Animal Reproduction Science 211 (2019) 106217

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Heterosis for morphometric characteristics of sperm cells from Duroc x Pietrain crossbred boars

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ARTICLE INFO

Keywords:
Crossbred boars
Heterosis
Spermatozoa

ABSTRACT

Morphometric studies of spermatozoa were conducted on 30 ejaculates collected from 15 boars represented by five Duroc boars, five Pietrain boars and five Duroc x Pietrain crossbred boars. Spermatozoa were stained using two methods: eosin-nigrosin and eosin-gentian. Values for morphometric measurements of sperm cells including head length, head width, head area, head perimeter, tail length and total sperm cell length were collected, and indices characterizing sperm cell morphological structure were calculated. The effects of heterosis on dimensions and shape of sperm cells from Duroc x Pietrain boars were evaluated. The effects of genetic effects on sperm head dimensions and shape were determined. Duroc x Pietrain crossbred boars produced spermatozoa with larger heads than purebred Duroc and Pietrain boars. The heads of sperm cells from crossbred boars were more rounded in shape, whereas sperm heads from purebred boars were more elongated. There were marked effects of heterosis on sperm head size and shape. Spermatozoa from Duroc boars also had larger and more rounded heads and longer tails than sperm cells from Pietrain boars. In addition, staining method affected the outcome from evaluation of heterosis on dimensions and shape of sperm cells. There were larger heterotic effects based on morphometric measurements of heads of spermatozoa stained using the eosin-nigrosin method than with use of the eosin-gentian dye technique.

1. Introduction

Spermatozoa are cells with different structures than other animal or plant cells. There is considerable structural variability in spermatozoa particularly in terms of the size and shape of the sperm head among species and breeds within species. Sperm cell dimensions and shape can be indicators of quality and predictors of the conception rate when these sperm are used for insemination. Sperm cell dimensions have a large amount of variability among males of different species (Gage and Morrow, 2003; Downing-Meisner et al., 2005) and among different breeds of the same species (Saravia et al., 2007). There are also considerable differences in sperm cell shape among males of the same population (Thurston et al., 2001; Maroto-Morales et al., 2010).

Variability of sperm cell dimensions are assessed using staining methods. In research conducted with humans (Maree et al., 2010) and animal semen (Łącka et al., 2016; Andraszek et al., 2018), staining method was determined to effect the measurements for sperm variables (*e.g.*, head shape), therefore staining methods can affect results when there are sperm cell measurements (Maree et al., 2010). The staining technique used can contribute to variability in sperm measurement outcomes as a result of the action of the chemical reagents used for staining (Brito et al., 2011; Czubaszek et al., 2019). Staining reagents can induce shrinkage or swelling of

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https://doi.org/10.1016/j.anireprosci.2019.106217
Received 15 February 2019; Received in revised form 12 October 2019; Accepted 23 October 2019
Available online 01 November 2019

the sperm heads (Banaszewska et al., 2015). A choice of the appropriate staining method, therefore, is important when assessing sperm cell dimensions. A popular staining method, recommended by the WHO for the examination of human sperm, is an eosin-nigrosin method. It is applied in sperm morphology assessment and enables identification of live and dead sperm cells (Frenau et al., 2010). Another method, widely used in sperm morphology and morphometry assessment, especially of breeding animals males, is the eosin-gentian method (Łącka et al., 2016; Kondracki et al., 2017).

Sperm head shape can be affected by genetic factors affecting the process of spermatogenesis (Pena et al., 2005). There is also an association between sperm dimensions and shape, and ejaculate characteristics (Rijsselaere et al., 2004; Wysokińska et al., 2009a; Kondracki et al., 2013; Górski et al., 2016). Importantly, sperm cell dimensions are associated with male fertility. Sperm head dimensions of spermatozoa of lesser-fertility boars differ from those of boars with relatively greater fertility (Gravance et al., 1996; Brito et al., 2011). Ejaculate characteristics vary with breed of boar (Kondracki et al., 2012). Ejaculates obtained from crossbred boars differed from those of purebred males in terms of both qualitative and quantitative characteristics (Ciereszko et al., 2000; Kawęcka et al., 2008). Ejaculates collected from crossbred boars usually have features that are associated with greater fertility when used for insemination (Wysokińska and Kondracki, 2013), which creates an opportunity for more efficient use of such boars for artificial insemination (AI). The outcomes from crossbreeding for production of boars, however, are not always as expected with regard to enhanced ejaculate quality.

Effects of crossbreeding largely depend on breeds selected for this purpose. Crossbred boars are usually characterized by having ejaculates with relatively greater quality than those of purebred boars as a result of heterosis and there are resulting enhanced fertility outcomes when crossbred boar semen is used for insemination (Smital, 2009; Wolf and Smital, 2009; Wysokińska and Kondracki, 2013). Sperm cells of crossbred boars, especially multi-breed crossbred boar have greater viability when preservation methods are imposed because of greater cell membrane integrity than spermatozoa of purebred boars (Wysokińska and Kondracki, 2014). In some cases, however, ejaculates obtained from crossbred boars have lesser sperm quality than those of purebred boars (Wierzbicki et al., 2010) or the characteristics of the sperm of the crossbred boars are intermediate compared with parent purebred boars (Wysokińska et al., 2009b). Two-breed crossbred boars are often preferred for reproduction because boars of this breeding have greater libido, and ejaculates can be easily obtained which is particularly important when the boars are used for artificial insemination. This is why the use of crossbred boars in pig artificial insemination is greater and continuing to increase as compared with use of purebred boars. Knowledge of advantages of using semen from crossbred boars for artificial insemination is insufficient and is usually limited to the ease of sperm collection and basic ejaculate characteristics. Relatively few studies have been conduct to evaluate quality and morphological and morphometric characteristics of spermatozoa from crossbred compared with purebred boars.

