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Frequency of Cytoplasmic Droplets Depends on the Breed and Age of Insemination Boars

Dorota BANASZEWSKA, Barbara BIESIADA-DRZAZGA and Katarzyna ANDRASZEK

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In this study an attempt was made to analyse morphological changes in sperm with particular attention to sperm with a cytoplasmic droplet, taking into account the age and breed of the boar. The material for the study consisted of ejaculates of insemination boars of five breeds. Morphological examination of sperm was carried out in 30 randomly selected boars – 6 individuals from each breed. The morphology of 500 spermatozoa was evaluated in each slide. The percentage of sperm with normal morphology in the semen of the boars varied between breeds and was dependent in varying degrees on the age of the boar. The primary defects in sperm occurred more frequently in Duroc and Pietrain boars than in other breeds and were more dependent on the age of the boar. The high percentage of primary defects in the sperm of young Duroc boars was determined by the frequency of sperm cells with a proximal cytoplasmic droplet on the midpiece. A particularly high percentage of sperm cells with secondary defects was noted in the initial stage of the reproductive life of the boars. The high number of sperm cells with secondary defects noted in the semen of the Duroc and Pietrain boars and the changes occurring with age in the frequency of secondary morphological defects were mainly determined by the frequency of sperm cells with a distal protoplasmic droplet in the middle piece and to a lesser degree by that of sperm cells with a pseudodroplet.

Key words: Boar, sperm morphology, cytoplasmic droplet, age, breed.

Dorota BANASZEWSKA, Barbara BIESIADA-DRZAZGA, Department of Breeding Methods and Poultry and Small Ruminant Breeding, Institute of Bioengineering and Animal Breeding, University of a Natural Sciences and Humanities in Siedlce, Poland.
banaszd@op.pl, andrasz@uph.edu.pl
Katarzyna ANDRASZEK, Department of Animal Genetics and Horse Breeding, Institute of Bioengineering and Animal Breeding, University of a Natural Sciences and Humanities in Siedlce, Poland.

In insemination practice most boars produce semen with values in the normal range, indicating a high-quality ejaculate. In some cases, however, reduced fertility is observed in breeding boars and the cause is not fully known. Classic morphological evaluation of semen taking into account changes in sperm which occur with age with varying frequency in boars of different genotypes can be useful in predicting fertility. Analyses of these changes conducted on a variety of material allow for the determination of certain threshold values that can be used to predict the fertilizing capacity of the semen of animals of a particular age and known genotype. Fertilization is the result of many mutually-determined events taking place in a strict order, and the normal course of each stage depends on full maturity of the gametes. While evaluation of ejaculate characteristics and sperm morphology provides general information on the possibility of fertilization, a more thorough analysis of morphological changes reveals the pathogenesis of reduced fertility. Many studies have demonstrated that an elevated percentage of immature sperm in ejaculates negatively affects fertilizing capacity (AMMAN *et al.* 2000; KUSTER *et al.* 2004; CHENOWETH 2005; FRENAU *et al*. 2010). Such sperm, due to abnormal spermatogenesis, may contain cytoplasmic droplets (BAKER *et al*. 1996). In this study an attempt was made to analyse morphological changes in sperm with particular attention to sperm with a cytoplasmic droplet, taking into account the age and breed of the boar.

Material and Methods

The material for the study consisted of ejaculates of insemination boars of five breeds used in Polish

sow insemination stations: Large White, Landrace, Hampshire, Duroc and Pietrain. The time in which full reproductive maturity was achieved and the suitability of boars for insemination was estimated based on examinations of changes in sperm morphology occurring with age.

Morphological examination of sperm was carried out in 30 randomly selected boars: 6 individuals from each breed. Young boars aged 7-8 months, before they had begun to be used for breeding, were selected for analysis. All boars selected were in good health, with no visible developmental defects, and had normal libido. One ejaculate sample per month was collected from each boar for the first 1.5 years of its reproductive life, for morphological examination of the sperm. The ejaculates were collected by the manual method (KING & MACPHERSON 1973) once a month at about 6:00 a.m. Microscope slides were prepared from the samples using a method described in a previous study by KONDRACKI *et al* (2006). The slides were examined under a light microscope using a 100x immersion lens. The morphology of 500 spermatozoa was evaluated in each slide. Numbers of sperm with normal morphology and with morphological abnormalities were recorded and the latter were categorized into sperm with primary and secondary defects according to the Blom classification system (BLOM 1981). The material was divided into 10 subgroups according to the age of the boar on the day the semen was collected. Altogether the study was carried out on 570 ejaculates: 114 ejaculates from each breed.

