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ASSOCIATION OF EJACULATE SPERM COUNTS WITH THEIR MORPHOLOGICAL AND MORPHOMETRIC CHARACTERISTICS IN HYPOR BOARS*

Krzysztof Górski♦, Stanisław Kondracki, Karolina Strachocka, Anna Wysokińska

Institute of Bioengineering and Animal Breeding, Siedlce University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland
♦Corresponding author: krzysztof.gorski@uph.edu.pl

Abstract

Reproductive efficiency in pigs is largely dependent on the fertility of the boar. Boars used at insemination stations should produce a large amount of semen with high fertilization capacity. The sperm count influences the conception rate and the number of insemination doses produced. The aim of this study was to evaluate the morphological and morphometric characteristics of boars of the Hypor breed in relation to the total sperm count in the ejaculate. An analysis was performed of 120 ejaculates collected from 12 Hypor boars used at three insemination stations. The ejaculate sperm count was found to affect the morphometric characteristics of spermatozoa from boars. In the ejaculates with the lowest sperm count the spermatozoa heads were significantly shorter and narrower and had a smaller surface area. The sperm from ejaculates with the lowest sperm count had relatively small head dimensions in relation to the tail length and total sperm length. In the ejaculates with the most sperm cells, the proportion of spermatozoa with major defects was significantly greater than in the ejaculates from other groups. In the ejaculates with the lowest sperm count the frequency of sperm with progressive motility was significantly lower. The fewest insemination doses can be prepared from these ejaculates.

Key words: boar, ejaculate, sperm count, sperm morphology

Boars used at insemination stations should produce a large amount of semen with high fertilization capacity (Kondracki et al., 2014). The sperm count influences the conception rate and the number of insemination doses produced (Kowalewski et al., 2016). The quality and viability of the spermatozoa are important as well. The quality of sperm and their fertilization capacity are linked to their morphology (Hirai et al., 2001). The frequency of morphological abnormalities in sperm is significant, as are their dimensions and shape. This determines the motility of the spermatozoa and their ability to penetrate the ovum (Gil et al., 2009). Changes taking place in the

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structure of the sperm cell membrane during capacitation and the acrosome reaction are of fundamental importance for the fusion of the spermatozoon and oocyte (Wysokińska and Kondracki, 2014 a). Both capacitation and the acrosome reaction are influenced by the presence of sperm with abnormal morphology that affects fertility in males (Long et al., 1996).

Production of sperm of high biological value depends on numerous factors, including the breed, age, and intensity of exploitation of the animal, time of year, semen collection conditions (Smital et al., 2004; Wysokińska and Kondracki, 2013; Knecht et al., 2014; Kowalewski et al., 2016) and the libido of the male (Kondracki et al., 2013; Wysokińska and Kondracki, 2014 b). Semen quality can be evaluated by examining sperm morphology, which makes it possible to assess whether the sperm structure is normal and to specify the type of morphological anomalies present (Górski et al., 2017). Ejaculates of males vary in terms of the frequency of sperm with abnormal morphology (Pinart et al., 1998; Ruiz-Sanchez et al., 2006; Górski et al., 2016).

The suitability of semen for artificial insemination may be influenced by the dimensions and shape of the sperm (Gage and Morrow, 2003), as correlations have been demonstrated between sperm dimensions and male fertility (Casey et al., 1997). Morphological characteristics may depend on the intensity of sperm production in the testicular tissue and thus the number of sperm stored in the epididymis and discharged in the ejaculate. The aim of this study was to evaluate the relationships between ejaculate traits, sperm morphological and morphometric characteristics, and sperm count in Hypor boars.

