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Original article

Effect of sperm concentration in an ejaculate on morphometric traits of spermatozoa in Duroc boars

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Abstract

The experimental material consisted of 75 ejaculates collected form 8 Duroc boars. The ejaculates were divided into three groups according to sperm concentration in an ejaculate. An ejaculate was obtained from each boar monthly and it was used to make microscopic preparations to examine spermatozoa morphology. In each preparation morphometric measurements were taken of fifteen randomly selected spermatozoa characterized by normal morphology. The following measurements of spermatozoa were taken: length and width of the spermatozoa head, head area, length of the flagellum, perimeter of the spermatozoon head and total spermatozoon length. The results were used to calculate indicators of spermatozoa morphology. Moreover, assessments were made of frequency of morphological defects to isolate spermatozoa with primary and secondary abnormalities following the Blom classification system. It was found that the concentration of spermatozoa in the ejaculate influenced the morphometric characteristics of spermatozoa. Ejaculates with low sperm concentrations are characterized by larger spermatozoa as compared to ejaculates with high sperm concentrations. However, sperm concentration in the ejaculate does not much influence the shape of spermatozoa.

Key words; boar, ejaculate, sperm concentration, morphometric characteristics, spermatozoa

Introduction

Boar infertility is a very important problem in mammal reproduction. Boar prolificacy depends on the quality of generated reproductive cells which can be determined by examining sperm morphology. The results of such an examination make it possible to check if a male is fertile or not (Soderquist et al. 1991, Casey et al. 1997, De Vos et al. 2003, Philips et al. 2004). A high frequency of spermatozoa with morphological abnormalities is an important indicator of declining boar fertility. Males can differ with respect to the frequency of spermatozoa with morphological abnormalities (Pinart et al. 1998, Kondracki and Wysokińska 2005). That the ability of individual spermatozoa to penetrate an oocyte is associated with spermatozoa size and shape, is conceivable. It has been found that sperm dimensions are highly variable in males belonging to different animal species (Gage and Morrow 2003, Downing et al. 2005), and breeds within a species (Saravia et al. 2007). Substantial differences have been reported

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between individuals of one and the same population (Thurston et al. 2001, Maroto-Morales et al. 2010). Many workers have been examining effects of sperm morphometric characteristics on male fertility. An association of sperm head dimensions and male prolificacy has been reported. Spermatozoa head dimensions of males with decreased fertility differ from the head dimensions of spermatozoa of highly fertile men (Katz et al. 1986, Azis et al. 1998) and stallions (Gravance et al. 1996, Brito et al. 2007). There has also been confirmed an association of sperm dimensions and shape with ejaculate characteristics (Rijsselare et al. 2004, Banaszewska et al. 2009, Wysokińska et al. 2009). Basic ejaculate traits examined in sow artificial insemination centers depend on boar breed. Duroc males deserve special attention as they produce low-volume ejaculates characterized by high sperm concentrations. It seems to be a genetically-determined characteristic of this breed, which has already been demonstrated in many works (Castro et al. 1996, Park and Yi 2002, Kondracki 2003, Smital et al. 2004, Smital 2009). Moreover, it has also been confirmed that the breed is characterized by a completely different pattern of sexual development of males, compared with other pig breeds. The development manifests itself in increasing ejaculate output of Duroc males up to 21-22 months of age, although at a much lower rate compared with other breeds (Kondracki et al. 2007). It appears that Duroc is a distinctive breed in terms of male properties compared with other pig breeds utilized at present. As a result, both the ejaculate output and traits of Duroc sperm morphology raise more and more interest.

This work is an attempt to estimate the relationship between sperm morphological characteristics and sperm concentration in ejaculates of Duroc boars.

Materials and Methods

Seventy-five ejaculates were collected from 8 Duroc boars at two sow artificial insemination centres owned by the Mazovian Centre of Animal Breeding and Reproduction in Łowicz (Poland). The boars were young, 7-9 months of age, at the initial stage of their reproductive utilization. The ejaculates were collected using a manual collection method (King and Macpherson 1973). Evaluation was made of ejaculates collected monthly from each boar during the period of 10 months.