Statistical differences between the samples were determined using Tukey's test and ANOVA (STATISTICA version 10.0, StatSoft Inc., PL). The level of significance was set at $P \leq 0.05$ or $P \leq 0.01$.

Results

The percentage of sperm with normal morphology in the semen of the boars varied between breeds and was dependent in varying degrees on the age of the boar (Table 1). The semen of Large White and Hampshire boars was of very good quality, indicated by a very high percentage of sperm cells with flawless morphology, generally exceeding 95% in boars of these breeds. The frequency of sperm cells with normal structure in these breeds was not found to depend on the age of the boar, as a high percentage of sperm with normal morphological structure was noted in the semen collected from the youngest boars (under 9 months) and in the older age groups. The high

Table 1

The frequency of occurrence of normal spermatozoa in semen of purebred boars in relation to the age of boars (%)

Age of boar (months)		Number of ejaculates	Breed of boar				
			Large white	Landrace	Hampshire	Duroc	Pietrain
<9	$\bar{x}$ Sd	30	97.57 2.60	92.77 5.01	94.17 4.12	87.90 7.17	86.50 11.47
9-10	$\bar{x}$ Sd	60	97.65 2.18	95.77 5.32	96.00 2.78	89.45 20.32	92.77 2.34
11-12	$\bar{x}$ Sd	60	95.63 5.53	95.77 3.30	96.73 1.60	87.30 11.82	91.23 12.98
13-14	$\bar{x}$ Sd	60	96.60 2.05	96.83 3.14	97.00 2.18	83.53 29.27	94.93 4.22
15-16	$\bar{x}$ Sd	60	97.73 1.95	95.50 3.28	98.02 1.46	91.45 9.20	97.07 2.17
17-18	$\bar{x}$ Sd	60	98.60 1.43	96.75 2.39	97.65 2.59	93.45 3.87	96.93 2.55
19-20	$\bar{x}$ Sd	60	96.58 2.70	94.85 3.10	96.93 2.18	94.82 4.17	97.15 3.29
21-22	$\bar{x}$ Sd	60	96.30 3.34	94.12 4.22	96.95 3.46	96.12 2.34	96.67 2.35
23-24	$\bar{x}$ Sd	60	97.75 1.93	91.33 10.71	96.93 1.64	94.18 6.72	92.40 8.41
25-26	$\bar{x}$ Sd	60	96.65 4.50	91.97 3.24	96.83 1.50	91.98 9.07	94.37 6.57
Total	$\bar{x}$ Sd	570	97.08 3.10	94.66 5.08	96.86 2.42	91.18 13.34	94.40 6.79

$LSD_{0.05} = 2.578$; $LSD_{0.01} = 3.068$

morphological quality of the semen of these breeds is underscored by the high repeatability of the results and the low variability (the standard deviation generally did not exceed 5% of the arithmetic mean).

Very different observations arise from analysis of the data concerning the sperm morphology of boars of the breeds Landrace, Duroc and Pietrain. The percentage of sperm cells with normal morphology in the semen of these boars was substantially more varied, depending not only on the breed of the boar but also on its age and on individual variation. Substantially fewer sperm cells with normal morphological structure were noted in semen collected from young individuals (under 9 months) and from those about 2 years and older, than in the middle period of reproductive life ($P \leq 0.01$). This tendency was particularly pronounced in Duroc boars, in whose semen the percentage of sperm with normal morphology was generally lower than in the other breeds and did not exceed 90% up to the age of about 15 months. The typical frequency of sperm with normal morphology in the other breeds (about 96%) was not attained by the Duroc boars until the age of 21-22 months, after which at the age of over 2 years a significant reduction was observed in the percentage of sperm without morphological changes.

Semen quality depends on how many of the ejaculated sperm have morphological defects preventing penetration of the egg cell. Of particular importance are primary defects, which occur during spermatogenesis. Data on the prevalence of primary defects in the semen of boars of different ages and breeds are presented in Table 2.

