insemination fitness of ejaculates is affected by the breed of the boar (Phetudomsinsuk et al., 2008). Sires of different breeds or crosses can produce ejaculates of different volumes, different sperm concentrations and different sperm motility and fertilisation capacity (Kunavongkrit et al., 2005).

Semen assessments play an important role in early detection of developmental disorders in domestic animals (Smital et al., 2004). Apart from classical methods of semen assessment, biochemical tests and morphological and morphometric analyses of spermatozoa are also performed. What is also assessed is sperm cell membrane damage, sperm chromatin structure, oxidative sperm damage, apoptotic sperm modifications and ATP levels (Gadea, 2005).

The view is dominant of particular insemination fitness of high-volume ejaculates enabling the preparation of numerous insemination doses. High volume ejaculates are produced by Pietrain boars (Wysokiñska et al., 2009; Kondracki et al., 2012). It was proved that ejaculate volume plays an essential part in fertilization and embryo survival. This may stem from the fact that elevated ejaculate volumes positively affect semen transport by stimulating the middle matrix wall layer to contract quicker and inducing the pituitary to release hor-

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mones responsible for contractions of unstriated muscles in the matrix (Stratman and Self, 1960).

Pietrain boars are used as sires in cross-breeding programs in order to improve the meat content of pigs (Kawêcka et al., 2008). Disadvantageous correlations between production and reproduction parameters have been identified in pig breeds with high meat content (Banaszewska and Kondracki, 2012). Considerable numbers of Pietrain sires have lowered or lacking libido, insufficient physical prowess and weak extremities (Young et al., 1986). Boars of this breed are also characterised by high stress vulnerability caused by the presence of the recessive halothane sensitivity gene (Orzechowska et al., 2004).

Materials and Methods

Eighty-seven ejaculates were collected from 10 Pietrain boars at three sow artificial insemination centres owned by the Mazovian Centre of Animal Breeding and Reproduction in Łowicz, Poland. The boars were young (approximately 7-8 months of age), at the initial stage of their reproductive utilization. All the boars selected for analyses were in good shape, without apparent developmental defects and with a correct sexual urge. The ejaculates were collected using a manual collection method (King and Macpherson, 1973). Evaluation was made of ejaculates collected monthly from each boar. Freshly collected ejaculates were used to determine the following physical characteristics: ejaculate volume (in ml) – measured after filtering out the gelatinous fraction, sperm concentration the photometric method, percentage of spermatozoa displaying normal motility determined on the basis of microscopic examination of sperm motility in a droplet of fresh semen, total number of spermatozoa in an ejaculate and the number of insemination doses obtained from one ejaculate calculated using the SYSTEM SUL v.6.1 software. Immediately after collection, a microscopic slide was prepared from each ejaculate. The slides were prepared and stained by means of a method presented previously in the work by Kondracki et al. (2006). All the slides underwent analysis under a microscope Nikon-400 using 100x immersion lens. Morphometric measurements were taken of fifteen randomly selected morphologically normal spermatozoa. The following sperm measurements were recorded: sperm head perimeter, head area, sperm head length, sperm head width, flagellum length and overall sperm length. The measurements were done by means of computer image analysis software (Screen Measurement v. 4.1, Laboratory Imaging S. r. o. LIM Czech Republic, Praha) following methodology prepared by Kondracki et al. (2005).

The collected material was grouped following the criterion of ejaculate volume into three sets (Tab.1): ejaculates with the volume of 211 ml and below (Group I), ejaculates with the volume of 211-300 ml (Group II), and ejaculates with the volume of 300 ml and above (Group III) (Table 1).

The following parameters of sperm morphology were calculated on the basis of the results of the morphometric measurements: sperm head width/length ratio, head length/overall sperm length ratio, sperm head length/flagellum length ratio, flagellum length/overall sperm length ratio, sperm head perimeter/overall sperm length ratio, sperm head area/overall sperm length ratio and the product of sperm head length and width/overall sperm length ratio. Additionally, in each preparation the morphometry of 500 spermatozoa was evaluated, numbers of spermatozoa with normal morphology and morphological abnormalities were recorded and these were categorized into spermatozoa with major and minor defects following the Blom classification system (Blom, 1981). The results were statistically processed using analysis of variance according to the following mathematical model:

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

where: Y_{ij} – trait value, μ - population mean, a_i – ejaculate volume effect, e_{ij} – error.

Differences between means were tested using Tukey's test at $P \leq 0.05$ and $P \leq 0.01$

Results

Table 2 shows the results of the assessment of all the physical parameters of the Pietrain ejaculates in relation to ejaculate volume.