The ejaculates were arbitrarily categorized, according to sperm concentration in an ejaculate, into:

– group I – ejaculates which had the sperm concentration of less than 500 x 10^3/mm^3 (23 ejaculates),

– group II – ejaculates which had the sperm concentration of between 500 and 600 x 10^3/mm^3 (32 ejaculates),

– group III – ejaculates which had the sperm concentration of more than 600 x 10^3/mm^3 (20 ejaculates).

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.6.35; Gogosystem, Poland) computer program.

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 E-400 using 100x immersion lens. Morphometric measurements were taken of fifteen randomly selected morphologically normal spermatozoa. The following sperm measurements were recorded: sperm head length, sperm head width, head area, tail length, sperm head perimeter and total sperm length. The methodology followed guidelines presented by Kondracki et al. (2005) and used a software for computer image analysis (Screen Measurement v. 4. 1, Laboratory Imaging S.r.o., Praha, Czech Republic). The measurements were used to calculate the following indicators of sperm morphology:

– 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 had area to total sperm length,

– ratio of a product of sperm head length and width to total sperm length.

In addition, morphology of 500 spermatozoa was evaluated and a division was made, according to Blom's classification system (1981), of spermatozoa into morphologically normal forms and the forms with primary and secondary abnormalities.

Study results were statistically processed using the 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 – sperm concentration effect, e_{ij} – error.

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

Results

Table 1 contains basic physical characteristics of Duroc ejaculates according to semen concentration. The greatest was the volume of ejaculates in the group of ejaculates whose concentration ranged between 500 and 600 x 10^3/mm^3. The volume was by over 30 ml greater than in the group of ejaculates with the sperm concentration of over 600 x 10^3/mm^3 ($P \leq 0.05$). Group I ejaculates contained by 21 and almost 24 mld spermatozoa less than group II and III ejaculates, respectively ($P \leq 0.01$). Less insemination doses were prepared from ejaculates with the sperm concentration of over 500 x 10^3/mm^3, compared with group II and III ejaculates ($P \leq 0.05$).

Table 2 demonstrates data on morphometric characteristics of spermatozoa according to sperm concentration in an ejaculate. The data indicate that there were some relationships of spermatozoa dimensions with sperm concentration in an ejaculate. The spermatozoa in the ejaculates with the sperm concentration of over 600 x 10^3/mm^3 (group III) had smaller dimensions, that is by 0.21 fm shorter heads ($P \leq 0.01$), than the spermatozoa in the ejaculates of the remaining groups (group I and II). Moreover, group III spermatozoa had narrower heads and shorter tails compared with the ejaculates characterized by lower sperm concentration ($P \leq 0.05$). Group III ejaculates contained spermatozoa whose heads had lower perimeter compared with group I ejaculates, and a lower sperm head area compared with group II ejaculates ($P \leq 0.05$).

Data reflecting spermatozoa shape in ejaculates with different sperm concentrations are presented in Table 3. They indicate that sperm shape is only slightly dependent upon sperm concentration in an ejaculate. On the whole, inter-group differences were very small and statistically insignificant with the sole exception of group II ejaculates, containing between 500 and 600 x 10^3/mm^3 spermatozoa, in which the ratio of a product of sperm head length and width to total sperm length was higher compared with group III ejaculates ($P \leq 0.05$).

Table 1. Physical traits of ejaculates (means ± SD) as related to the sperm concentration in an ejaculate.

Variables	Sperm concentration [x 10^3/mm^3]		
	group I < 500	group II 500-600	group III > 600
Number of ejaculates	23	32	20
Sperm concentration [x 10^3/mm^3]	399.69 ± 80.28^A	550.93 ± 37.00^B	689.15 ± 62.88^C
Ejaculate volume [ml]	194.34 ± 69.07^a	197.81 ± 35.44^a	167.00 ± 40.66^b
Percentage of spermatozoa with normal motility [%]	79.56 ± 2.08^a	79.37 ± 2.45^a	80.00 ± 2.62^a
Total number of spermatozoa [x 10^9]	67.40 ± 31.64^A	88.50 ± 18.52^B	91.25 ± 19.35^B
Number of insemination doses per ejaculate	20.39 ± 8.84^a	26.71 ± 5.08^b	24.10 ± 5.71^b

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 2. Morphometric traits of sperms (means ± SD) with regard to the sperm concentration in an ejaculate.