The data indicate that primary defects in sperm occurred more frequently in Duroc and Pietrain boars than in the other breeds and were more dependent on the age of the boar. The highest percentage of morphologically defective sperm cells was observed in young boars of these breeds, at the beginning of their reproductive life. In the ejaculates of Pietrain boars under the age of 9 months about 2.5% of sperm had primary defects, i.e. over 1% more than in the other breeds ($P \leq 0.01$), with the exception of Duroc. The ejaculates of the Duroc boars collected before the age of 9 months contained over twice as many sperm cells with primary morphological defects than the ejaculates of the Pietrain boars ($P \leq 0.01$) and many times more than the ejaculates of the other breeds ($P \leq 0.01$) collected at the same age. The percentage of sperm cells with primary morphological defects in the semen of Duroc and Pietrain boars decreased with age, but up to the end of the first year of life it was significantly higher than in the breeds Large

Table 2

The frequency of occurrence of spermatozoa with major abnormalities in semen of purebred boars in relation to the age of boars (%)

Age of boar (months)		Number of ejaculates	Breed of boar				
			Large white	Landrace	Hampshire	Duroc	Pietrain
<9	$\bar{x}$ Sd	30	0.33 0.41	0.80 0.91	1.43 1.07	5.10 6.74	2.47 1.60
9-10	$\bar{x}$ Sd	60	0.58 0.62	0.55 0.26	0.92 0.97	1.25 2.42	1.73 1.71
11-12	$\bar{x}$ Sd	60	0.97 2.25	0.88 0.94	0.37 0.37	2.35 3.54	2.27 4.54
13-14	$\bar{x}$ Sd	60	0.88 0.93	0.50 0.42	0.42 0.39	0.82 1.01	1.15 1.28
15-16	$\bar{x}$ Sd	60	0.70 0.82	0.42 0.32	0.95 0.92	1.57 1.61	0.80 0.66
17-18	$\bar{x}$ Sd	60	0.33 0.33	0.42 0.30	0.60 0.73	1.42 1.33	0.82 1.69
19-20	$\bar{x}$ Sd	60	0.65 0.75	1.22 1.92	0.35 0.31	1.28 1.36	0.48 0.40
21-22	$\bar{x}$ Sd	60	1.07 0.94	1.03 0.95	0.62 0.62	1.10 0.95	0.67 0.83
23-24	$\bar{x}$ Sd	60	0.65 0.74	0.82 1.06	0.67 0.99	0.95 1.17	1.85 4.17
25-26	$\bar{x}$ Sd	60	0.58 0.78	1.10 0.62	0.85 0.95	1.23 1.82	1.68 1.94
Total	$\bar{x}$ Sd	570	0.69 1.00	0.77 0.92	0.68 0.78	1.53 2.43	1.34 2.33

$LSD_{0.05} = 0.586$; $LSD_{0.01} = 0.697$

White, Landrace and Hampshire. It is worth noting that the high percentage of primary defects in the sperm of young Duroc boars was determined by the frequency of sperm cells with a proximal cytoplasmic droplet in the middle piece (Fig. 1a). In the semen of Duroc boars collected before the age of 9 months the percentage of sperm cells with a proximal cytoplasmic droplet was 4.73 % (Fig. 2), while the total percentage of sperm with primary defects in the semen of these boars was 5.1 % (Table 3). Furthermore, in semen from Duroc boars aged 11-12 months a high degree of frequency of sperm cells with a pseudodroplet (1.6%) was observed (Fig. 3). Secondary morphological defects were also more frequently observed in the semen of Duroc and Pietrain boars and were more age-dependent than in the case of the other breeds (Table 3).

A particularly high percentage of sperm cells with secondary defects was noted in the initial stage of the reproductive life of the boars. In the semen of the Pietrain boars collected before the age of 9 months over 11% of sperm cells had secondary defects, i.e. about 4-9% more than in the semen of the other breeds collected at the same age ($P \leq 0.01$). In the semen of Pietrain boars at the age of 9-12 months the prevalence of sperm with secondary defects decreased to about 6%, and at the age of over one year was substantially lower and in general remained at a level similar to that noted in the sperm of the boars of the other breeds. A high percentage of sperm with secondary defects was also noted in the semen of the young Duroc boars. The high frequency of sperm with secondary morphological defects in boars of this breed persisted, and even significantly increased, up to the age of 13-14 months, reaching a very high level of 15.65%. At this age the frequency of sperm with secondary morphological defects was about five times higher than in the boars of the other breeds