The data contained in Table 2 indicate that the physical parameters of the Pietrain ejaculates are correlated with ejaculate volume. Rising ejaculate volume is accompanied with decreasing sperm concentration and falling sperm motility. What clearly grows is however the ejaculate sperm count and the number of insemination doses obtained from an ejaculate. Group I, the one with lowest-volume ejaculates, had the highest sperm concentration. It averagely amounted to 498.84 thousand/mm^3, i.e. over 95 thousand/mm^3 more than the sperm concentration in Group II ($P \leq 0.01$) and over 144 thousand/mm^3 more than in Group III, the one with the lowest ejaculate volume ($P \leq 0.01$).

Table 1
Number of ejaculates depending on the ejaculate volume

Specification	Volume of ejaculate, ml			
	group I < 211	group II 211-300	group III > 300	Including
Number of ejaculates	26	29	32	87

Group I, with the lowest ejaculate volume, was found to have the highest sperm motility. The percentage of motile spermatozoa in this group was over 4% higher than in Group II (P≤0.01) and almost 3% higher than in Group III (P≤0.05). The highest sperm count was identified in Group III ejaculates. The ejaculates of this group contained over 102 billion progressively motile spermatozoa, over 22 billion sperm cells more than in the ejaculates of Group II (P≤0.01) and approximately 32 billion spermatozoa more than in the ejaculates of Group I, the one with the lowest volumes (P≤0.01). The number of spermatozoa affects the number of insemination doses that can be prepared from one ejaculate. The highest number of insemination doses was prepared from Group III ejaculates, with the greatest volumes. Each ejaculate in this group provided over 34 insemination doses, around 8 insemination doses more than the ejaculates of Group II and over 11 insemination doses more than the ejaculates of Group I (P≤0.01).

Table 3 presents the results of the measurements of the incidence of morphological abnormalities in the spermatozoa in relation to ejaculate volume.

The percentage of normal spermatozoa in the particular groups ranged from 93.4 to 95.08%. The highest sperm quality was identified in Group I ejaculates, those with the lowest volume. These ejaculates were found to contain over 95% morphologically normal spermatozoa, i.e. roughly 1.7% more than in the ejaculates of boars in Group II and 1.42% more than in the ejaculates of Group III boars. These differences were not however confirmed statistically. Based on the data of Table 3, it can be concluded that the quality of Pietrain semen was very high. The share of spermatozoa with major abnormalities in the particular groups ranged from 0.6 to 1.73%. The differences between the groups were insignificant and were not confirmed statistically. The highest percentage of spermatozoa with major abnormalities was identified in the highest-volume ejaculates.

Major morphological abnormalities in the spermatozoa mostly referred to spermatozoa with a protoplasm drop in the proximal position. The percentage of spermatozoa with this defect was however small, only slightly above 1% (Figure 1).

The highest numbers of spermatozoa with minor morphological abnormalities were identified in the semen of Group II boars (5.45%). The differences between these groups were however insignificant and were not confirmed statistically. The data of Table 3 show then that the volume of ejaculates

Table 2
Physical traits of ejaculates (means ± SD) as related to the volume of ejaculate

Variables	Ejaculate volume, ml		
	group I < 211	group II 211-300	group III > 300
Number of ejaculates	26	29	32
Ejaculate volume, ml	182.69±26.76^{A}	260.34±26.11^{B}	380.31±65.52^{C}
Sperm concentration, x 10^3/mm^3	498.84±80.96^{B}	403.07±76.65^{Aa}	351.06±87.51^{Ab}
Percentage of spermatozoa with normal motility, %	79.61±1.96^{B}	75.52±5.06^{A}	76.87±4.71^{AB}
Total number of spermatozoa, x 10^9	70.77±17.39^{A}	80.35±16.02^{A}	102.38±32.46^{B}
Number of insemination doses per ejaculate	23.19±3.43^{A}	26.58±5.84^{A}	34.56±10.69^{B}

Different superscripts mean significant differences among means within particular rows;
lower-case letters: P≤0.05, upper-case letters: P≤0.01.

Table 3
Frequency of occurrence of spermatozoa morphologically changes (means ± SD) as related to the volume of ejaculate

Variables	Ejaculate volume, ml		
	group I < 211	group II 211-300	group III > 300
Number of ejaculates	26	29	32
Ejaculate volume, ml	182.69±26.76^{A}	260.34±26.11^{B}	380.31±65.52^{C}
Percentage of normal spermatozoa, %	95.08±3.43^{a}	93.40±6.04^{a}	93.66±6.95^{a}
Sperm with major abnormalities, %	0.68±0.56^{a}	1.15±2.81^{a}	1.73±2.58^{a}
Sperm with minor abnormalities, %	4.24±3.38^{a}	5.45±5.50^{a}	4.61±5.15^{a}

Different superscripts mean significant differences among means within particular rows;
lower-case letters: P≤0.05, upper-case letters: P≤0.01.