Variables	Sperm concentration [x 10^3/mm^3]		
	group I < 500	group II 500-600	group III > 600
Head length (µm)	9.50 ± 0.31^A	9.50 ± 0.27^A	9.31 ± 0.25^B
Head width (µm)	4.87 ± 0.26^a	4.89 ± 0.23^a	4.76 ± 0.10^b
Perimeter of the head (µm)	24.01 ± 1.00^a	23.89 ± 0.78^{ab}	23.55 ± 0.58^b
Head area (µm2)	41.12 ± 1.53^{ab}	41.33 ± 1.58^a	40.41 ± 1.50^b
Flagellum length (µm)	44.48 ± 1.80^a	44.51 ± 2.04^a	43.51 ± 0.81^b
Total length (µm)	53.98 ± 1.94^a	54.01 ± 2.15^a	53.01 ± 1.04^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 3. Morphometric indexes of sperm (means ± SD) as related to the sperm concentration in an ejaculate.

Variables (%)	Sperm concentration [x 10^3/mm^3]		
	group I < 500	group II 500-600	group III > 600
Head width/head length	51.35 ± 2.05^a	51.57 ± 2.30^a	51.15 ± 1.05^a
Head length/total length	17.60 ± 0.57^a	17.60 ± 0.66^a	17.31 ± 0.65^a
Head length/flagellum length	21.37 ± 0.83^a	21.37 ± 0.99^a	20.94 ± 0.95^a
Head area/total length	76.18 ± 2.10^a	76.57 ± 2.26^a	75.56 ± 3.05^a
Head length x width/total length	85.87 ± 4.87^{ab}	86.17 ± 3.78^a	83.77 ± 3.08^b
Perimeter of the head/total length	44.49 ± 1.44^a	44.27 ± 1.77^a	44.22 ± 1.13^a
Flagellum length/total length	82.39 ± 0.57^a	82.39 ± 0.66^a	82.68 ± 0.65^a

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

Table 4. Frequency of occurrence of spermatozoa morphologically changes (means± SD) as related to the sperm concentration in an ejaculate.

Variables (%)	Sperm concentration [x 10^3/mm^3]		
	group I < 500	group II 500-600	group III > 600
Percentage of normal spermatozoa [%]	94.61 ± 4.05^a	94.68 ± 4.36^a	94.66 ± 4.12^a
Sperm with major abnormalities [%]	1.58 ± 2.51^a	1.05 ± 1.55^a	0.71 ± 1.72^a
Sperm with minor abnormalities [%]	3.80 ± 3.51^a	4.25 ± 3.83^a	4.63 ± 3.93^a

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

Table 4 shows data on frequency of sperm morphological abnormalities according to sperm concentration in an ejaculate. The data indicate that sperm concentration did not significantly influence the frequency of sperm morphological abnormalities. Inter-group differences between frequencies of primary and secondary morphological abnormalities were small and not confirmed by statistical analysis.

Discussion

The present results suggest that sperm morphometric characteristics depend on spermatozoa concentrations. There were confirmed significant differences between sperm dimensions of groups with different sperm concentrations. Ejaculates categorized as a group with the greatest sperm concentrations had smaller spermatozoa compared with the ejaculates characterized by a low sperm concentration. The spermatozoa had narrower heads than the spermatozoa in the ejaculates with low sperm concentrations. Sperm head shape is conditioned mainly by the shape and size of the nucleus and acrosome. Studies by Saravia et al. (2007) have demonstrated that Duroc boars have got larger and more elliptical heads than boars of other breeds. Determining differences is sperm head dimensions facilitates more precise assessment of the boar's spermiogram (Severa et al. 2010). Sperm head abnormalities make it possible to distinguish fertile males from boars with decreased fertility (Gravance et al. 1996). Spermatozoa of boars with poorer fertilizing effectiveness have got larger and more elongated heads than those of highly fertile males (Hirai et al. 2001).