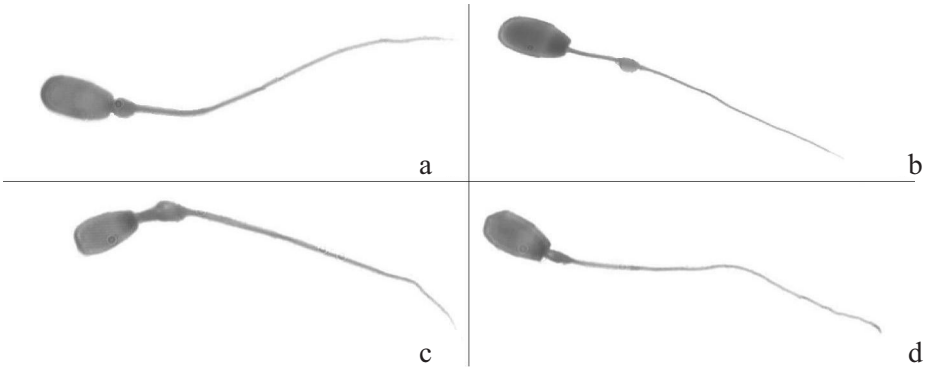

Fig. 1. Photograph of cytoplasmic droplets from the sperm cells of proximal (a) and distal (b) regions, and sperm with pseudodroplets (c, d).

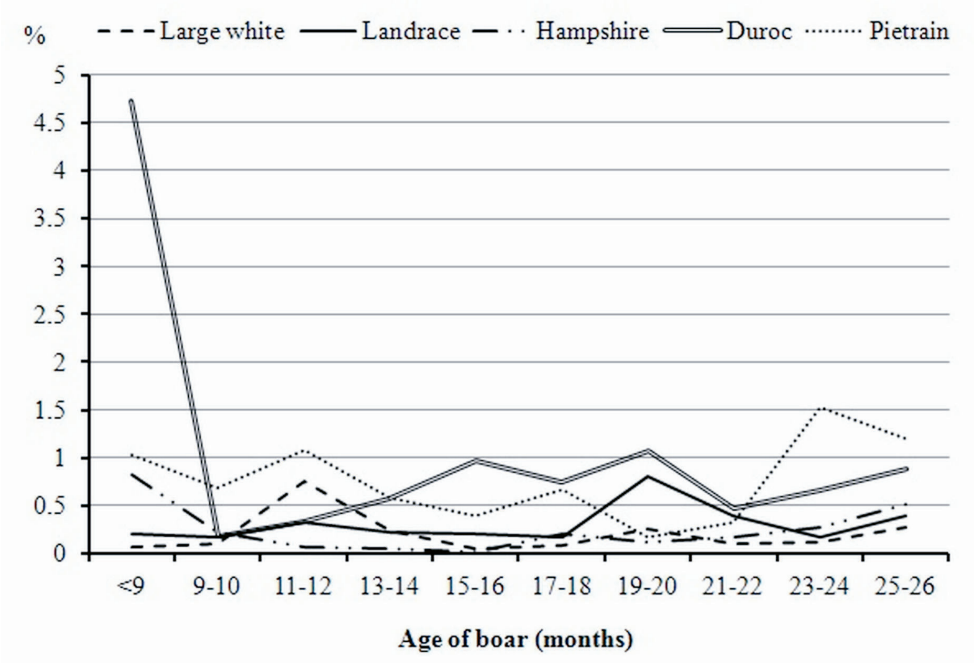

Fig. 2. Frequency of proximal cytoplasmic droplets depending on the breed and age of boars.

Table 3

The frequency of occurrence of spermatozoa with minor abnormalities in semen of pure-bred boars in relation to the age of boars (%)

Age of boar (months)		Number of ejaculates	Breed of boar				
			Large white	Landrace	Hampshire	Duroc	Pietrain
<9	$\bar{x}$ Sd	30	2.10 2.75	6.53 4.88	4.40 3.69	7.00 5.11	11.03 11.62
9-10	$\bar{x}$ Sd	60	1.77 2.09	3.68 5.29	3.08 2.46	9.30 20.35	5.50 2.60
11-12	$\bar{x}$ Sd	60	3.40 4.00	3.35 3.07	2.90 1.54	10.35 9.36	6.50 10.19
13-14	$\bar{x}$ Sd	60	2.52 1.84	2.67 3.13	2.58 1.94	15.65 28.76	3.92 3.86
15-16	$\bar{x}$ Sd	60	1.57 1.64	4.08 3.21	1.03 1.04	6.98 8.30	2.13 2.38
17-18	$\bar{x}$ Sd	60	1.07 1.33	2.83 2.41	1.75 2.07	5.13 3.65	2.25 2.34
19-20	$\bar{x}$ Sd	60	2.77 2.33	3.93 3.13	2.72 2.19	3.90 3.28	2.37 3.16
21-22	$\bar{x}$ Sd	60	2.63 3.32	4.85 3.82	2.43 2.92	2.78 2.34	2.67 2.09
23-24	$\bar{x}$ Sd	60	1.60 1.76	7.85 10.78	2.40 1.60	4.87 5.70	5.75 7.73
25-26	$\bar{x}$ Sd	60	2.77 3.85	6.93 3.24	2.32 1.60	6.62 7.78	3.95 5.76
Total	$\bar{x}$ Sd	570	2.22 2.63	4.57 4.99	2.46 2.16	7.27 12.75	4.27 5.89