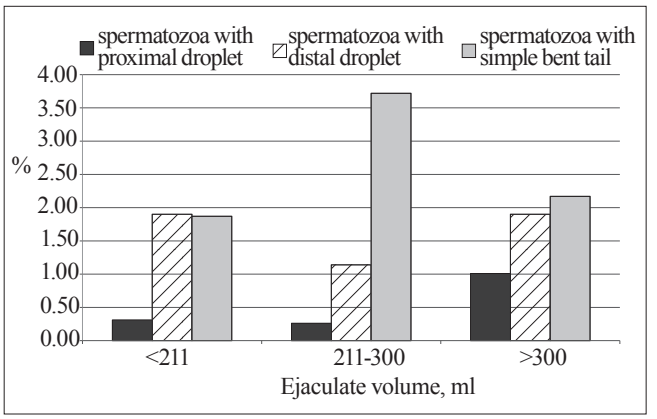

Fig. 1. Frequency of occurrence of chosen of anomaly of morphological sperms depending on the ejaculate volume

collected from Pietrain boars only slightly affects the incidence of morphological abnormalities in spermatozoa.

Table 4 contains data on the dimensions of the spermatozoa in relation to ejaculate volume.

The data of Table 4 reveal that the ejaculates with the highest volume in excess of 300 ml (Group III) contained spermatozoa with clearly larger dimensions as compared with the average- and low-volume ejaculates (Groups I and II). The sperm head length evidently grew along with rising ejaculate volume. The heads of spermatozoa from the most voluminous ejaculates were 0.28 μm longer than the heads of Group I spermatozoa. The difference was considerable and highly statistically significant (P≤0.01). Also, the heads of spermatozoa form Group III ejaculates were averagely 0.14 μm and 0.24 μm broader than the heads of spermatozoa from Group I (P≤0.05) and II (P≤0.01) ejaculates. As a result the

Table 4
Morphometric traits of sperms (means ± SD) with regard to the volume

Variables	Ejaculate volume, ml		
	group I < 211	group II 211-300	group III > 300
Number of ejaculates	26	29	32
Ejaculate volume, ml	182.69 ± 26.76^{A}	260.34 ± 26.11^{B}	380.31 ± 65.52^{C}
Head length, μm	9.14 ± 0.34^{A}	9.29 ± 0.36^{AB}	9.42 ± 0.35^{B}
Head width, μm	4.64 ± 0.25^{a}	4.54 ± 0.19^{A}	4.78 ± 0.24^{Bb}
Head perimeter, μm	23.84 ± 0.90^{a}	23.83 ± 1.01^{a}	24.55 ± 1.01^{b}
Head area, μm^2	39.84 ± 2.81^{A}	39.68 ± 2.24^{A}	42.30 ± 2.23^{B}
Flagellum length, μm	44.31 ± 1.43^{ab}	43.70 ± 2.79^{b}	45.20 ± 1.65^{a}
Total length, μm	53.45 ± 1.47^{AB}	52.99 ± 2.85^{A}	54.62 ± 1.64^{B}

Different superscripts mean significant differences among means within particular rows;
lower-case letters: P≤0.05, upper-case letters: P≤0.01.

Table 5
Morphometric indexes of sperm (means ± SD) as related to the volume

Variables	Ejaculate volume, ml		
	group I < 211	group II 211-300	group III > 300
Number of ejaculates	26	29	32
Ejaculate volume, ml	182.69 ± 26.76^{A}	260.34 ± 26.11^{B}	380.31 ± 65.52^{C}
Head width/head length	50.88 ± 3.30^{b}	48.88 ± 1.95^{a}	50.79 ± 3.10^{b}
Head length/total length	17.11 ± 0.69^{a}	17.59 ± 1.20^{a}	17.26 ± 0.77^{a}
Head length/flagellum length	20.64 ± 1.01^{a}	21.37 ± 1.84^{a}	20.87 ± 1.12^{a}
Head area/total length	82.89 ± 0.69^{a}	82.41 ± 1.20^{a}	82.74 ± 0.77^{a}
Head length x width/total length	44.63 ± 1.68^{a}	45.08 ± 2.91^{a}	44.98 ± 1.99^{a}
Perimeter of the head/total length	74.54 ± 4.87^{a}	75.08 ± 5.94^{a}	77.49 ± 4.19^{a}
Flagellum length/total length	79.42 ± 4.82^{a}	79.86 ± 6.43^{a}	82.42 ± 4.73^{a}