Material and methods

The analysis was performed on 120 ejaculates collected from 12 Hypor boars. The animals were housed at three AI stations: C, G and Z. Station C is located at latitudes 52°N and 52.54°N and longitudes 20°E and 36.38°E. Station G is located at latitudes 51°N and 58.27°N and longitudes 19°E and 01.17°E. Station Z is located at latitudes 52°N and 05.28°N and longitudes 19°E and 59.01°E. Young boars aged 7–9 months, in their initial stage of reproductive activity, were selected for the study. All the boars from which semen was collected were raised in the same conditions, in accordance with current animal welfare regulations (Regulation of the Minister of Agriculture and Rural Development, 2010). Food intake was individualized for each boar according to nutrition requirements (Swine Nutrition Requirements, 1993), with *ad libitum* access to water. The animals had no infectious or reproductive diseases and were covered by a routine disease prevention program and regular veterinary care. Ejaculates were collected every four days by the manual method (King and Macpherson, 1973). Ten ejaculates from each boar, collected at one-month intervals, were evaluated. The fresh ejaculates were evaluated for physical traits, including the ejaculate volume, spermatozoa concentration, percentage of spermatozoa with proper motility, total number of spermatozoa in one ejaculate, and number of AI doses. The ejaculate volume (without the gelatinous fraction) was measured and

its ratio to the ejaculate weight was calculated. Ejaculates were weighed using an electronic balance. Spermatozoa concentration was assessed using the colorimetric method. The method was based on the measurement of light intensity passing through the suspended spermatozoa in an isotonic solution of chlorine or sodium citrate. Sperm motility was determined by microscopic examination using a light microscope (Nikon Eclipse 50i, Japan). Magnification of 200x was used to determine the percentage of spermatozoa with appropriate motility at 37°C in the total number of spermatozoa visible under the microscope. The total sperm count was calculated based on ejaculate volume and sperm concentration in relation to the percentage of spermatozoa with progressive motility. The total number of semen doses per ejaculate was calculated from the volume, motility and sperm concentration. The number of insemination doses was established following dilution such that one insemination dose contained about three billion spermatozoa. The total number of sperm cells per ejaculate and number of AI doses were estimated using SYSTEM SUL software, designed for use by insemination centres.

The ejaculates were divided into three groups according to the criterion of total sperm count in the ejaculate: group I – ejaculates with less than 88 billion spermatozoa (47 ejaculates), group II – ejaculates with 88 to 115 billion spermatozoa (38 ejaculates), group III – ejaculates with more than 115 billion spermatozoa (35 ejaculates).

Microscope slides were prepared from the ejaculate samples. The slides were prepared and stained according to the method described in Kondracki et al. (2006). In each slide the morphological structure of 500 spermatozoa was evaluated, specifying the number of morphologically normal sperm and the number with morphological abnormalities, and distinguishing forms with major and minor defects according to the classification by Blom (1981). The following anomalies were classified as major: spermatozoa with a proximal droplet, spermatozoa with a strongly coiled tail, double forms, underdeveloped sperm and sperm with a pear-shaped head. Minor anomalies included spermatozoa with free heads, spermatozoa with distal droplets and spermatozoa with a simple bent tail. Microscopic analysis of sperm morphology was carried out using immersion lenses at ×100 magnification, with a Nikon-E 50i light microscope. Morphometric analysis of the sperm was performed as well.

In each slide morphometric measurements were performed on 15 randomly selected morphologically normal sperm cells using image analysis software (Screen Measurement v. 4.1), according to the method developed by Kondracki et al. (2005). The following morphometric sperm cells measurements were performed: head length, head width, head perimeter, head area, flagellum length and total sperm length. 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 × width/total length.

The experimental data were subjected to a statistical analysis with STATISTICA ver. 10 software (StatSoft, Tulsa, USA), using analysis of variance ANOVA for different N (N = number of subjects in groups). The material obtained was statistically analysed according to the following mathematical model:

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

where:

Y_{ij} – value of the parameter under analysis,
μ – overall mean,
a_i – effect of sperm count in boar ejaculate,
e_{ij} – random error.

The significance of the differences between the groups was assessed with the Tukey's post hoc test at P≤0.05.

Results

Table 1 presents data characterizing the basic physical characteristics of the ejaculates in relation to the ejaculate sperm count.