$LSD_{0.05} = 2.427; LSD_{0.01} = 2.889$

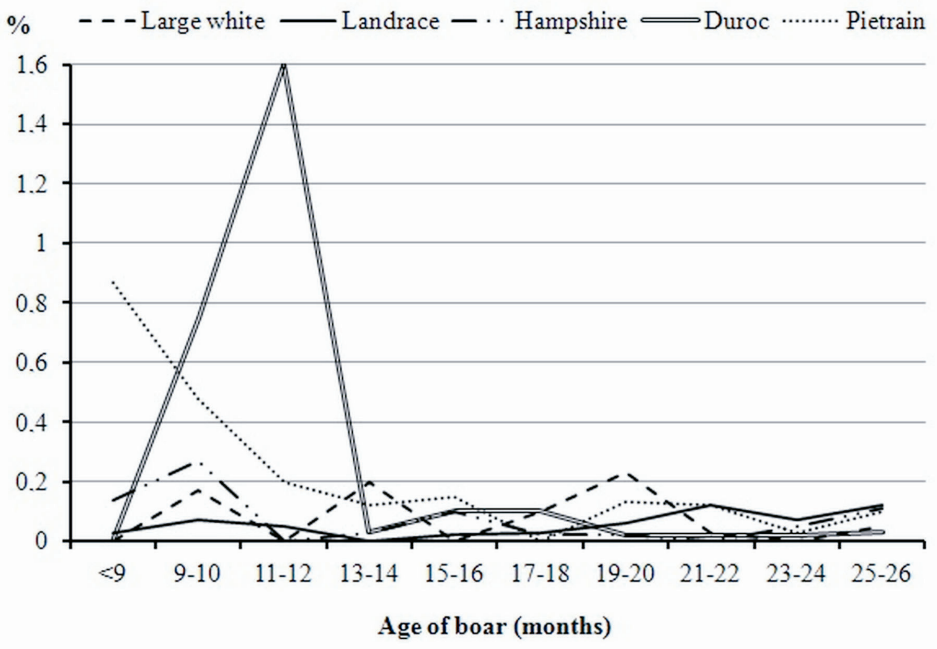

Fig. 3. Frequency of pseudodroplets depending on the breed and age of boars.

($P \leq 0.01$). The high number of sperm cells with secondary defects noted in the semen of the Duroc and Pietrain boars (Fig. 4) and the changes occurring with age in the frequency of secondary morphological defects were mainly determined by the frequency of sperm cells with a distal protoplasmic droplet in the middle piece (Fig. 1b) and also in the lower level of sperm with pseudodroplets (Fig. 1c, d).

