

Morphometrical characteristics of spermatozoa in Polish Landrace boars with regard to the number of spermatozoa in an ejaculate

Anna Wysokińska, Stanisław Kondracki¹, Dorota Banaszewska
Department of Animal Reproduction and Hygiene, University of Podlasie,
Siedlce, Poland

Received: 27 April 2009; accepted: 26 October 2009

SUMMARY

Ejaculates (n=344) were collected from 35 Polish Landrace boars. The ejaculates were assigned to one of four groups according to the total number of spermatozoa in an ejaculate. Morphometrical measurements of spermatozoa with proper morphology were performed. Spermatozoa with smaller head length and head area were found in ejaculates with the greatest total sperm number (more than 120×10^9 spermatozoa) than in ejaculates with total number of spermatozoa of $70-90 \times 10^9$. The results of the present study suggest that the number of spermatozoa in an ejaculate influences morphometrical characteristics of the spermatozoa in Polish Landrace boars. *Reproductive Biology* 2009 **9** 3: 271-282.

Key words: boar, ejaculate, total number of spermatozoa, morphometry

¹Corresponding author: University of Podlasie, Department of Animal Reproduction and Hygiene, 14 Prusa St., 08-110 Siedlce, Poland, email: sk@ap.siedlce.pl

INTRODUCTION

The main criterion for keeping a boar at an insemination station is the production of boar ejaculates containing a high quantity of spermatozoa with a high fertilization ability. The optimal production of semen of high biological value is influenced by numerous factors, including: breed [23, 32], season of the year [8, 33], age of the boar [10] and frequency of sperm collection. Examination of sperm morphology and determining the number of spermatozoa with morphological defects plays a significant role in semen quality assessment.

Many studies indicate that individual animals differ in the number of spermatozoa characterized by morphological defects [23, 28, 30]. The mammalian spermatozoon is characterized by a morphology responsible for passing on genetic information during the process of egg fertilization. The sperm head plays a significant role in the fertilization process. The shape of sperm head is conditioned by the nucleus and acrosome size [3]. The semen usefulness for insemination may be influenced by sperm morphometric characteristics resulting from semen size and shape. Relationships between sperm size and sire fertility have been documented [6, 12]. Some reports indicate that sperm morphometric characteristics of boars are not closely associated with the frequency of incidence of morphological abnormalities or with physical parameters of the ejaculate [20]. Morphological characteristics of spermatozoa can be influenced by the intensity of their production in the testes as well as the number of spermatozoa stored in epididymises and excreted in an ejaculate. Since the issue had not been fully examined yet, we attempted to assess sperm morphometric characteristics of the Polish Landrace boars in relation to the total number of spermatozoa in an ejaculate.

MATERIALS AND METHODS

Ejaculates and semen assessment

Ejaculates (n=344) were collected from 35 Polish Landrace boars kept two in two sow insemination stations at the Mazovian Centre of Animal

Breeding and Reproduction, Łowicz, Poland. The ejaculates were collected using the gloved-hand technique [19] from young boars (7-8 months) in the initial stage of their breeding utilization. Ejaculates collected from each boar were assessed for about 10 months at monthly intervals. Immediately after collection, the volume (ml) was determined after the gel fraction was removed, and sperm concentration ($\times 10^3/\text{mm}^3$) was measured using the photometric method. The percentage of spermatozoa with normal motility was estimated under a microscope with a warmed stage (37°C) at a magnification of $\times 200$. The total number of spermatozoa and the number of insemination doses obtained from one ejaculate were calculated using the SYSTEM SUL (v. 6.35; Gogosystem, Poland) computer program. The ejaculates were divided into four groups according to the total number of spermatozoa in an ejaculate: I/ less than 70×10^9 , II/ $70-90 \times 10^9$, III/ $90.1-120 \times 10^9$, and IV/ more than 120×10^9 spermatozoa.

Immediately after semen collection, smears were prepared and slides were stained using the Bydgoska method [22]. The slides were subjected to microscopic examinations using a Nikon E-400 microscope with immersion at $\times 100$ magnification. In each slide, morphometric measurements were performed of 15 randomly selected spermatozoa with normal morphology. The measurements were taken using an image computer analysis package (Screen Measurement v. 4.1, Laboratory Imaging S.r.o. LIM Czech Republic, Praha). The following sperm morphometric measurements were taken: sperm head length, sperm head width, head area, sperm head perimeter, flagellum length and total sperm length [20]. On the basis of these results the following indices of sperm morphology were calculated: width-to-length ratio of sperm head (%), ratio of head length to total sperm length (%), ratio of head length to sperm tail length (%), ratio of tail length to total sperm length (%), ratio of sperm head perimeter to total sperm length (%), ratio of sperm head area to total sperm length (%), ratio of sperm head length and width to total sperm length (%). Moreover, the morphology of 500 spermatozoa was assessed for each slide. Spermatozoa with major and minor abnormalities were classified according to Blom [5]. Results were processed using one-way analysis of variance followed by Tukey's post hoc test at $p \leq 0.05$ and $p \leq 0.01$.