Table 1. Basic characteristics of ejaculates related to the total number of spermatozoa in an ejaculate (Least Square Mean (LSM) ± Standard Error (SE))

Variable	Total number of spermatozoa (× 10^9)		
	group I < 88	group II 88–115	group III > 115
Total number of spermatozoa (× 10^9)	61.88 a±16.38	102.10 b±8.12	130.44 c±11.46
Ejaculate volume (ml)	244.56 a±46.24	305.86 b±22.71	339.82 c±85.60
Sperm concentration (×10^6)	412.10 a±156.32	499.14 b±94.78	363.45 a±85.70
Percentage of spermatozoa with progressive motility (%)	73.09 a±4.65	79.48 b±2.23	77.82 b±4.17
Number of insemination doses per ejaculate	24.59 a±8.28	38.83 c±7.19	33.20 b±11.33

Different letters denote significant differences among means within particular rows; lower-case letters: P≤0.05.

The data in Table 1 indicate that an increased ejaculate sperm count is accompanied by greater ejaculate volume. The average ejaculate volume in the group of ejaculates with the fewest sperm cells (group I) was 244.56 ml, which was 95.26 ml smaller than in the group with the highest sperm count (group III) and 61.3 ml smaller than in the ejaculates containing between 88 and 115 billion sperm (group II) (P≤0.05). The highest sperm concentration was noted for the group II ejaculates. In these ejaculates the sperm concentration was 87.04 ×10^6 higher than in the ejaculates with the lowest sperm count (group I) and 135.69 ×10^6 higher than in the ejaculates with the highest sperm count (group III) (P≤0.05). The most insemination doses were prepared also from the ejaculates in group II, which contained from 88 to 115 billion sperm over 5 insemination doses more than were prepared from the ejaculates with the highest sperm count (group III) and over 14 insemination doses more than in the case of the group I ejaculates, with the lowest sperm count (P≤0.05). In the ejaculates with the lowest sperm count the sperm exhibited relatively low motility. In this

group of ejaculates normal progressive movement was observed in only 73.09% of spermatozoa, which was 4.73% less than in the group III ejaculates and 6.39% less than in the ejaculates of group II (P≤0.05).

Table 2 presents the results of the morphometric measurements of the sperm in relation to the ejaculate sperm count.

Table 2. Morphometric characteristics of spermatozoa related to the number of spermatozoa in an ejaculate (Least Square Mean (LSM) ± Standard Error (SE))

Variable	Total number of spermatozoa ($\times 10^9$)		
	group I < 88	group II 88–115	group III >115
Head length (µm)	8.49 a±0.72	8.81 b±0.77	8.92 b±0.55
Head width (µm)	4.45 a±0.41	4.68 b±0.43	4.74 b±0.41
Perimeter of the head (µm)	22.82 a±1.77	23.28 a±1.53	23.29 a±1.08
Head area (μm^2)	35.24 a±5.46	37.70 b±5.69	37.71 b±4.20
Flagellum length (µm)	44.05 a±1.25	43.91 a±1.27	43.72 a±0.93
Total length (µm)	52.54 a±1.35	52.72 a±1.66	52.64 a±1.02

Different letters denote significant differences among means within particular rows; lower-case letters: P≤0.05.

In the ejaculates with less than 88 billion sperm (group I), the sperm had smaller head dimensions than in the case of ejaculates with more sperm (groups II and III). In the ejaculates of group I the sperm heads were significantly shorter and narrower and had a smaller surface area (P≤0.05). In the group I ejaculates, the sperm had longer tails than the sperm from the other two groups, but these differences were not confirmed statistically. The data in Table 2 indicate a correlation between sperm dimensions and ejaculate sperm count.

Table 3 presents indices illustrating differences in the shape of the sperm from ejaculates with different sperm counts.

Table 3. Morphometric indexes of spermatozoa related to the number of spermatozoa in an ejaculate (Least Square Mean (LSM) ± Standard Error (SE))

Variable (%)	Total number of spermatozoa ($\times 10^9$)		
	group I < 88	group II 88-115	group III > 115
Head width/head length	52.64 a±4.88	53.16 a±3.26	53.15 a±3.64
Head length/total length	16.16 a±1.26	16.70 b±1.16	16.93 b±0.96
Head length/flagellum length	19.30 a±1.80	20.07 b±1.67	20.40 b±1.38
Perimeter of the head /total length	43.45 a±3.37	44.14 a±2.32	44.25 a±1.75
Head area/total length	67.05a±10.11	71.38 b±9.51	71.02 b±7.49
Head length × width/total length	72.24 a±10.95	78.47 b±11.76	80.45 b±10.15
Flagellum length/total length	83.84 b±1.26	83.30 a±1.16	83.07 a±0.96

Different letters denote significant differences among means within particular rows; lower-case letters: P≤0.05.