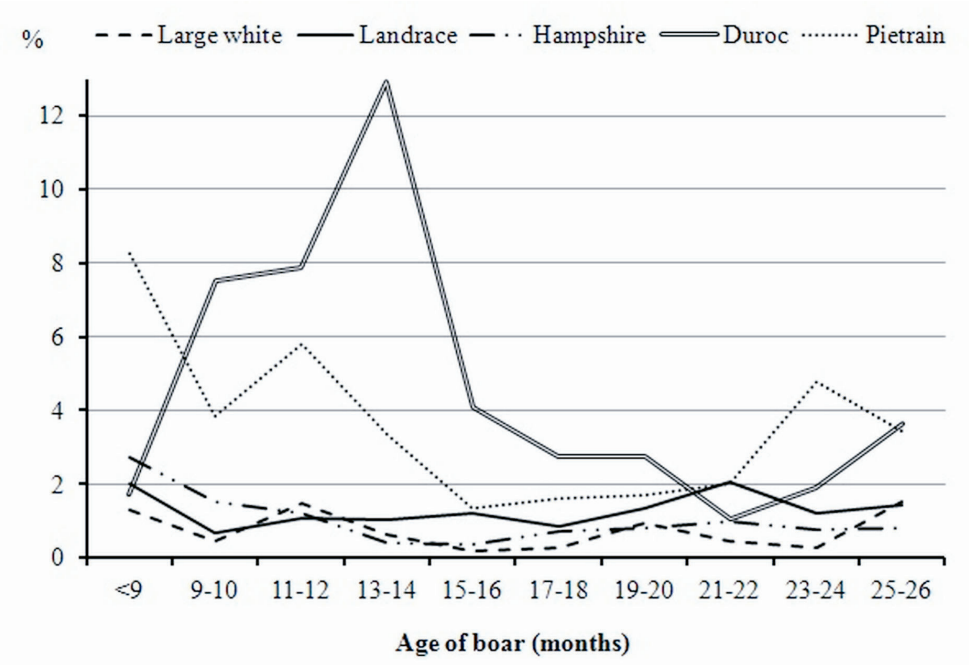

Fig. 4. Frequency of distal cytoplasmic droplets depending on the breed and age of boars.

Discussion

The purpose of the analyses was to determine threshold values for semen parameters that can define ranges for predicting fertilizing capacity in boars of different genotypes. The morphological characteristics of semen, however, are influenced by many factors. For this reason it is worth determining which morphological defects are most significant in predicting fertilization of the egg cell, which would suggest the possibility of supplementing basic evaluation of sperm morphology with an evaluation of selected morphological changes reducing fertility in the male.

Classification of defective sperm involves distinguishing sperm cells with primary defects which have been correlated with reduced fertility (KUBO-IRIE *et al.* 2005), and those exhibiting secondary defects, which do not necessarily indicate disturbances in spermatogenesis, but whose presence in large proportions in the ejaculate may significantly reduce fertility (BARTH 1997). All hereditary and environmental disorders in spermatogenesis and the sperm maturation process in the epididymis are reflected in the production of sperm with abnormal morphology, some of which may have damaged chromatin structure or have led to an abnormally high level of DNA damage (ENCISO 2011). Many studies indicate a relationship between elevated frequency of morphological changes in sperm and chromatin instability (KUBO-IRIE *et al.* 2005), increased frequency of chromosomal abnormalities (CALOGERO *et al.* 2001; SUN *et al.* 2006) or aberrant chromatin structure (FISCHER *et al.* 2003). The fertilizing capacity of sperm cells depends on a combination of many traits, such as high mitochondrial membrane potential to produce ATP, which determines motility, an intact acrosomal membrane, or chromatin structure integrity (BENCHAIB *et al.* 2003; CELEGHINI *et al.* 2004).

The results of the morphological evaluation of sperm presented in this study indicate that semen obtained from the youngest breeders may contain substantially more sperm cells with morphological anomalies than semen from more mature boars aged over one year. This tendency is less pronounced, however, in boars of the breeds Large White and Hampshire, while it is very marked in Duroc and Pietrain boars. The Duroc breed merits particular attention in this regard. In the semen of boars of this breed a high percentage of sperm with defective morphology persists for a relatively long time, up to the age of about 13-14 months, and is much higher than in boars of other breeds. Ejaculates of Duroc boars are characterized by small volume and a large number of sperm cells, which means a high sperm concentration significantly exceeding that of the ejaculates of other breeds (BANASZEWSKA & KONDRACKI 2012). This raises the question of whether the increased number of morphological defects is determined exclusively by the course of spermatogenesis or is generated as a secondary effect in the seminal tract and during

semen collection. This is a possibility because due to their high concentration the sperm of boars of this breed have less hospitable conditions for development after leaving the seminiferous tubules. The large number of secondary defects shown in the semen of the young Duroc boars indicates that this may be the case.

Comparison of the frequency of morphological abnormalities in the sperm of boars of different breeds showed substantial differences in the prevalence of cytoplasmic droplets. At the same time the percentage of sperm with abnormal morphological structure was shown to decrease with age. This also finds confirmation in analyses conducted on bulls in which ejaculates obtained from young individuals that had not reached reproductive maturity had a high percentage of sperm with a distal protoplasmic droplet, while in mature bulls the presence of such sperm in the semen is considered an indication of abnormalities in spermatogenesis and maturation of the sperm in the epididymis (AMANN *et al.* 2000; CHACÓN 2001; THUNDATHIL *et al.* 2001).

