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**Effect of breed and age on the morphology
of A.I. boars spermatozoa**

Wpływ rasy i wieku knurów stacyjnych na morfologię plemników

Summary. The aim of the paper was the analysis and assessment of the influence of the breed and age of breeding boars on sperm morphology of spermatozoa. 90 ejaculates were analysed, taken from Polish Large White boars, Polish White Lop-eared boars, Pietrain boars and hybrid boars: Pietrain × Duroc, Duroc × Pietrain, Hampshire × Pietrain. The boars were divided into three age groups: group I – under 24 months of age, group II – under 36 months of age, and group III – under 36 months of age. The sperm assessment comprised: normally developed sperm, sperm with secondary changes (with a protoplasmic droplet, with a distal tail loop or a middle-piece, free heads), sperm with primary changes (with an underdeveloped head and middle-piece). It was found that the breed/crossing variant had a significant influence on the percentage of basic defects in boar semen. It was observed that there was a tendency for fewer spermatozoa with a distal tail loop, free head and “pseudo-droplet” in the ejaculates of younger boars. On the other hand, the total number of sperm with defects of spermatozoa in the semen of boars of different age groups did not differ much.

Key words: breed, age, boar, semen, sperm, morphology of spermatozoa, defects of spermatozoa

INTRODUCTION

One of the main indicators at the microscopic assessment of boar semen is sperm motility. However, a decrease in sperm fertilization ability is not always connected with sperm motility disorders [Sławeta and Strzeżek 1982, Johnson *et al.* 2000]. That is why in assessing the quality of boar semen it is recommended that other tests be taken into account, for example, sperm morphology of spermatozoa and acrosome condition [Udała *et al.* 2005]. Many authors [Bronicka and Dembiński 1999, Kondracki *et al.* 2000, Pruneda *et al.* 2005, Wysokińska and Kondracki 2005, Kawęcka *et al.* 2008, Pokrywka *et al.* 2009] observe that the intensity of sperm morphological changes in boar semen depends on different genetic and environmental factors. It proves that the gonadal sperm

production depends above all on the breed, level of physical and sexual development (the boar's age), season of the year, feeding, and intensity of exploitation. Sperm morphological assessment is especially important since there is a proven correlation between sperm structure and sperm ability to inseminate an oocyte [Soderquist *et al.* 1991, Johnson *et al.* 2000]. Thus, it is justified that sperm morphology of spermatozoa tests are recommended and that the results of the tests are taken into account when the quality of boar semen is assessed.

The objective of the paper was an analysis and assessment of the influence of the breed and age of breeding boars on sperm morphology of spermatozoa.

MATERIAL AND METHODS

In sperm morphological tests 90 ejaculates taken from boars kept in the Sow Insemination Station in Kraśnik, Lubelskie Province were analysed. The assessment of sperm in the semen comprised: normally developed sperm, sperm with secondary changes (with a protoplasmic droplet, with a distal tail loop or a middle-piece, free heads), and sperm with primary changes (with an underdeveloped head and middle-piece).

To carry out the morphological assessment of sperm smears were stained with nigrosin and eosin produced in England. The classification and count of normal sperm and sperm morphologically changed was carried out using the method introduced by Bennett and O'Hagan [1967] and Bielański [1977]. In every smear 500 spermatozoa were assessed under a light microscope with immersion at 1 000 × magnification.

Smears for microscopic tests were prepared from the samples of analysed ejaculates. The smears were stained using the Bydgoska method: on the basic slide a thin smear of semen was made, which was fixed for 5 minutes in a 96% ethanol solution. The fixed smear was rinsed in distilled water, and then tinted in a 10% water solution of eosin over a period of 20–60 seconds. The tinted smear was rinsed in distilled water and stained with gentian violet for 3–5 minutes. After staining, the smears were rinsed, dried and then observed under a microscope.

In the sperm morphological analysis in semen the following sperm morphological defects were taken into account: single tail loop, free normal head, "pseudo-droplet", small normal head, stricture of head base, distal tail loop, diadem defect or head abnormal outline, abaxial head and total defects of spermatozoa.