The present study was conducted to compare morphometric features of sperm cells from Duroc x Pietrain crossbred and purebred Duroc and Pietrain boars, and to evaluate the effects of heterosis on dimensions and shape of spermatozoa produced from crossbreeding of the Duroc and Pietrain breeds.

2. Materials and methods

2.1. Animals and ejaculate collection

The studies were conducted on 30 ejaculates collected from 15 boars, represented by five purebred Duroc, five purebred Pietrain and five two-breed Duroc x Pietrain crossbred boars. Semen from these boars was being routinely collected at an insemination station for AI. The boars were 18 to 24 months of age. Boars were housed in similar environmental conditions with water was available *ad libitum*. Ejaculates were collected using a manual method once a week. Immediately after collection, the semen was filtered through four layers of sterile gauze into a pre-warmed beaker to remove gel particles. Two consecutive ejaculates were collected from each boar. Semen was only used in the study when the ejaculates had at least 70% of spermatozoa that were highly motile and sperm concentration was at least 100,000/ml of semen. Sperm motility was evaluated using 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 37 °C. Using 200x magnification, the percentage of normally motile spermatozoa was determined based on the number of sperm present in the field of vision of the microscope. Sperm concentration in the ejaculates was determined using a photometric method, utilizing a spectrophotometer (IMV Technologies, France). The method was based on the measurement of light intensity passing through the suspended spermatozoa in an isotonic solution of chlorine or sodium citrate. There were 90% of the spermatozoa in those ejaculates that did not have any morphological changes during the course of the experiment. Immediately after collection, two microscopic slides were prepared from each ejaculate, of which one was stained using the eosin-nigrosin method and the other using the eosin-gentian dye staining technique.

2.2. Staining methods

2.2.1. Eosin-nigrosin staining method (differential staining)

The preparations for analyses were made according to the following methodology: a drop of four samples of semen was placed on a slide preheated to 36 °C and mixed with twice the volume of the dye mixture (one sample of 5% bluish eosin solution (Carl Roth Gmbh + Co. KG, Karlsruhe, Germany) containing 10% nigrosin aqueous solution (Sigma-Aldrich, USA) using a glass rod to produce a smear on the slide. The samples were air-dried at room temperature.

2.2.2. Eosin-gentian staining method

Thin, fat-free semen smears, heated to 36 °C, were prepared. After drying, the smear was fixed in 96% ethanol. The smear sample was subsequently rinsed in water and counterstained using 10% blue eosin solution (Carl Roth Gmbh + Co. KG, Karlsruhe, Germany) for 20–60 seconds. The slides were again rinsed with water, and stained for 3 min with gentian pigment (Sigma-Aldrich, USA). After staining, the slides were washed and dried. The use of this procedure resulted in a clean background, and thus a relative ease of contrasting of the stained spermatozoa.

2.3. Microscopic analysis and morphometric measurements

Microscopic slides, prepared using the procedures previously described in this manuscript, were used for conducting morphometric evaluations of sperm cells. The morphometric evaluations of sperm cells were conducted using an analysis system consisting of a Nikon E50i light microscope, the Moticam PRO 282B digital camera and a computer. The equipment comprised a microscope (Nikon 50i, Japan) equipped with a 100x bright-field Nikon objective. A video digital camera Moticam PRO 282B was mounted on the microscope to obtain the images and transmit these to the computer. The array size of the video frame grabber was 2592 × 1944 pixels. Resolution of images was 0.0345 μm per pixel in both the horizontal and vertical axes. Each sperm cell was evaluated using a software Motic Images Advanced 3.2 connected to an image analysis system. There were morphometric evaluations using the graphics tablet Wacom Intuos driver 6.3.17 (Germany) equipped with very sensitive digital pen. For each boar, 40 morphologically normal spermatozoa were evaluated, including: 20 sperm cells stained using the eosin-nigrosin technique and 20 sperm cells stained using the eosin-gentian technique. In total, 600 spermatozoa were evaluated. Morphologically normal spermatozoa were considered to have oval shaped heads, no acrosome defects, no bent tails and no cytoplasmic droplets. The following measurements of each sperm cell were taken: head length – estabilished as the length of a–b line where a is the point where the head and the connecting piece (the tail neck) adjoin, and b is the farthest point a at the tip of the head; head width – the length of c–d line where c and d are the farthest sticking points from each other on the edge of sperm cell head, and the line was drawn perpendicular to the long axis of the sperm head at the height of ½ an acrosome; head area – measuring the area limited by a curve extending along the perimeter of the sperm head; head perimeter – length of the boundary limiting the sperm head; tail length – the length of a–e curve drawn along the long axis of the tail, where a is defined as above, and e is the tail tip and total sperm cell length – the length tail and head (Fig. 1). Measurements were performed using the methodology developed by Kondracki et al. (2005). Based on the measurement results, the following indices characterizing morphological structure of sperm cells were calculated:

- head width / head length × 100,
- head length / sperm length × 100,
- head length / tail length × 100,

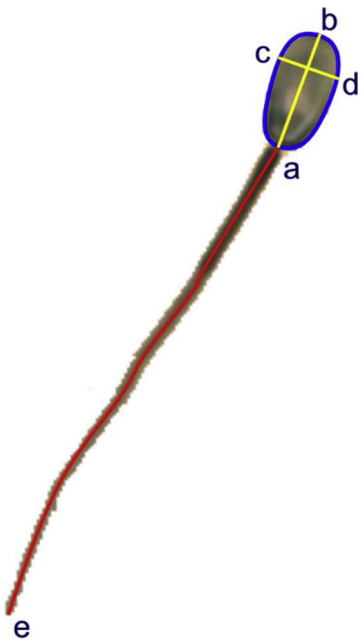

Fig. 1. Approaches for measuring morphometric sperm dimensions: head length – segment a–b (the yellow line), head with – segment c–d (the yellow line), head area – the area of the region limited by the blue line, head perimeter – the length of the blue line, tail length – the length of the curve a–e (signed with the red color) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

- tail length / total length × 100,
- head perimeter / total length × 100,
- head area / total length × 100,
- head length × width / total length × 100.