Reduced fertility in males has many causes. One cause known in humans is the presence in the sperm of excess residual cytoplasm (GOMEZ *et al.* 1996), which in contrast to cytoplasmic droplets is visible in a considerable length of the middle piece due to incomplete cytoplasmic extrusion during spermiogenesis (GOMEZ *et al.* 1996). In boar semen residual cytoplasm is visible in the form of a pseudodroplet. These changes take the form of round or elongated nodules of granulation tissue surrounded by mitochondria within the middle piece. In the present study the highest frequency of sperm cells with a pseudodroplet on the middle piece was noted in the semen of young Duroc boars aged about 11-12 months. The excess residual cytoplasm, in contrast with a typical cytoplasmic droplet, contains elevated levels of certain enzymes that produce pathological quantities of reactive oxygen species (BAKER *et al.* 1996; GOMEZ *et al.* 1996), which may lead to oxidative stress, and in consequence reduce sperm motility (ZINI *et al.* 1998) and fertilization capacity (ERGUR *et al.* 2002). Data obtained by BLOM (1968) indicate that when there is a high occurrence of pseudodroplets sperm motility is reduced. This is thought to be an inherited defect reducing fertility in the male (BLOM 1968) and may indicate gamete immaturity (GERGELY *et al.* 1999; AMANN 2010). Some authors suggest that diminished sperm maturity is linked to anomalies in the shape of the sperm head (HUSZAR *et al.* 1994).

The data obtained in the present study show that proximal or distal protoplasmic droplets in the middle piece are frequently encountered morphological defects in boar sperm. Proximal cytoplasmic droplets can be identified as regularly shaped residual cytoplasm under the cell membrane in the neck of the sperm (AMANN *et al.* 2000). The presence in the semen of sperm with a distal droplet reflects the functioning of the ductus epididymidis and accessory genital glands. The location of the cytoplasmic droplet in the middle piece changes during the maturation process. The droplet is formed in the last stage of spermatogenesis, during maturation of the sperm in the ductus epididymidis, when male reproductive cells differentiate into fully functional spermatozoa (AITKEN 2004; ZHENG *et al.* 2007). In contrast with the first two stages of spermatogenesis, significant morphological changes occur in its final stage, when large, spherical spermatids are transformed into an organized gamete. At this time the sperm tail and acrosome are formed, chromatin condensation leads to a substantial decrease in the size of the cell nucleus, the mitochondria form a spiral along the neck and the middle piece, and the remainder of the cytoplasm and organelles should be removed to facilitate motility (ZHENG *et al.* 2007). Most of the cytoplasm of reproductive cells is phagocytized by the Sertoli cells in the seminiferous tubules of the testes, and the remainder migrates down the tail in the form of a droplet (HUSZAR *et al.* 1998; COOPER 2005). This is a natural physiological process in which a droplet of cytoplasm first occupies a proximal position and then moves to the distal position, where it is finally shed during ejaculation (OYEYEMI & UBIOGORO 2005; COOPER 2011). This takes place owing to peristaltic movements of the ductus epididymidis, stimulated by a high concentration of sperm, which may cause the droplet to migrate along the sperm tail (COOPER 2011). Droplets around the neck of the sperm in the region of the caput have been found to be less regular in shape and somewhat larger than in other regions of the epididymis, where they were smaller and more spherical (DATTA *et al.* 2010). A study by ZHENG *et al.* (2007) showed that proper cytoplasm removal is genetically regulated by a process requiring the participation of a certain gene (*Spem1*). The absence of this gene impedes the process of cytoplasm removal, probably preventing it from detaching from the head and neck of the sperm during tail formation. Retention of the droplet may impede the development of normal sperm forms; e.g. the neck may bend or the middle piece and tail can wrap around the head, which has been observed in mice (ZHENG *et al.* 2007). The behaviour of the droplet may result in angulation of the tail, whereas the loss of the droplet allows the tail to remain straight, which facilitates the migration of sperm in the female genital tract (COOPER 2011). Retention of the droplet results in increased frequency of sperm with cytoplasmic droplets in the ejaculate, which is linked to re-