Table 1. Basic characteristics (means±SD) of ejaculates related to the total number of spermatozoa in an ejaculate

Variable	Total number of spermatozoa ($\times 10^9$)			
	group I <70	group II 70–90	group III 90.1–120	group IV >120
Number of ejaculates	85	86	93	80
Total number of spermatozoa ($\times 10^9$)	59.36±8.27 ^A	79.22±6.41 ^B	103.49±8.69 ^C	149.68±24.79 ^D
Ejaculate volume (ml)	192.60±56.64 ^A	228.95±56.02 ^B	286.77±68.51 ^C	375.37±79.89 ^D
Sperm concentration ($\times 10^3/\text{mm}^3$)	427.99±113.65 ^a	463.21±95.45 ^b	481.21±92.89 ^b	522.06±90.91 ^c
Percentage of spermatozoa with normal motility (%)	77.06±4.58 ^a	78.49±3.60 ^a	77.53±4.34 ^a	77.50±4.36 ^a
Number of insemination doses per ejaculate	19.93±3.27 ^A	26.86±3.28 ^B	34.06±4.35 ^C	48.91±9.62 ^D

Different superscripts mean significant differences among means within particular rows; lower-case letters: $p \leq 0.05$, upper-case letters: $p \leq 0.01$

RESULTS

Table 1 presents the basic characteristics of ejaculates. The number of spermatozoa in an ejaculate increased along with an increase in ejaculate volume. Ejaculates characterized by a higher total spermatozoa number also had higher sperm concentrations. Moreover, ejaculates with a total spermatozoa number of more than 120×10^9 yielded 28 insemination doses more than ejaculates with the total number of spermatozoa of less than 70×10^9 . There were no differences in sperm motility among the examined groups.

Table 2 presents sperm morphometric characteristics in relation to the total number of spermatozoa in an ejaculate. In ejaculates with a total number of spermatozoa of $70-90 \times 10^9$ (group II), the spermatozoa had or tended to have larger dimensions than those from the remaining groups. Although some of the differences were not significant, the group II spermatozoa usually had longer flagella and heads, as well as a larger head area and head perimeter. The lowest sperm dimensions were usually recorded in the ejaculates with the greatest total number of spermatozoa (group IV).

Morphometric indexes of spermatozoa are presented in Table 3. Ratios of flagellum length to total length, head area to total length, and head length \times width/total length were larger in group II ejaculates than in groups I and IV. Moreover, a ratio of sperm head perimeter to total length was lower in group II than in groups I and IV. Data illustrating the frequency of occurrence of morphologically changed spermatozoa are presented in Table 4. It appears that sperm morphology was only vaguely affected by the number of spermatozoa in an ejaculate. Only one significant difference was observed for the percentage of spermatozoa with major abnormalities; this parameter was the highest in the group with the smallest total number of spermatozoa in ejaculate (less than 70×10^9).

DISCUSSION

The results obtained in the present study suggest that the number of spermatozoa in an ejaculate influences morphometrical characteristics of the sper-

Table 2. Morphometric characteristics (means±SD) of spermatozoa related to the number of spermatozoa in an ejaculate.

Variable	Total number of spermatozoa ($\times 10^9$)			
	group I <70	group II 70–90	group III 90.1–120	group IV >120
Head length (μm)	9.15±0.36 ^{AB}	9.27±0.40 ^B	9.07±0.38 ^A	9.09±0.30 ^A
Head width (μm)	4.75±0.26 ^a	4.73±0.32 ^a	4.70±0.29 ^a	4.67±0.22 ^a
Head area (μm^2)	40.79±2.53 ^{ab}	41.53±2.53 ^b	40.43±2.28 ^{ab}	39.94±1.92 ^a
Perimeter of the head (μm)	23.47±0.95 ^{AB}	23.79±1.04 ^B	23.36±0.90 ^A	23.54±0.86 ^{AB}
Flagellum length (μm)	45.65±1.53 ^b	45.71±1.79 ^b	45.11±1.52 ^a	44.92±1.55 ^a
Total length (μm)	54.81±1.77 ^{AB}	54.98±2.09 ^B	54.20±1.78 ^A	54.01±1.71 ^{AC}

Different superscripts mean significant differences among means within particular rows; lower-case letters: $p \leq 0.05$, upper-case letters: $p \leq 0.01$

Table 3. Morphometric indexes (means±SD) of spermatozoa related to the number of spermatozoa in an ejaculate.