Sperm head shape has got a marked effect on sperm hydrodynamics. Spermatozoa with elongated heads are faster than those with round heads (Malo et al. 2006). Research in dogs by Rijsselaere et al. (2004) has shown that sperm concentration influences sperm dimensions. Ejaculates with lower sperm concentrations contained spermatozoa with shorter and narrower heads, smaller head area and perimeter than ejaculates with higher sperm concentrations. An influence of sperm concentration in an ejaculate on their morphometric characteristics has also been found in stallions (Davis et al. 1993). Stallions which produced ejaculates with high sperm concentrations contained spermatozoa with smaller heads which were less elongated in shape than spermatozoa of ejaculates with lower sperm concentrations. The narrowest and most elongated sperm heads were typical of spermatozoa in ejaculates with low sperm concentrations.

The data presented in this paper indicate that in ejaculates characterized by high sperm concentrations (over 600 x 10^3/mm^3), spermatozoa have got shorter and narrower heads than in ejaculates with lower sperm concentration. Studies conducted by Banaszewska et al. (2009) in Pietrain boars have revealed a slight relationship of sperm concentration and characteristics of sperm morphometry. Ejaculates with lower sperm concentrations had a bit longer heads than ejaculates with high sperm concentrations. Studies on Polish Landrace boars have demonstrated that ejaculates with high sperm counts contained spermatozoa with smaller heads than ejaculates with low sperm counts (Wysokińska et al. 2009). Some abnormalities in the morphology of the sperm head may be associated with the condition of the chromatin structure. Even if heads seem to have a normal shape, they can have a disturbed chromatin structure in the nucleus or acrosome defects (Karabinus et al. 1997). It has been found that semen containing spermatozoa with head defects may reduce the quality of embryos (De Jarnette et al. 1992) and results in miscarriages in the first months of pregnancy (Chenoweth 2005). Larger sperm heads determined in the semen of stallions with decreased fertility may indicate disturbed spermatogenesis, and, first and foremost, changes in the chromatin structure taking place during sperm maturation and transportation in the duct of epididymis (Casey et al. 1997).

The data presented in this paper indicate that ejaculates with high sperm concentrations (group III) contain spermatozoa with shorter tails as compared to ejaculates with small sperm concentrations (groups I, II). A sperm tail (flagellum) is an important organelle which determines sperm velocity and potential ability of a spermatozoon to successfully fertilize an ovum. There are some works which demonstrate an association between sperm tail length, mid-piece in particular, and sperm motility (Katz and Drobnis 1990, Gil et al. 2009, Lüpold et al. 2009). According to Gomendio and Roldan (1991), sperm length is positively correlated with sperm velocity. Mid-piece length may influence the amount of energy produced in mitochondria (Bierła et al. 2007). Studies by Malo et al. (2006) have shown that spermatozoa with a shorter mid-piece swim faster than spermatozoa whose mid-piece is longer. The authors suggest that both the mid-piece and the remaining sperm tail part should be considered together because it is possible that sperm motility is also conditioned by the energy generated by other than mid-piece sperm tail components. In low-motility semen, spermatozoa have got shorter tails compared with semen containing a large percentage of progressively motile spermatozoa (Noorafshan and Karbalay-Doust 2010). The results of the present study indicate that spermatozoa with the longest tails are found in ejaculates with low sperm concentrations. Similar findings have been reported by Rijsselare et al. (2004) who examined dogs and found that ejaculates with lower sperm concentrations contained spermatozoa with longer tails compared with ejaculates characterized by higher sperm concentrations. Presumably spermatozoa with longer tails are more competitive and can reach the egg cell more rapidly.

To sum up, sperm concentration in an ejaculate influences sperm morphometric characteristics. Ejaculates with low sperm concentrations contain spermatozoa with greater dimensions than ejaculates with high sperm concentrations. Sperm concentration in an ejaculate influences neither sperm shape nor sperm quality. Increased sperm concentration does not significantly influence their motility or frequency of spermatozoa with morphological abnormalities.

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