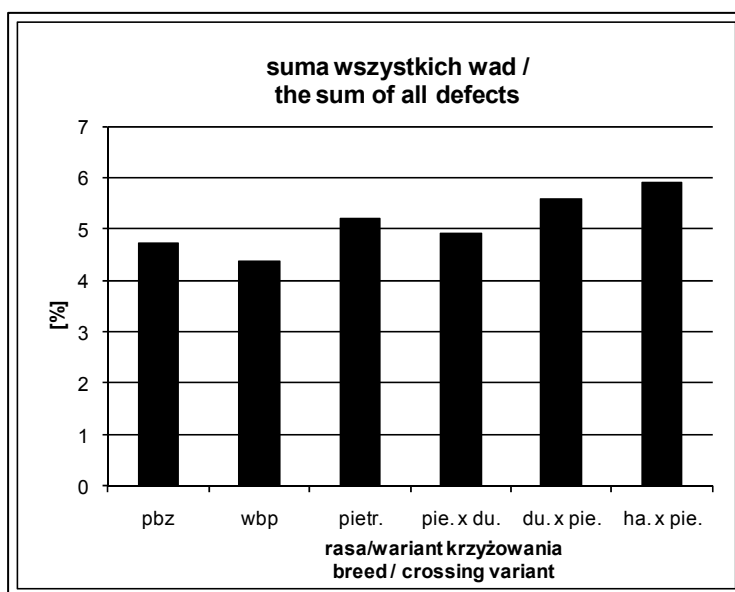
The sperm morphological analysis was carried out on the ejaculates of the following groups of boars: Polish Large White boars, Polish White Lob-eared boars, Pietrain boars and hybrid boars such as: Duroc × Pietrain boars, Pietrain × Duroc boars and Hampshire × Pietrain boars. The boars were divided into the following age groups:

Group I – under 24 months of age, group II – from 24 to 36 months old, group III – over 36 months of age.

The influence of breed/crossing variant and age of boars on the above enumerated properties was assessed using a single factor analysis of variance. The level of analysed properties was illustrated with arithmetic means, and the significance of differences between the groups was assessed using the student's t-test.

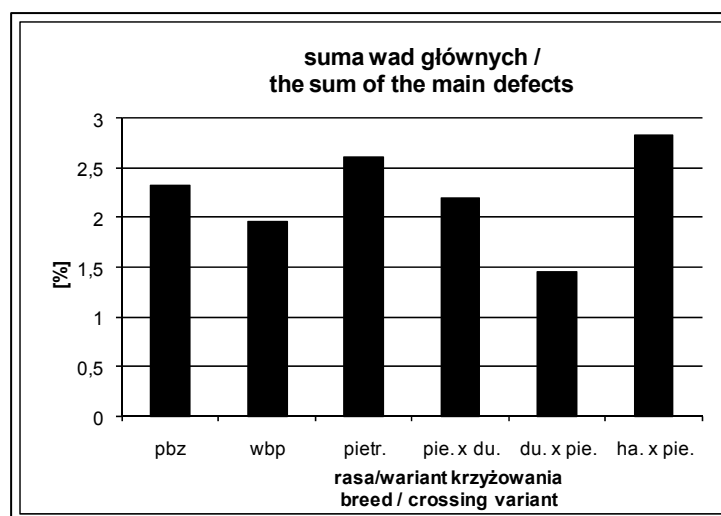
RESULTS AND DISCUSSION

The data illustrating the total number of all defects of spermatozoa found in boar semen in relation to the breed/crossing variant is shown in graph 1. The data analysis shows that boar semen from the analysed breed groups was characterized by a low percentage of sperm morphological defects of spermatozoa. The number of spermatozoa with defects in ejaculates of purebred boars ranged from 4.4% (in the semen of Polish Large White boars) to 5.22% (in the semen of Pietrain boars). A bit higher percentage of all sperm defects was found in the semen of hybrid boars: Duroc × Pietrain boars (5.6%) and Hampshire × Pietrain boars (5.9%). However, the found differences between the semen of boars from the analysed groups were not confirmed statistically. Wysokińska and Kondracki [2005] found that a similar number of spermatozoa with morphological defects was found in Pietrain boars (5.3%) and a smaller number in hybrid boars: Duroc × Pietrain boars and Hampshire × Pietrain boars (about 2%). Kawęcka's research [2002] also found that Pietrain boars were characterized by a big share of morphologically changed sperm.



Graph 1. The influence of breed and crossing variant on the sum of all defects
Wykres 1. Wpływ rasy i wariantu krzyżowania na sumę wszystkich wad

Graph 2 compares the total number of primary sperm defects of spermatozoa in the semen of breeding boars in relation to the breed. The semen of Hampshire × Pietrain hybrids was characterized by the greatest number of spermatozoa with primary defects, and it was 2.8%. The smallest number of defects of spermatozoa (1.45%) was found in the ejaculates of Duroc × Pietrain hybrids. A statistical analysis using a student's t-test for independent samples confirmed significant differences between groups of boars: Polish White Lop-eared boars – Duroc × Pietrain boars ($p \leq 0.05$), Pietrain boars – Duroc × Pietrain boars ($p \leq 0.05$), and Hampshire × Pietrain boars – Duroc × Pietrain boars ($p \leq 0.05$).