The slides were prepared and assessed microscopically by the same person.

2.4. Statistical analysis

Experimental data were analyzed using a program STATISTICA 13.1 Pl (StatSoft, Tulsa, USA). All results are expressed as mean ± standard deviation (SD). The data were statistically analyzed using the following mathematical model: $Y_{ijk} = \mu + a_i + bj + ab_{ij} + e_{ijk}$ where: Y_{ijk}– value of the analyzed parameter, μ - populational mean, a_i – the effect of boar breed, bj - the effect of staining method, ab_{ij} – the effect of interaction between factors, e_{ijk} – error. The significance of the differences between the groups was assessed using the Tukey test at $P < 0.05$.

The effects of heterosis on morphometric measurements of crossbred boar sperm cells in relation to the mean value of a given trait of the parent breeds were calculated using the following formula:

$$VR = \frac{X_{F1} - X_{MP}}{X_{MP}} \times 100$$

where:

VR – the effect of heterosis

X_{F1} – mean value of a given trait in crossbred boars

X_{MP} – mean value of the trait in boars of parent breeds

3. Results

3.1. Head dimensions

The sperm heads stained with eosin-nigrosin of crossbred Duroc x Pietrain boars had larger dimensions than sperm heads of purebred boars (Table 1). There were marked and positive effects of heterosis mostly for sperm head area (VR = 9.79), sperm head perimeter (VR = 4.69) and sperm head width (VR = 3.39). The head area of spermatozoa produced by crossbred boars was greater by 7.1% compared with sperm heads of Duroc boars and were as much as 12% greater than that of Pietrain boar sperm heads ($P < 0.05$). The heads of sperm cells produced by crossbred boars also had a larger perimeter ($P < 0.05$) and were longer and wider compared with Pietrain boars ($P < 0.05$).

The data in the Table 1 are indicative of the larger head dimensions of spermatozoa produced by crossbred than purebred boars. These data were also obtained using the eosin-gentian staining method. The values for inter-breed differences were much smaller than those values for sperm cell measurements obtained by staining with eosin-nigrosin. Also, the effects of heterosis on sperm head dimensions were smaller (by more than a half) than the values calculated based on measurements of sperm cells stained with eosin-nigrosin. The sperm cell staining method, therefore, had marked effects on the assessment of the effects of heterosis on sperm head dimensions.

3.2. Tail dimensions

In slides stained with the eosin-nigrosin method, the tails of spermatozoa of crossbred boars were of similar length as spermatozoa

Table 1
Dimensions of sperm cells from Duroc and Pietrain purebred and Duroc x Pietrain crossbred boars, and effects of heterosis on sperm dimensions in crossbred boars when measurements were made on slides stained using the eosin-nigrosin (EN) and eosin-gentian (EG) technique.

Variable	Breed						Effect of heterosis VR (%)	
	Duroc x Pietrain		Duroc		Pietrain			
	EN	EG	EN	EG	EN	EG	EN	EG
Number of analyzed cells	100	100	100	100	100	100		
Head length (μm)	9.01 ± 0.36^{a}	9.32 ± 0.41^{b}	8.99 ± 0.63^{a}	9.36 ± 0.58^{b}	8.78 ± 0.44^{c}	9.24 ± 0.37^{b}	1.35	0.22
Head with (μm)	4.58 ± 0.33^{a}	4.86 ± 0.29^{b}	4.52 ± 0.27^{c}	4.79 ± 0.32^{d}	4.35 ± 0.34^{e}	4.78 ± 0.27^{d}	3.39	1.67
Head perimeter (μm)	28.55 ± 1.77^{a}	29.16 ± 1.52^{b}	27.55 ± 1.74^{c}	28.64 ± 2.10^{a}	26.99 ± 1.70^{c}	28.39 ± 1.45^{a}	4.69	2.28
Head area (μm^2)	36.10 ± 3.43^{a}	38.22 ± 3.00^{b}	33.71 ± 3.42^{c}	37.34 ± 4.91^{d}	32.05 ± 3.23^{b}	36.71 ± 2.82^{ad}	9.79	3.24
Tail lenght (μm)	43.80 ± 1.64^{a}	44.68 ± 1.18^{b}	45.48 ± 1.63^{c}	46.24 ± 1.72^{d}	43.67 ± 2.21^{a}	44.53 ± 1.48^{b}	−1.75	−1.54
Total length (μm)	52.81 ± 1.71^{a}	54.01 ± 1.35^{b}	54.47 ± 1.88^{b}	55.60 ± 2.00^{c}	52.44 ± 2.36^{a}	53.77 ± 1.63^{b}	−1.21	−1.23

^{a,b,c,d}Values in rows marked with different letters differ $P < 0.05$.

Table 2
Morphometric indices for the eosin-nigrosinand eosin-gentian-stained sperm cells from Duroc and Pietrain purebred and Duroc x Pietrain crossbred boars, and effects of heterosis on morphometric indices of sperm cells in crossbred boars in relation to the mean value for a a specific variable in parent breeds.