Different superscripts mean significant differences among means within particular rows;
lower-case letters: P≤0.05, upper-case letters: P≤0.01.

spermatozoa from the most voluminous ejaculates also had a significantly greater head perimeter and area than the spermatozoa from Group I and II ejaculates ($P \leq 0.01$). The tails of the spermatozoa from Group III ejaculates were on average 1.5 μm longer than the tails of the spermatozoa from Group II ejaculates ($P \leq 0.05$) and 0.89 μm longer in comparison with the tails of Group I spermatozoa. Consequently, the total length of Group III spermatozoa was the greatest, owing to both head length and tail length.

Table 5 presents indices of sperm shape.

The data of Table 5 show that ejaculate volume has little influence on the shape of the spermatozoa of Pietrain boars. The majority of the morphological structure indices of the spermatozoa assumed similar values in the particular groups, and the observed differences generally remained within the limits of statistical error. The only fact that was proved was that the spermatozoa of Group II ejaculates, with the volume of 211-300 ml, had more elongated heads than the spermatozoa of Group I and III ejaculates. The width to length ratio of the sperm head in Group II ejaculates was significantly higher than in the case of Group I and III ejaculates ($P \leq 0.05$). A slightly lower ratio of head length to total sperm length and tail length also seems to account for the more elongated shape of the spermatozoa in Group II ejaculates.

Discussion

The obtained data show sperm motility in fresh ejaculates to remain at a relatively steady level. The percentage of progressively motile spermatozoa remained within the limits of 75-79%, with higher range values determined for the ejaculates with the lowest volumes. Boar ejaculates should contain at least 60% progressively motile spermatozoa (Martin-Rillo et al., 1996). According to Kuster and Althouse (1999), this index should assume a value of above 70%.

The data of the present paper show ejaculates with lower volumes to have greater sperm concentration. Sperm concentration falls, in turn, with rising ejaculate volume. This suggests the existence of an inversely proportional relationship between ejaculate volume and sperm concentration. The data presented in Table 2 quite clearly show this correlation in Pietrain boars. The existence of an inversely proportional correlation between ejaculate volume and sperm concentration was also confirmed by previous studies (Kondracki et al., 2012).

The total number of spermatozoa in the ejaculate depends on ejaculate volume and sperm concentration and motility. As results from the data of the present work, the total number of spermatozoa in the ejaculates of the Pietrain boars was very high, ranging on average from approximately 70 billion in Group I to over 102 billion in Group III ejaculates. Data confirming high numbers of spermatozoa in Pietrain boar ejaculates were also gathered in other studies (Wysokiñska et al., 2009).

The number of spermatozoa in the ejaculate also determines the number of insemination doses that can be prepared out of the ejaculate. The data of Table 2 show that the number of insemination doses prepared out of Pietrain boar ejaculates was very high. High numbers of insemination doses are characteristic of Pietrain boar ejaculates.

It should be noted that the ejaculate sperm count, and consequently the number of insemination doses obtained from the ejaculate clearly rose along with ejaculate volume (Table 2). The data of Table 2 reveal that a 100 ml increase in ejaculate volume entails a 16 billion increase in the ejaculate sperm count and enables the preparation of a greater number o insemination doses out of the ejaculate by approximately 5.8 units. This occurs despite the simultaneous fall in the sperm concentration and, to a lesser degree, the decline in sperm motility. It seems thus that the performance of Pietrain insemination boars mainly depends on the volume of produced ejaculates and this parameter should be regarded as the fundamental insemination fitness index in the case of boars of this breed.

Based on the results of the present work, we can conclude that there is a connection between the morphometric parameters of spermatozoa and ejaculate volume. Differences in sperm dimensions and shape were identified between the groups which differed in ejaculate volume. The present study identified a correlation between sperm head dimensions and ejaculate volume. Spermatozoa in ejaculates with the highest volumes had larger and longer heads with greater perimeters and areas than spermatozoa in ejaculates with lower volumes. The sperm head structure may be one of the factors that affect male fertility (De Paz et al., 2011). Sperm head defects are often the cause of reduced fertility, deteriorated embryo quality, lowered sperm capacity to fuse with the egg cell and miscarriages in the first period of pregnancy (Chenoweth, 2005). Some studies confirmed the correlation between male fertility and sperm head dimensions. In males with higher fertility, spermatozoa have narrower and shorter heads (Hirai et al., 2001).