Significance – Istotność:

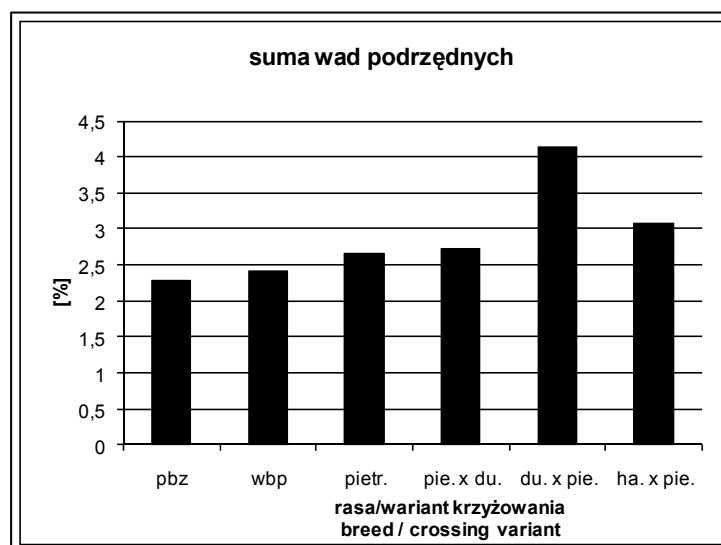
pbz – du. × pie.*

pietr. – du. × pie.*

ha. × pie. – du. × pie.*

* $p \leq 0.05$

Wykres 2. Wpływ rasy i wariantu krzyżowania na sumę wad głównych
Graph 2. The influence breed and crossing variant on the sum of the main defects



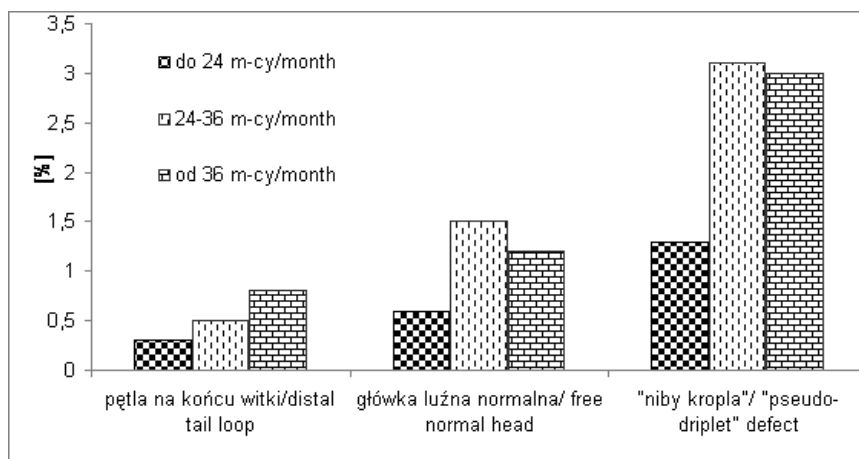
Significance – Istotność:

pbz – du. × pie.**

** $p \leq 0.01$

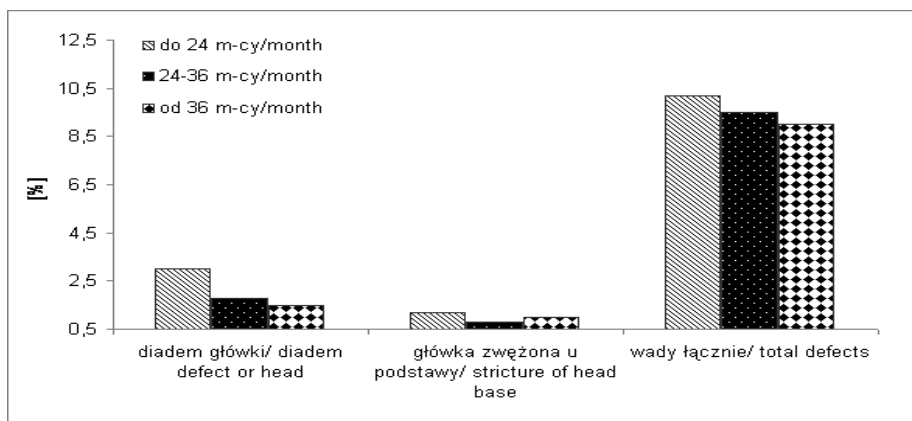
Graph 3. The influence breed and crossing variant on the sum second-rate defects
Wykres 3. Wpływ rasy i wariantu krzyżowania na sumę wad podrzędnych