Variable (%)	Total number of spermatozoa ($\times 10^9$)			
	group I <70	group II 70–90	group III 90.1–120	group IV >120
Head width/head length	51.94±3.29 ^a	51.07±3.45 ^a	51.89±3.57 ^a	51.36±2.74 ^a
Head length/total length	16.71±0.46 ^a	16.86±0.46 ^a	16.75±0.45 ^a	16.84±0.48 ^a
Head length/flagellum length	20.07±0.67 ^a	20.29±0.67 ^a	20.12±0.66 ^a	20.26±0.69 ^a
Head area/total length	71.32±11.01 ^A	75.20±4.83 ^B	72.62±8.95 ^{AB}	69.78±11.33 ^A
Head length x width/total length	75.35±13.07 ^a	79.46±7.70 ^b	76.32±10.91 ^{ab}	73.56±13.34 ^{ac}
Perimeter of the head/total length	47.66±13.17 ^b	43.78±4.62 ^a	46.13±10.81 ^{ab}	49.55±14.32 ^{bc}
Flagellum length/total length	75.83±20.52 ^A	82.39±6.89 ^B	78.43±16.85 ^{AB}	73.67±22.66 ^A

Different superscripts mean significant differences among means within particular rows; lower-case letters: $p \leq 0.05$, upper-case letters: $p \leq 0.01$.

Table 4. Frequency of occurrence of normal and abnormal spermatozoa (means \pm SD) related to the number of spermatozoa in an ejaculate.

Variable (%)	Total number of spermatozoa ($\times 10^9$)			
	group I <70	group II 70–90	group III 90.1–120	group IV >120
Percentage of normal spermatozoa	95.23 \pm 8.53 ^a	95.46 \pm 9.17 ^a	94.85 \pm 8.13 ^a	95.43 \pm 3.83 ^a
Sperm with major abnormalities	0.80 \pm 1.10 ^A	0.30 \pm 0.42 ^B	0.63 \pm 1.48 ^{AB}	0.32 \pm 0.54 ^B
Sperm with minor abnormalities	3.95 \pm 8.30 ^a	4.24 \pm 9.15 ^a	3.97 \pm 4.82 ^a	4.24 \pm 3.73 ^a

Different superscripts mean significant differences among means within particular rows; lower-case letters: $p \leq 0.05$, upper-case letters: $p \leq 0.01$.

matozoa. We demonstrated some differences between ejaculates characterized by a different spermatozoa number in relation to sperm dimensions and shape. Spermatozoa with longer flagella and heads as well as greater total length were found in group II compared to group III and IV. According to Gomendio and Roldan [13] sperm length is positively correlated with sperm velocity. Spermatozoa with longer mid-pieces and tails have stronger flagella [17]. Tail length and mid-piece length are associated with a different level of energy produced in mitochondria and, as a result, different sperm motility rates [4]. It is possible that spermatozoa with longer tails are more likely to reach an egg and are more competitive compared with other spermatozoa. In our study spermatozoa with the longest flagella were found in ejaculates with the lowest total spermatozoa number (groups I and II).

Some studies have demonstrated marked differences in sperm head dimensions between breeds and individual boars, and even between individual ejaculates [26, 27, 31]. Some authors associate sperm head shape with chromatin structure and integrity and, consequently, with the ability to fertilize the egg [18, 25]. High-fertility boar spermatozoa have smaller and shorter heads than spermatozoa that are less able to fertilize [16]. Rijselaere et al. [29] have found that dog ejaculates characterized by high sperm concentrations contained spermatozoa with shorter tails than ejaculates characterized by lower sperm concentration. We found that spermatozoa in groups III and IV had shorter heads than those in group II which suggests that differences in sperm morphometrical parameters in the pig are related to the total number of spermatozoa in an ejaculate.