duced fertility in animals (AMANN *et al.* 2000; KUSTER *et al.* 2004), as a large percentage of sperm cells with cytoplasmic droplets reduces the fertilizing capacity of semen (BLOM 1981; SŁAWETA & MORSTIN 1982; SYSA 1988; SÖDERQUIST *et al.* 1991; WABERSKI *et al.* 1994; MORSTIN 1996; AMMAN *et al.* 2000; KUSTER *et al.* 2004; CHENOWETH 2005; FRENAU *et al.* 2010), while a high frequency of proximal cytoplasmic droplets may cause early abortion (SAACKE *et al.* 2000). According to ALTHOUSE (1997) the acceptable threshold for the occurrence of sperm with a protoplasmic droplet is 15%. In semen collected from the head of the epididymis of 28-month-old Landrace boars, about 50% of the sperm had a proximal droplet in the middle piece, while in the body of the epididymis about 50% of sperm already had a distal droplet (BRIZ *et al.* 1995). As the sperm migrate through successive regions of the epididymis, the percentage of mature and male sex cells with normal structure increases, exceeding 80% in the tail of the epididymis. Sperm with a proximal protoplasmic droplet in the middle piece are not generally observed in the tail of the epididymis, while sperm with a distal droplet occur with a frequency of 18% (BRIZ *et al.* 1995). Similar observations have been reported by other authors (ANAND & ATREJA 1986; AWOJOBI & OYEYEMI 2001; DATTA *et al.* 2010). The data obtained in the present study show that sperm with a proximal protoplasmic droplet in the middle piece were most often noted in the semen of Duroc and Pietrain boars. Sperm with a proximal cytoplasmic droplet in the semen of boars of these breeds accounted for over half of all primary defects in the sperm of these animals. Relatively few sperm with proximal protoplasmic droplets in the middle piece were noted in the semen of the Large White, Hampshire and Landrace breeds (0.16-0.32% of sperm), which may indicate that the epididymis was functioning normally in these boars.

The presence of a cytoplasmic droplet in the middle piece can cause swelling and bending of the sperm tail (COOPER *et al.* 2004; FENTIC *et al.* 2006; COOPER 2011), which prevents normal movement and reduces fertility (RENGAN *et al.* 2012). The presence of a protoplasmic droplet can probably also indicate abnormalities in the structure of the middle piece (SŁAWETA & MORSTIN 1982), which together with the acrosome are a site of concentration of enzymatic proteins taking part in the fertilization process. Moreover, cytoplasmic droplets contain numerous enzymes and receptors (RENGAN *et al.* 2012) which in excessive quantities negatively affect male fertility. One of these is 15-lipoxygenase, which is responsible for maturation of sperm in the epididymis, removal of the droplet, and the formation of the mitochondrial spiral in the sperm middle piece (AITKEN 2004; RENGAN *et al.* 2012). CARREIRA *et al.* (2012) reported that in ejaculates with a high percentage of sperm with a distal droplet reduced motility was observed as well. This is dependent on the age of the individual, because in young bulls up to 6 weeks after sexual maturity is attained a dynamic increase was observed in the percentage of sperm exhibiting progressive motion, accompanied by a decrease in the frequency of sperm with proximal cytoplasmic droplets. Also present in cytoplasmic droplets are A-kinase anchoring proteins, which play a role in regulation of sperm motility and the acrosome reaction (CARR *et al.* 2001). In contrast to typical cytoplasmic droplets, excess residual cytoplasm has a surplus of numerous enzymes, which can negatively affect sperm function in the female genital tract, leading to reduced fertility (RENGAN *et al.* 2012). Taking this into account we can presume that in semen with a high percentage of morphological sperm defects, sperm with apparently normal morphology may exhibit some type of dysfunction as well. This is indicated by a study by THUNDATHIL *et al.* (2001), which suggests that morphologically normal sperm coexisting in the semen with sperm with a proximal droplet were not fully functional. This may explain the low percentage of blastocyst development after the use of semen with a high frequency of cytoplasmic droplets (about 40-85%) (THUNDATHIL *et al.* 2001; CARREIRA *et al.* 2012).

Given the important role of cytoplasmic droplets in determining the fertilizing capacity of semen it should be emphasized that they are more frequently observed in boars of some breeds than others. Moreover, sperm with a droplet occur with greater frequency in young individuals. Expanding routine examination of semen to include the frequency of sperm with a cytoplasmic droplet seems advisable, as this would improve prognostication of the fertility of the male used for insemination. Furthermore, production of lower-quality semen can be an individual trait. Such individuals should be diagnosed at an early stage in order to avoid unnecessary costs of keeping breeders whose sperm has unsatisfactory fertilizing capacity.

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