Variable	Breed						Effect of heterosis VR (%)	
	Duroc x Pietrain		Duroc		Pietrain			
	EN	EG	EN	EG	EN	EG	EN	EG
Number of analyzed cells	100	100	100	100	100	100		
Head width/head length	50.91 ± 4.27^{a}	52.12 ± 3.15^{b}	50.48 ± 5.02^{a}	51.30 ± 3.28^{ab}	49.64 ± 3.89^{a}	51.85 ± 3.40^{ab}	1.70	1.05
Head length/total length	17.08 ± 0.74^{abc}	17.26 ± 0.63^{a}	16.51 ± 1.01^{b}	16.83 ± 0.83^{c}	16.76 ± 0.93^{cb}	17.19 ± 0.62^{a}	2.64	1.47
Head length/tail length	20.61 ± 1.08^{ac}	20.88 ± 0.92^{a}	19.79 ± 1.42^{b}	20.24 ± 1.20^{cd}	20.14 ± 1.38^{d}	20.76 ± 0.90^{a}	3.20	1.85
Tail length/total length	82.92 ± 0.74^{a}	82.74 ± 0.63^{a}	83.49 ± 1.00^{b}	83.17 ± 0.83^{b}	83.24 ± 0.93^{b}	82.81 ± 0.62^{a}	−0.54	−0.30
Head perimeter /total length	54.14 ± 4.26^{a}	54.02 ± 2.95^{a}	50.60 ± 3.26^{b}	51.52 ± 3.54^{b}	51.58 ± 4.09^{b}	52.84 ± 3.10^{c}	5.97	3.53
Head area/total length	68.49 ± 7.56^{a}	70.78 ± 5.47^{b}	61.90 ± 6.10^{c}	67.12 ± 8.06^{a}	61.21 ± 6.40^{c}	68.27 ± 4.89^{a}	11.26	4.55
Head length × width/total length	78.24 ± 6.58^{a}	83.86 ± 6.46^{b}	74.63 ± 7.18^{c}	80.74 ± 7.85^{d}	72.97 ± 7.71^{e}	82.21 ± 5.22^{bd}	6.02	2.92

^{a,b,c,d,e}Values in rows marked with different letters differ $P < 0.05$.

of Pietrain boars but were shorter by 3.7% than Duroc boar spermatozoa ($P < 0.05$; Table 1). There were similar relationships for the total sperm length. The effects of heterosis on sperm tail length and total length were negative and relatively small.

Using the eosin-nigrosin staining method did not affect the values for heterosis effects on sperm tail length and total sperm length. The effects of heterosis on sperm tail length and total sperm length, with use of the eosin-gentian staining method, were VR = −1.54 and VR = −1.23, respectively, and were similar to the relative values when there was staining of spermatozoa using eosin-nigrosin method and determination of values for these variables.

3.3. Sperm morphometric indices

Results from analysis of the data contained in Table 2 indicate that with eosin-nigrosin and eosin-gentian staining there were differences in sperm cell shape between Duroc x Pietrain crossbred and purebred Duroc and Pietrain boars. Spermatozoa of crossbred boars differed from those of purebred boars in head shape. There was a larger head width-to-length ratio for spermatozoa produced by crossbred boars with the heads being more rounded in shape whereas purebred boars produced spermatozoa with more elongated heads, which was particularly obvious in sperm of Pietrain boars in preparations stained using the eosin-nigrosin method ($P < 0.05$).

In preparations stained using the eosin-nigrosin method, spermatozoa of crossbred boars were characterized by a greater head perimeter-to-total sperm length and head area-to-total sperm length ratio and a greater ratio of the product of head length and width to total sperm length than spermatozoa of purebred boars ($P < 0.05$). There were large effects of heterosis on these characteristics, ranging from VR = 5.97 to VR = 11.26. The effects of heterosis on the remaining morphometric indices were much less and ranged from VR = −0.54 for the sperm tail length-to-total sperm length ratio to VR = 3.20 for the sperm head length-to-sperm tail length ratio.

In Table 2, there are data characterizing morphometric indices of sperm cells stained using the eosin-gentian dye technique and the data for the effects of the calculated value for heterosis for these indices. These data indicated that with the use of eosin-gentian dye staining there were differences in sperm shape between Duroc x Pietrain crossbred and purebred Duroc and Pietrain boars. These differences were, however, much smaller than those when there was use of eosin-nigrosin staining. The effects of heterosis were also less and ranged from VR = −0.30 to VR = 4.25.

4. Discussion

The data from the present study indicate that crossbreeding affects the dimensions and shape of sperm cells of domestic pig males. Duroc x Pietrain crossbred boars produce spermatozoa with larger heads than purebred Duroc and Pitrain boars. The greatest differences are in the sperm head area and perimeter. These differences are confirmed by the effects of heterosis on these dimensions. The dimensions of sperm head can be associated with male fertility. Results of studies conducted on the sperm of humans (Obara et al., 2001; Gage and Morrow, 2003), boars (Pena et al., 2005; Saravia et al., 2007) and stallions (Hidalgo et al., 2008; Phetudomsinsuk et al., 2008) indicate sperm cells from lesser-fertility individuals differed in dimensions from spermatozoa obtained from individuals with greater fertility based on conception rate values. Males producing spermatozoa with smaller heads had greater fertility. Waheed et al. (2015) and Gravance et al. (1996) reported differences in sperm head dimensions between stallions with relatively lesser and greater fertility. Spermatozoa of stallions with lesser fertility had larger heads (Waheed et al., 2015).

Results of the present study unequivocally provide evidence that sperm cells from crossbred boars had larger heads compared with those from purebred boars. This morphological characteristic can adversely affect the fertilizing capacity of sperm cells. The majority of sperm morphometry research is based on using the CASA-Morph system (Computer Assisted Sperm Morphometry

Analysis; Soler et al., 2016, 2017a; Soler et al., 2017b; Yaniz et al., 2016; Valverde et al., 2019). Using the CASA-Morph system, there are measurements of a sperm head and acrosome but a sperm tail is not measured. In the present study, there was evaluation of both a sperm head and tail is made. Analysis of morphometric characteristics of sperm cells has to include both the sperm head, midpiece and tail (Gil et al., 2009), because there are significant associations between the sperm head and tail length (Gage, 1998). There is an inverse relationship between sperm length and the duration of sperm cell motility and thus its capacity for ova fertilization (Gomendio and Roldan, 1993). In a study conducted with primates and rodents, the spermatozoa with a longer length had a greater swim velocity but period during which the cells continued to be motile is shorter compared with sperm with a shorter length (Gomendio and Roldan, 1991). There have been similar results regarding sperm length and duration of motility when there were assessments of fish spermatozoa (Stockley et al., 1997). The data obtained in the present study indicate that the differences in sperm tail length between crossbred and purebred boars were slight but there are indications that there is a lesser tail length of sperm cells produced by crossbred boars. It can be expected, therefore, that motility of spermatozoa from crossbred boars will be less but that there will be a longer motility persistence than that of sperm from purebred boars. The longer period during which spermatozoa motility is sustained in sperm from crossbred boars contributes to the greater fertilizing capacity of sperm from these boars.