Studies conducted on different animal species point to differences in dimensions and shapes [2, 21, 24] and link these differences to sperm competitiveness in the female reproductive tract [11, 13]. Sperm dimensions and shape can be influenced by sperm preservation [1, 15]. Arruda et al. [1] showed that in frozen stallion semen, sperm head dimensions are smaller (lower length, lower perimeter and smaller area) than in fresh semen spermatozoa. These authors ascribe these differences to either loss of acrosome or changed chromatin structure of spermatozoa in frozen semen. On the basis of changes in sperm head dimension it is possible to distinguish fertile individuals or animals with limited fertility [14]. Spermatozoa with larger

heads were found in the semen of stallions with decreased fertility [6]. Smaller dimensions of the head may indicate disturbances in the process of spermatogenesis or changes in chromatin structure during maturation and transportation of spermatozoa. An incidence of spermatozoa with head defects may result in lower quality embryos [9] and miscarriages during the initial stages of pregnancy [7].

In our study, total sperm number in an ejaculate was related to quantitative characteristics of an ejaculate. As the number of spermatozoa increased, ejaculate volume, sperm concentration and the number of insemination doses increased as well. In order to effectively utilize all spermatozoa from ejaculates with the greatest total sperm number, one would have to use higher ejaculate dilutions. Studies carried out on Polish Large White boars showed a distinctive impact of sperm concentration on the total number of spermatozoa in an ejaculate [22]. As the sperm concentration increased, an increased total number of spermatozoa was observed, however a reduced ejaculate volume was recorded at the same time.

To conclude, we found that the number of spermatozoa in an ejaculate was related to morphometrical characteristics of spermatozoa. Spermatozoa with smaller head length and head area were found in ejaculates with the greatest total sperm number (more than 120×10^9 spermatozoa, group IV) than in ejaculates with a total number of spermatozoa of $70-90 \times 10^9$ (group II). The total number of spermatozoa in an ejaculate only slightly influenced the sperm morphology. An increase in the total number of spermatozoa was followed by increased ejaculate volume, sperm concentration and number of insemination doses.

REFERENCES

1. Arruda RP, Ball BA, Gravance CG, Garcia AR, Liu IKM **2002** Effects of extenders and cryoprotectants on stallion sperm head morphometry. *Theriogenology* **58** 253-256.
2. Ball BA, Mohammed HO **1995** Morphometry of stallion spermatozoa by computer-assisted image analysis. *Theriogenology* **44** 367-377.
3. Bierła JB, Gizejewski Z **2007** Differences in spermatozoon: physiology or pathology? (in Polish) *Medycyna Weterynaryjna* **63** 1408-1411.

4. Bierła JB, Gizejewski Z, Leigh CM, Ekwall H, Söderquist L, Rodriguez-Martinez H, Zalewski K, Breed WG **2007** Sperm morphology of the Eurasian beaver *Castor fiber*: an example of a species of rodent with highly derived and pleiomorphic sperm populations. *Journal of Morphology* **268** 683-689.
5. Blom E **1981** The morphological estimation of the spermatozoa defects of bull II. The proposal of new classification of spermatozoa defects (in Polish). *Medycyna Weterynaryjna* **37** 239-242.
6. Casey PJ, Gravance CG, Davis RO, Chabot DD, Liu IKM **1997** Morphometric differences in sperm head dimensions of fertile and subfertile stallions. *Theriogenology* **47** 575-582.
7. Chenoweth PJ **2005** Genetic sperm defects. *Theriogenology* **64** 457-468.
8. Ciereszko A, Ottobre JS, Glogowski J **2000** Effects of season and breed on sperm acrosin activity and semen quality of boars. *Animal Reproduction Science* **64** 89-96.
9. De Jarnette JM, Saake RG, Barne J, Volger CJ **1992** Accessory sperm: Their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. *Journal of Animal Science* **70** 484-491.
10. Deka D, Goswami RN, Mili DC, Nath DR **2002** Effect of age of the sow and boar on reproduction performance. *Indian Veterinary Journal* **79** 615-616.
11. Gage MJG, Cook PA **1994** Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera:Pyralidae). *Functional Ecology* **8** 594-599.
12. Gage MJG, Morrow EH **2003** Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. *Current Biology* **13** 754-757.
13. Gomendio M, Roldan ERS **1991** Sperm competition influences sperm size in mammals. *Proceedings of the Royal Society of London, Series B - Biological Science* **243** 181-185.
14. Gravance CG, Liu IKM, Davis RO, Hughs JP, Casey PJ **1996** Quantification of normal stallion sperm-head morphometry. *Journal of Reproduction and Fertility* **108** 41-46.
15. Hidalgo M, Rodriguez I, Dorado JM **2007** The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Animal Reproduction Science* **100** 61-72.
16. Hirai M, Boersma A, Hofflich A, Wolf E, Föll J, Aumüller R, Braun AJ **2001** Objectively measured sperm motility and sperm head morphometry in boars (*Sus scrofa*): relation to fertility and seminal plasma growth factors. *Journal of Andrology* **22** 104-110.
17. Katz DF, Drobnis EZ **1990** Analysis and interpretation of the forces generated by spermatozoa. in: Fertilization in mammals (Bavister, B.D., Cummins, J., Roldan, E.R.S., and Norwell, M.A.) *Serono Symposia* 125-137.
18. Karabinus DS, Vogler CJ, Saacke RG, Evenson DP **1997** Chromatin structural changes in sperm after scrotal insulation of Holstein bulls. *Journal of Andrology* **18** 549-555.
19. King GJ, Macpherson JW **1973** A comparison of two methods for boar semen collection. *Journal of Animal Science* **36** 563-565.
20. Kondracki S, Banaszewska D, Mielnicka C **2005a** The effect of age on the morphometric sperm traits of domestic pigs. *Cellular and Molecular Biology Letters* **10**, **1** 3-13.
21. Kondracki S, Banaszewska D, Wysokińska A, Iwanina M **2005b** The estimation of sperm morphometric traits of Polish Large White and Polish Landrace young boars