The shape indices of the sperm were found to depend on the ejaculate sperm count. Sperm from the ejaculates with the fewest sperm (group I) had smaller ratios of head dimensions to tail length or to total sperm length than sperm from the ejaculates of groups II and III. This is indicated by the significantly smaller ratios of head length/total sperm length and head length/tail length ($P \leq 0.05$). Sperm from the ejaculates with the fewest sperm were also found to have significantly smaller ratios of head area/total sperm length and head length × width/total length than sperm from ejaculates in groups II and III ($P \leq 0.05$). Sperm from the ejaculates with the lowest sperm count had a higher tail length/total sperm length ratio than sperm from ejaculates of groups II and III, with higher sperm counts ($P \leq 0.05$). These data show that sperm from the ejaculates with the fewest sperm had a relatively smaller head and a relatively longer tail, which gave them a different shape.

Table 4 presents data on the frequency of morphological sperm abnormalities in relation to the total sperm count in the ejaculate of Hypor boars.

Table 4. Frequency of occurrence of normal and abnormal spermatozoa related to the number of spermatozoa in an ejaculate (Least Square Mean (LSM) ± Standard Error (SE))

Variable (%)	Total number of spermatozoa ($\times 10^9$)		
	group I < 88	group II 88–115	group III > 115
Sperm with major abnormalities	0.95 a±1.47	0.71 a±0.90	1.72 b±2.20
Sperm with minor abnormalities	3.09 a±2.91	2.67 a±2.80	2.97 a±2.09
Percentage of normal spermatozoa	95.98 a±3.53	96.61 a±3.38	95.31 a±3.10

Different letters denote significant differences among means within particular rows; lower-case letters: $P \leq 0.05$.

The ejaculates with the most sperm cells (over 115 billion) had the highest percentage of sperm with major abnormalities. In the group III ejaculates the proportion of spermatozoa with major defects was significantly greater than in the ejaculates from groups I and II, containing fewer sperm ($P \leq 0.05$). Irrespective of the sperm count, however, the frequency of sperm with major morphological abnormalities was very low, typical of breeding boars with high fertility. The percentage of sperm with minor defects was about 3%. Thus it was relatively low, and as shown in Table 4, did not depend on the number of sperm in the ejaculate. Thus the data in Table 4 indicate that ejaculate sperm count has little significance for the occurrence of morphological sperm defects.

Discussion

Our study was carried out with the use of the ejaculates of Hypor boars, a hybrid line of pigs, not yet recognized in terms of the morphological characteristics of the sperm but commonly used in the production of hybrid fatteners. Research indicates the existence of a link between spermatozoa dimensions and the sperm number.

These data indicate that in ejaculates containing a small number of sperm, the sperm also display low motility. The results of the study suggest that ejaculate sperm count affects the morphometric characteristics of the sperm. Differences were shown in the dimensions and shape of sperm cells from ejaculates with different sperm counts. A study by Arnaud et al. (2001) showed that within a certain reproductive potential the number of sperm may compensate for their size. The present study showed that in the ejaculates containing the fewest sperm, the sperm cells had smaller head dimensions than those from ejaculates with a higher sperm count (groups II and III). According to Noorafshan and Karbalay-Doust (2010), sperm length is positively correlated with sperm velocity. Spermatozoa with longer midpieces and tails have greater tail strength. The length of the midpiece may be associated with the level of energy produced in the mitochondria (Bierła et al., 2007). Sperm with longer tails are more competitive than other sperm because they can reach the ovum more quickly. The results obtained did not confirm a correlation between tail length and the number of sperm in the ejaculate, although the data in Table 2 indicate that tail length decreases slightly as ejaculate sperm count increases. This correlation has been more clearly confirmed in research on Landrace boars (Wysokińska et al., 2009).