Motility of sperm cells is one of the main indicators of sire fertility. A larger percentage of progressively motile spermatozoa is associated with a greater fertilizing capacity. There are only a few studies in which there has been investigation of associations of sperm motility and dimensions and shape of these cells. There, however, was an association between sperm head shape and the motion hydrodynamics of spermatozoa. Spermatozoa with elongated heads had a greater swim velocity than spermatozoa with rounded heads (Malo et al., 2006). Based on results from the present study, it can be expected that sperm cells of purebred males, having more elongated heads, will have more rapid motility than spermatozoa of crossbred boars with more rounded heads. Spermatozoa which are longer can produce more energy in the midpiece (Cardullo and Baltz, 1991) and can generate a greater force for movement (Tourmente et al., 2011). In the present study, spermatozoa of crossbred Duroc x Pietrain boars were slightly shorter than sperm cells of Duroc boars but longer than spermatozoa of Pietrain boars. The calculated values for effects of heterosis on sperm length and sperm tail length were negative (-1.75% and -1.21% with eosin-nigrosin and -1.54% and -1.23 with eosin-gentian staining).

The effects of heterosis on morphometric dimensions of spermatozoa have not been previously evaluated. There were relatively greater heterosis effects, however, for quantitative and qualitative characteristics of semen from crossbred boars (Smital et al., 2004; Wysokińska and Kondracki, 2013). In the studies of Smital et al. (2004), there were positive and distinct effects of heterosis on ejaculate volume and number of spermatozoa per ejaculate in Duroc x Pietrain crossbred boars. There were slightly different results in the studies of Czarnecki et al. (1999). Results of those studies indicated there was a positive effect of heterosis in Duroc x Pietrain crossbred boars only in terms of ejaculate volume. Crossbred Duroc x Pietrain boars were usually characterized as having a greater quality of ejaculate which contained more spermatozoa with greater motility than ejaculates of purebred Duroc and Pietrain boars (Wysokińska and Kondracki, 2004; Kawęcka et al., 2008). Results in the studies of Wierzbicki et al. (2010) indicated purebred Duroc, Hampshire and Pietrain boars produced ejaculates with a larger volume and more spermatozoa than crossbred boars. For this reason, these boars were often retained in herds for longer periods and had a greater copulation performance while in the herds. The results from the present study indicate spermatozoa of Duroc x Pietrain crossbred boars have a larger head perimeter and area than spermatozoa of purebred Duroc and Pietrain boars. For these characteristics of sperm head, there are positive effects of heterosis with the use of both staining methods utilized in the present study.

There were more obvious effects of heterosis on sperm head dimensions on slides stained using the eosin-nigrosin method. Heterosis effects on sperm head dimensions as determined with the use of the eosin-nigrosin staining method were more than twice as great as the effects calculated based on data with eosin-gentian dye staining. Sperm head shape is determined primarily by shape and size of the nucleus and acrosome. Thurston et al. (2001) suggested that there were effects of genetic factors on sperm head shape. Also, the results of the present study indicate there is a genetic effect on variability of sperm head dimensions. These results indicate there is a marked breed and crossbreeding effect on dimensions and shape of sperm cells. Saravia et al. (2007) reported that sperm head length, width, area and perimeter were larger for Duroc boars than in the other breeds evaluated in this study (Risco-hybrid, Large With, Landrace, Yorker-hybrid). The results of the present study also indicated that Duroc boars produced spermatozoa with larger heads than Pietrain boars. This is consistent with the thought that sperm of Duroc boars have relatively large heads. The data obtained in the present study also indicate that sperm cells produced by Duroc boars have longer tails than spermatozoa from Pietrain boars. It can be hypothesized, therefore, that Duroc boars produce relatively larger spermatozoa than Pietrain boars. Furthermore, the data of Saravia et al. (2007) indicated that Duroc boars produced larger spermatozoa than boars of other breeds. Duroc boars, however, had sperm cells with smaller heads than Duroc x Pietrain crossbred boars which was particularly obvious when there were evaluations using differential staining with eosin-nigrosin in the present study (Table 1). Hence, crossing the Duroc and Pietrain breeds resulted in two-breed crosses with a marked heterosis effect manifested by increased dimensions of the sperm head.

Results of some studies indicate there is a significant variability of sperm head dimensions not only in boars of different breeds but also among boars of the same breed and even between ejaculates of the same boar (Pena et al., 2005, 2006; Saravia et al., 2007; Kondracki et al., 2012). These data indicate that sperm head dimensions are also affected by breed-independent factors. Sperm shape and structural proportions have a marked variability. The data obtained in the present study indicate there are effects of heterosis on sperm cell shape, and especially on the proportion of sperm head size to total sperm length in sperm from crossbred boars. These differences were most obvious when there was use of the differential staining technique with the eosin-nigrosin (Table 2). There has been some thought that the differences in sperm head shape are associated with integrity of nuclear chromatin (Aziz et al., 1998; Ostermeier et al., 2001). These differences in sperm head shape can result from abnormal chromatin condensation and DNA fragmentation and from the presence of nuclear vacuoles. These differences in morphological characteristics negatively affect fertilizing

capacity of sperm cells and fetus development (Gandini et al., 2000; Prisant et al., 2007; Auger, 2010). There are results of studies which indicate that sperm heads are larger is semen when there is a greater presence of sperm heads with morphological defects than in semen with normal sperm morphology (Katz et al., 1986; Casey et al., 1997). In this context, evaluation of a sperm head structure is of particular importance because the size and shape of the sperm head is an important criterion of spermatozoa classification and evaluation for morphological anomalies. Morphometric characteristics of sperm cells are important because of associations of these characteristics with the functioning of cellular structures in these cells (Maroto-Morales et al., 2016).