The analysis of the morphometric parameters of the spermatozoa in Pietrain boar ejaculates differing in volume authorizes the conclusion that the spermatozoa contained in the highest-volume ejaculates were the longest. This fact was confirmed both in relation to total sperm length and tail length. The tail length may be associated with physical ejaculate parameters. Spermatozoa in low sperm concentration ejaculates were found to have longer tails than spermatozoa

in highly concentrated ejaculates (Kondracki et al., 2011). That, the ability of individual spermatozoa to penetrate an oocyte is associated with spermatozoa velocity, is conceivable (Suarez, 2007). Some observations show that sperm velocity may depend on tail length. Spermatozoa with longer tails can negotiate the distance to the egg cell faster (Lüpold et al., 2009). Helfenstein et al. (2010) suggest the existence of a correlation between head and tail length and sperm velocity. Spermatozoa with a lower ratio of head length to tail length move faster. It is a common belief, however, that such spermatozoa have a lower survival rate, which results from a faster depletion of mitochondrial ATP energy (Gage, 1998). According to Cardullo and Baltz (1991), the amount of energy produced by spermatozoa largely depends on the size of sperm midpiece.

The present study found the highest number of morphologically abnormal spermatozoa in ejaculates with a volume ranging from 211 to 300 ml. This was largely determined by a high frequency of minor abnormalities. This group is specific as it contains a high percentage of spermatozoa with tail abnormalities. Sperm morphology studies are of considerable importance for the assessment of sperm quality. The determination of the extent of acceptable morphological modifications in boar sperm structure is not easy, though. Boars with normal fertility always have a certain percentage of morphologically abnormal spermatozoa (Bonet, 1990). According to Blom (1981), the presence of maximally 15% of spermatozoa with major abnormalities and 10-15% of spermatozoa with minor abnormalities is acceptable. Kuster and Althouse (1999) claim that the share of morphologically abnormal spermatozoa in boar semen should not exceed 20%.

The presented data show that the greatest number of spermatozoa with major abnormalities is contained in ejaculates of the highest volumes. The percentage of spermatozoa with major abnormalities in the ejaculates of Group III was more than twice higher than in the ejaculates with a volume below 211 ml. Among major morphological abnormalities, the most frequent one was a proximal protoplasmic droplet on the midpiece. It is commonly believed that this abnormality results from incorrect sperm maturation (Bonet, 1990). Among others, they may be caused by a shorter time spent by spermatozoa in the epididymal duct (Pruneda et al., 2005) as a result of excessively frequent ejaculation (Bonet et al., 1992). Briz et al. (1995) have reported that spermatozoa with this defect are mostly identified in the head of the epididymis. According to Martin-Rillo et al. (1996), it is acceptable that the ejaculate contains 20% spermatozoa with a proximal droplet at the maximum. A higher percentage results in reduced male fertility (Soderquist et al., 1991). The presence of spermatozoa with a cytoplasmic droplet negatively affects semen potential for long-term storage. The proportion of spermatozoa with a proximal and distal protoplasmic droplet should not exceed 15% (Waberski et al., 1994).

The presented data show that the most common minor morphological abnormality concerned spermatozoa with a simple bent tail. The number of spermatozoa with this defect was however inconsiderable, below 4% in any of the subgroups. According to Martin-Rillo et al. (1996), a share of spermatozoa with a coiled tail in boar semen in excess of 5% disqualifies the semen. Tail loops most often arise in the tail of the epididymis and are associated with a persisting distal cytoplasmic droplet. As a result, a bend in the tail is observed in the vicinity of the mid-piece ring (Bonet et al., 1992). Such abnormalities negatively affect the fertilization potential of the semen (Bonet, 1990).

Conclusions

Morphometric sperm parameters depend on the volume of ejaculates obtained from Pietrain boars. A rise in ejaculate volume is accompanied with increasing head and tail length, as well as greater head perimeter and area. As a result, this causes an increase in the total sperm length, with the longest sperms identified in the most voluminous ejaculates. No correlation between structural abnormality incidence and ejaculate volume was found. The quality of the semen of the analyzed Pietrain boars was very high, with the percentage of morphologically abnormal spermatozoa below 6%. It was shown that a rise in ejaculate volume is accompanied with an increase in the total sperm count in the ejaculate and with a decrease in sperm concentration. The higher the ejaculate volume, the more insemination doses can be prepared out of the ejaculate.

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