The present study indicates that in ejaculates with a low sperm count the sperm have smaller head dimensions. The heads of these sperm were significantly shorter and narrower than the heads of sperm from ejaculates with a high sperm count. Research conducted on Landrace boars showed that sperm from ejaculates with a high sperm count have smaller head dimensions than those from ejaculates with a small number of sperm (Wysokińska et al., 2009). The study cited, as well as the data obtained in the present study (Table 3), also shows that ejaculate sperm count may differentiate the shape of sperm heads and the proportions of the dimensions of individual organelles. The shape of the sperm head may be determined by the organization of DNA (Steinholt et al., 1994). Even slight deviations in the shape of the sperm head may presumably be caused by changes in chromatin structure in the cell nucleus (Ostermeier et al., 2001), and this may lead to reduced fertility (Evenson and Wixon, 2006). The sperm of boars with lower fertilization capacity have been shown to have larger and more elongated heads than the sperm of boars of high fertility (Hirai et al., 2001). The shape of the sperm head affects the hydrodynamics of the spermatozoon. According to Malo et al. (2006), sperm with elongated heads move faster than those with round heads. According to Thurston et al. (2001), the form of sperm movement depends on the shape of the head. Spermatozoa with higher values for ellipticity (head length/head width) exhibit lower ability to move forward in a straight line (Gil et al., 2009).

The data presented in our study indicate that the frequency of morphological sperm abnormalities depends to a small degree on the total number of sperm in the ejaculate. In the ejaculates containing the most sperm (over 115 billion), somewhat more sperm with major defects were noted than in the ejaculates from groups I and II ($P \leq 0.05$). This may indicate a tendency towards a higher frequency of morphological anomalies in ejaculates containing large numbers of spermatozoa. A high frequency of sperm with head defects may be the cause of reduced embryo quality (De Jarnette et al., 1992) and abortion during the initial period of pregnancy (Chenoweth, 2005).

Impairment of spermatogenesis and sperm maturation in the epididymis results in the production of sperm with abnormal morphology. Some of these cells may have damaged chromatin structure or a high level of DNA damage (Enciso et al., 2011). Some authors indicate that the frequency of morphological sperm abnormalities is linked to chromatin instability and increased frequency of chromosome abnormalities (Calogero et al., 2001; Fischer et al., 2003; Kubo-Irie et al., 2005; Sun et al., 2006).

Semen with a high frequency of morphological abnormalities is highly sensitive to factors accompanying semen dilution and the preparation of insemination doses, which is manifested in a high frequency of sperm with a damaged cell membrane (Wysokińska et al., 2015). The occurrence of morphologically abnormal cells indicates reduced functionality of the germinal epithelium and leads to decreased fertility in the male. A particularly unfavourable phenomenon is the presence in the semen of spermatozoa with major defects arising during spermatogenesis. Significant morphological abnormalities occur in the final stage of spermatogenesis, when large, spherical spermatids are transformed into an organized gamete (Zheng et al., 2007). A large percentage of sperm cells with major abnormalities, particularly acrosome defects, substantially reduces the chance of fertilization. The data obtained in the present study indicate that the percentage of spermatozoa with major defects was small, and did not exceed 1.72% in any group of ejaculates.

To sum up, the ejaculate sperm count affects the morphometric characteristics of spermatozoa. In ejaculates with a low sperm count the spermatozoa have smaller head dimensions than those of ejaculates with a high sperm count. In the ejaculates with the fewest sperm, the sperm cells had shorter and narrower heads and a smaller head area. Sperm from ejaculates with the lowest sperm count have relatively small head dimensions (length, area, product of length and width) in relation to the length of the tail and of the entire spermatozoon, which gives them a different shape as compared to sperm from ejaculates with a high sperm count. The frequency of morphological sperm abnormalities depends to a slight degree on the number of sperm in the ejaculate. The frequency of sperm with major defects is somewhat higher in ejaculates containing the most sperm. The sperm count is correlated with the quantitative characteristics of the ejaculate. A markedly lower frequency of sperm with progressive motility was noted in the ejaculates with the fewest sperm. The fewest insemination doses can be prepared from these ejaculates.

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