5. Conclusion

In conclusion, there are genetic effects on the dimensions and shape of sperm heads. Spermatozoa from crossbred Duroc x Pietrain boars have larger and more rounded heads than sperm heads of purebred boars. There are marked effects of heterosis on the sperm dimensions and shape. Spermatozoa of purebred Duroc boars have larger and more rounded heads as well as longer tails than spermatozoa from Pietrain boars. The staining method has an effect on the values determined for the effects of heterosis on sperm head dimensions and shape. Estimated heterotic effects on sperm dimensions and shape are greater when there is use of eosin-nigrosin staining than with use of the eosin-gentian staining technique. The staining method, however, does not affect the evaluation of heterosis effects on sperm tail length and total sperm length.

Author contributions

A.W. collected and analyzed the samples and wrote the manuscript; S.K. author of the concept, performed the statistical analyses and wrote the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

None.

References

Andraszek, K., Banaszewska, D., Biesiada-Drzazga, B., 2018. The use of two staining methods for identification of spermatozoon structure in roosters. Poult. Sci. 97, 2575–2581. https://doi.org/10.3382/ps/pey056.
Auger, J., 2010. Assessing human sperm morphology: top models, underdogs or biometrics? Asian J. Androl. 12, 36–46. https://doi.org/10.1038/aja.2009.8.
Aziz, N., Fear, S., Taylor, C., Kingsland, C.R., Lewis-Jones, D.I., 1998. Human sperm head morphometric distribution and its influence on human fertility. Fertil. Steril. 70, 883–891. https://doi.org/10.1016/S0015-0282(98)00317-3.
Banaszewska, D., Andraszek, K., Czubaszek, M., Biesiada-Drzazga, B., 2015. The effect of selected staining techniques on bull sperm morphometry. Anim. Reprod. Sci. 159, 17–24. https://doi.org/10.1016/j.anireprosci.2015.06.019.
Brito, L.F.C., Greene, L.M., Kelleman, A., Knobbe, M., Turner, R., 2011. Effect of method and clinician on stallion sperm morphology evaluation. Theriogenology 76, 745–750. https://doi.org/10.1016/j.theriogenology.2011.04.007.
Cardullo, R.A., Baltz, J.M., 1991. Metabolic regulation in mammalian sperm: mitochondrial volume determines sperm length and flagellar beat frequency. Cell Motil. Cytoskeleton 19, 180–188. https://doi.org/10.1002/cm.970190306.
Casey, P.J., Gravance, C.G., Davis, R.O., Chabot, D.D., Liu, I.K., 1997. Morphometric differences in sperm head dimensions of fertile and subfertile stallions. Theriogenology 47 (2), 575–582.
Ciereszko, A., Ottobre, J.S., Glogowski, J., 2000. Effects of season and breed on sperm acrosin activity and semen quality of boars. Anim. Reprod. Sci. 64, 89–96. https://doi.org/10.1016/S0378-4320(00)00194-9.
Czarnecki, R., Różycki, M., Udała, J., Kawęcka, M., Kamyczek, M., Pietruszka, A., Delikator, B., 1999. The growth rate, meatiness value and reproductive performance of young duroc boars and their hybrids with the pietrain breed. Rocz. Nauk. Zoot. 105–110.
Czubaszek, M., Andraszek, K., Banaszewska, D., Walczak-Jędrzejewska, R., 2019. The effect of the staining technique on morphological and morphometric parameters of boar sperm. PLoS One 14 (3), 1–17. https://doi.org/10.1371/journal.pone.0214243.
Downing-Meisner, A., Klaus, A.V., O'Leary, M.A., 2005. Sperm head morphology in 36 species of artiodactylans, perissodactylans, and cetaceans (Mammalia). J. Morphol. 263, 179–202. https://doi.org/10.1002/jmor.10297.
Frenau, G.E., Chenoweth, P.J., Ellis, R., Rupp, G., 2010. Sperm morphology of beef bulls evaluated by two different methods. Anim. Reprod. Sci. 118, 176–181. https://doi.org/10.1016/j.anireprosci.2009.08.015.
Gandini, L., Lombardo, F., Paoli, D., Caponecchia, L., Familiari, G., Verlengia, C., Dondero, F., Lenzi, A., 2000. Study of apoptotic DNA fragmentation in human spermatozoa. Hum. Reprod. 15, 830–839. https://doi.org/10.1093/humrep/15.4.830.
Gage, M.J.G., 1998. Mammalian sperm morphometry. Proc. R. Soc. Lond. B 265, 97–103. https://doi.org/10.1098/rspb.1998.0269.
Gage, M.J.G., Morrow, E.H., 2003. Experimental evidence for the evolution of numerous, tiny sperm via sperm competition. Curr. Biol. 13, 754–757. https://doi.org/10.1016/S0960-9822(03)00282-3.
Gil, M.C., Garcı´a-Herreros, M., Baro´n, F.J., Aparicio, I.M., Santos, A.J., Garcı´a-Marı´n, L.J., 2009. Morphometry of porcine spermatozoa and its functional significance in relation with the motility parameters in fresh semen. Theriogenology 71, 254–263. https://doi.org/10.1016/j.theriogenology.2008.07.007.
Gomendio, M., Roldan, E.R.S., 1991. Sperm competition influences sperm size in mammals. Proc. R. Soc. Lond. B 243, 181–185. https://doi.org/10.1098/rspb.1991.0029.
Gomendio, M., Roldan, E.R.S., 1993. Coevolution between male ejaculates and female reproductive biology in eutherian mammals. Proc. R. Soc. Lond. B 252, 7–12. https://doi.org/10.1098/rspb.1993.0039.
Górski, K., Kondracki, S., Wysokińska, A., Nazaruk, A., 2016. The importance of ejaculate volume for the physical parameters of ejaculates and sperm morphology of hypor boars. Kafkas Univ. Vet. Fak. Derg. 22, 493–501. https://doi.org/10.9775/kvfd.2015.14772.
Gravance, C.G., Liu, I.K.M., Davis, R.O., Hughes, J.P., Casey, P.J., 1996. Quantification of normal head morphometry of stallion spermatozoa. J. Reprod. Fertil. 108, 41–46.
Hidalgo, M., Rodríguez, I., Dorado, J., Soler, C., 2008. Morphometric classification of Spanish thoroughbred stallion sperm heads. Anim. Reprod. Sci. 103, 374–378. https://doi.org/10.1016/j.anireprosci.2007.06.001.
Katz, D.F., Overstreet, J.W., Samuels, S.J., Niswander, P.W., Bloom, T.D., Lewis, E.L., 1986. Morphometric analysis of spermatozoa in the assessment of human male fertility. J. Androl. 7, 203–210. https://doi.org/10.1002/j.1939-4640.1986.tb00913.x.
Kawęcka, M., Pietruszka, A., Jacyno, E., Czarnecki, R., Kamyczek, M., 2008. Quality of semen of young boars of the breeds Pietrain and Duroc and their reciprocal