- used in the insemination (in Polish). *Scientific Annals of Polish Society of Animal Production* **1**, **3** 509-519.
22. Kondracki S, Banaszewska D, Wysokińska A, Sadowska A **2006** Ejaculate traits and spermatozoa morphology as related to spermatozoa concentration in ejaculates of Polish Large White boars. *Animal Science Papers and Reports* **24** (**3**) 111-119.
 23. Kondracki S, Wysokińska A, Banaszewska D, Iwanina M **2007** Application of spermogram classification for evaluation of the semen morphology of a boar or a group of boars (in Polish). *Scientific Annals of Polish Society of Animal Production* **1** (**3**) 79-89.
 24. Morrow EH, Gage MJG **2001** Consistent significant variation between individual males in spermatozoal morphometry. *Journal of Zoology* **254** 147-153.
 25. Ostermeier GC, Sargeant GA, Yandell BS, Evenson DP, Parrish JJ **2001** Relationship of bull fertility to sperm nuclear shape. *Journal of Andrology* **22** 595-603.
 26. Peña FJ, Saravia F, García-Herreros M, Núñez-Martínez I, Tapia JA, Johannisson A **2005** Identification of sperm morphometric subpopulations in two different portions of the boar ejaculate and its relation to post-thaw quality. *Journal of Andrology* **26** 716-723.
 27. Peña FJ, Saravia F, Núñez-Martínez I, Johannisson A, Wallgren M, Rodriguez-Martínez H **2006** Do different portions of the boar ejaculate vary in their ability to sustain cryopreservation? *Animal Reproduction Science* **93** 101-113.
 28. Pinart E, Camps R, Briz MO, Bonet S, Egozcue J **1998** Unilateral spontaneous abdominal cryptorchidism: structural and ultrastructural study of sperm morphology. *Animal Reproduction Science* **49** 247-268.
 29. Rijsselaere T, Soom A, Hoflack G, Meas D, Kruif A **2004** Automated sperm morphometry and morphology analysis of canine semen by the Hamilton-Thorne analyser. *Theriogenology* **62** 1292-1306.
 30. Ruiz-Sanchez AI, O'Donoghue R, Novak S, Dyck MK, Cosgrove JR, Dixon WT, Foxcroft GR **2006** The predictive value of routine semen evaluation and IVF technology for determining relative boar fertility. *Theriogenology* **66** 736-748.
 31. Saravia F, Núñez-Martínez I, Morán JM, Soler C, Muriel A, Rodriguez-Martínez H, Peña FJ **2007** Differences in boar sperm head shape and dimensions recorded by computer-assisted sperm morphometry are not related to chromatin integrity. *Theriogenology* **68** 196-203.
 32. Smital J, De Sousa LL, Mohnsen A **2004** Differences among breeds and manifestation of heterosis in AI boar sperm output. *Animal Reproduction Science* **80** 121-130.
 33. Wysokińska A, Kondracki S, Banaszewska D **2005** The influence of the season on the semen quality of Duroc, Hampshire and Pietrain purebred boars and crossbreds of Duroc x Pietrain and Hampshire x Pietrain (in Polish). *Scientific Annals of Polish Society of Animal Production* **1** 535-544.