crosses. Arch. Tierz. 51 (1), 42–54. https://doi.org/10.5194/aab-51-42-2008.
Kondracki, S., Banaszewska, D., Mielnicka, C., 2005. The effect of age on the morphometric sperm traits of domestic pig (Sus scrofa domestica). Cell. Mol. Biol. Lett. 10 (1), 3–13.
Kondracki, S., Banaszewska, D., Bajena, M., Komorowska, K., Kowalewski, D., 2013. Correlation of frequency of spermatozoa morphological alterations with sperm concentration in ejaculates of Polish Landrace boars. Acta Vet. Beograd 63 (5–6), 513–524.
Kondracki, S., Iwanina, M., Wysokińska, A., Huszno, M., 2012. Comparative analysis of Duroc and Pietrain boar sperm morphology. Acta. Vet. Brno 81 (2), 141–145. https://doi.org/10.2754/avb201281020195.
Kondracki, S., Wysokińska, A., Kania, M., Górski, K., 2017. Application of two staining methods for sperm morphometric evaluation in domestic pigs. J. Vet. Res. 61, 345–349. https://doi.org/10.1515/jvetres-2017-0045.
Łącka, K., Kondracki, S., Iwanina, M., Wysokińska, A., 2016. Assessment of stallion semen morphology using two different staining methods, microscopic techniques, and sample sizes. J. Vet. Res. 60, 99–104. https://doi.org/10.1515/jvetres-2016-0014.
Malo, A.F., Gomendio, M., Garde, J., Lang-Lenton, B., Soler, A.J., 2006. Sperm design and sperm function. Biol. Lett. 2, 246–249. https://doi.org/10.1098/rsbl.2006.0449.
Maree, L., du Plessis, S.S., Menkveld, R., van der Horst, G., 2010. Morphometric dimensions of the human sperm head depend on the staining method used. Hum. Reprod. 25 (6), 1369–1382. https://doi.org/10.1093/humrep/deq075.
Maroto-Morales, A., Ramón, M., García-Álvarez, O., Soler, A.J., Esteso, M.C., Martinez-Pastor, F., Pérez-Guzmán, M.D., Garde, J.J., 2010. Characterization of ram (Ovis aries) sperm head morphometry using the Sperm-Class Analyzer. Theriogenology 73, 437–448. https://doi.org/10.1016/j.theriogenology.2009.10.003.
Maroto-Morales, A., Garcia-Alvarez, O., Ramon, M., Martinez-Pastor, F., Fernandez-Santos, M.R., Soler, A.J., Garde, J.J., 2016. Current status and potential of morphometric sperm analysis. Asian J. Androl. 18, 863–870. https://doi.org/10.4103/1008-682X.187581.
Obara, H., Shibahara, H., Tsunoda, H., Taneichi, A., Fujiwara, H., Takamizawa, S., Idei, S., Sato, I., 2001. Prediction of unexpectedly poor fertilization and pregnancy outcome using the strict criteria for sperm morphology before and after sperm separation in IVF-ET. Int. J. Androl. 24, 102–108. https://doi.org/10.1046/j.1365-2605.2001.00275.x.
Ostermeier, G.C., Sargeant, G.A., Yandell, B.S., Evenson, D.P., Parrish, J.J., 2001. Relationship of bull fertility to sperm nuclear shape. J. Androl. 22, 595–603. https://doi.org/10.1002/j.1939-4640.2001.tb02219.x.
Pena, F.J., Saravia, F., Garcia–Herreros, M., Nunez-Martinez, I., Tapia, J.A., Johannisson, A., 2005. Identification of sperm morphometric subpopulations in two different portions of the boar ejaculate and its relation to post-thaw quality. J. Androl. 26, 716–723. https://doi.org/10.2164/jandrol.05030.
Pena, F.J., Saravia, F., Nunez-Martinez, I., Johannisson, A., Wallgren, M., Rodriguez-Martinez, H., 2006. Do different portions of the boar ejaculate vary in their ability to sustain cryopreservation? Anim. Reprod. Sci. 93, 101–113. https://doi.org/10.1016/j.anireprosci.2005.06.028.
Phetudomsinsuk, K., Sirinarumitr, K., Laikul, A., Pinyopummin, A., 2008. Morphology and head morphometric characters of sperm in Thai native crossbred stallions. Acta Vet. Scand. 50 (41), 1–9. https://doi.org/10.1186/1751-0147-50-41.
Prisant, N., Escalier, D., Soufir, J.C., Morillon, M., Schoevaert, D., Misrahi, M., Tachdjian, G., 2007. Ultrastructural nuclear defects and increased chromosome aneuploidies in spermatozoa with elongated heads. Hum. Reprod. 22, 1052–1059. https://doi.org/10.1093/humrep/del481.
Rijsselaere, T., Van Soom, A., Hoflack, G., Meas, D., de Kruif, A., 2004. Automated sperm morphometry and morphology analysis of canine semen by the Hamilton-Thorne analyzer. Theriogenology 62, 1292–1306. https://doi.org/10.1016/j.theriogenology.2004.01.005.
Saravia, F., Núñez-Martínez, I., Morán, J.M., Soler, C., Muriel, A., Rodríguez-Martínez, H., Peña, F.J., 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. https://doi.org/10.1016/j.theriogenology.2007.04.052.
Smital, J., De Sousa, L.L., Mohnsen, A., 2004. Differences among breeds and manifestation of heterosis in AI boar sperm output. Anim. Reprod. Sci. 80, 121–130. https://doi.org/10.1016/S0378-4320(03)00142-8.
Smital, J., 2009. Effect influencing boar semen. Anim. Reprod. Sci. 110, 335–346. https://doi.org/10.1016/j.anireprosci.2008.01.024.
Soler, C., Cooper, T.G., Valverde, A., Yaniz, J.J., 2016. Afterword to Sperm morphometrics today and tomorrow special issue in Asian Journal of Andrology. Asian J. Androl. 18, 895–897. https://doi.org/10.4103/1008-682X.188451.
Soler, C., Alambiaga, A., Marti, M.A., Garcia-Molina, A., Valverde, A., Contell, J., Campos, M., 2017a. Dog sperm head morphometry: its diversity and evolution. Asian J. Androl. 19, 149–153. https://doi.org/10.4103/1008-682X.189207.
Soler, C., Contell, J., Bori, L., Sancho, M., Garcia-Molina, A., Valverde, A., Segarvall, J., 2017b. Sperm kinematic, head morphometric and kinetic-morphometric subpopulations in the blue fox (*Alpex lagopus*). Asian J. Androl. 19, 154–159. https://doi.org/10.4103/1008-682X.188445.
Stockley, P., Gage, M.J.G., Parker, G.A., Møller, A.P., 1997. Sperm competition in fish: the evolution of testis size and ejaculate characteristics. Am. Nat. 149, 933–954. https://doi.org/10.1086/286031.
Thurston, L.M., Watson, P.E., Mileham, A.J., Holt, W.V., 2001. Morphologically distinct sperm subpopulations defined by Fourier shape descriptors in fresh ejaculates correlate with variation in boar semen quality following cryopreservation. J. Androl. 22, 382–394. https://doi.org/10.1002/j.1939-4640.2001.tb02194.x.
Tourmente, M., Gomendio, M., Roldan, E.R.S., 2011. Mass-specific metabolic rate and sperm competition determine sperm size in marsupial mammals. PLoS One 6, 1–7. https://doi.org/10.1371/journal.pone.0021244.
Valverde, A., Madrigal-Valverde, M., Castro-Morales, O., Gadea-Rivas, A., Johnston, S., Soler, C., 2019. Kinematic and head morphometric characterization of spermatozoa from Brown Caiman (Caiman crocodilus fuscus). Anim. Reprod. Sci. 207, 9–20. https://doi.org/10.1016/j.anireprosci.2019.06.011.
Waheed, M.M., Ghoneim, I.M., Abdou, M.S.S., 2015. Morphometric characteristics of spermatozoa in the Arabian horse with regard to season, age, sperm concentration, and fertility. J. Equine Vet. Sci. 35, 244–249.
Wierzbicki, H., Górska, I., Macierzyńska, A., Kmieć, M., 2010. Variability of semen traits of boars used in artificial insemination. Med. Wet. 66 (11), 765–769.
Wolf, J., Smital, J., 2009. Quantification of factors affecting semen traits in artifical insemination boars from animal model analyses. J. Anim. Sci. 87, 1620–1627. https://doi.org/10.2527/jas.2008-1373.
Wysokińska, A., Kondracki, S., 2004. Heterosis effects on physical traits of ejaculate in Duroc x Pietrain and Hampshire x Pietrain crossbred boars. Anim. Sci. Pap. Rep. 22 (4), 595–601.
Wysokińska, A., Kondracki, S., Banaszewska, D., 2009a. Morphometrical characteristics of spermatozoa in Polish Landrace Boars with regard to the number of spermatozoa in an ejaculate. Reprod. Biol. 9 (3), 271–282.
Wysokińska, A., Kondracki, S., Kowalewski, D., Adamiak, A., Muczyńska, E., 2009b. Effect of seasonal factors on the ejaculate properties of crossbred Duroc x Pietrain and Pietrain x Duroc boars as well as purebred Duroc and Pietrain boars. Bull. Vet. Inst. Pulawy 53, 677–685.
Wysokińska, A., Kondracki, S., 2013. Assessment of the effect of heterosis on semen parameters of two-breed crossem of Duroc, Hampshire and Pietrain boars. Arch. Tierz. 56 (7), 65–74. https://doi.org/10.7482/0003-9438-56-007.
Wysokińska, A., Kondracki, S., 2014. 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. https://doi.org/10.4141/cjas2013-095.
Yaniz, J.L., Capistros, S., Vincente-Fiel, S., Hidalgo, C.O., Santolaria, P., 2016. A comparative study of the morphometry of sperm head components in cattle, sheep, and pigs with a computer-assisted fluorescence method. Asian J. Androl. 18, 840–843. https://doi.org/10.4103/1008-682X.186877.