Graph 3 illustrates a total number of secondary defects in boar semen in relation to the breed and crossing variant. The semen of Duroc × Pietrain hybrid boars was characterized by the greatest mean total number of secondary defects of spermatozoa (4.14%). The smallest number of secondary defects of spermatozoa was found in the semen of Polish White Lob-eared boars – 2.9%. Statistically, a significant difference in the number of secondary defects of spermatozoa was found between Polish White Lop-eared boars and Duroc × Pietrain hybrid boars ($p \leq 0,01$). Many authors [Kondracki *et al.* 2005, Kondracki and Wysokińska 2005, Udała *et al.* 2005, Wysokińska and Kondracki 2005] found significant differences in the morphology of sperm produced by boars of different origin.



Graph 4. The influence of age on the following morphological defects of sperm: distal tail loop, free normal head, "pseudo-droplet" defect

Wykres 4. Wpływ wieku na wady morfologiczne plemników, takie jak: pętla na końcu witki, główka luźna normalna, „niby-kropla”



Graph 5. The influence of age of boar on the amount sperm with defects of: diadem defect or head, stricture of head base, all of defects

Wykres 5. Wpływ wieku knura na ilość plemników z takimi wadami, jak: diadem główki, główka zwężona u podstawy, wady łącznie

Graph 4 illustrates differences in the frequency of occurrence of spermatozoa with a distal tail loop, a free normal head and a “pseudo-droplet” in boar semen in relation to boar age. The greatest number of spermatozoa with a distal tail loop was found in boars over 36 months of age. The smallest number of spermatozoa with a normal loose head was found in boars under 24 months of age. Spermatozoa with a “pseudo-droplet” were mostly produced by boars aged from 24 to 36 months. The smallest number of spermatozoa with a “pseudo-droplet” was found in the semen of boars under 24 months.

Graph 5 presents differences in the number of spermatozoa with a diadem defect, with a stricture of the head base and the total number of spermatozoa with morphological defects in the semen of boars of different ages. The greatest number of spermatozoa with a diadem defect was found in the semen of boars under 24 months of age. The smallest number of such spermatozoa was produced by older boars, aged over 36 months. Spermatozoa with a stricture of the head base were most often produced by young boars under 24 months of age. Whereas the smallest number of spermatozoa with this defect was produced by boars aged from 24 to 36 months. Based on the research carried out it may be stated that the biggest number of defective spermatozoa was produced by gonads of young boars, under 24 months of age. The smallest number of defective spermatozoa was produced by boars over 36 months of age. Similar observations result from the research carried out by Kondracki *et al.* [2005] and Udała *et al.* [2005], who found favourable sperm morphology parameters in young boars, under 12 months of age. Ejaculates of these boars were substantially different as regards the number of spermatozoa with a correct morphological structure and with primary changes from the ejaculates obtained from boars aged 13–18 months and over 24 months.

The research carried out proved that mean values of the main parameters characterising sperm morphology were different from one another, depending on the boar origin. The total number of spermatozoa with morphological defects in the semen of analysed boars of different origins was low and was within the norm adopted for boars used in breeding [Wierzbowski 1999, Strzeżek 2002]. This research did not confirm the opinion of some authors, pointing out that morphological defects were rarer in the semen of hybrid boars as compared to purebred boars [Wysokińska and Kondracki 2005, Udała *et al.* 2005]. However, the findings of the enumerated above authors were confirmed with regard to a worse sperm quality in the semen of Pietrain boars.

In the conducted research the morphological state of sperm (the total number of sperm defects of spermatozoa) in different age groups was similar, with a more favourable tendency in older breeding boars. The analysis of the morphological state with regard to the occurrence of primary and secondary changes in the ejaculates of breeding boars in different age groups showed that the best sperm characterized young boars. The sperm taken from young breeding boars (under 24 months of age) was characterized by the smallest number of spermatozoa with a distal tail loop. Moreover, the semen of young boars as compared to older boars (from 24 to 36 months old) had the smallest number of sperm with such defects as a “pseudo-droplet” or a free head. The greatest number of abnormalities was found in the ejaculates of older breeding boars. The biggest number of spermatozoa with a distal tail loop was found in the semen of boars aged 36 months and older. As regards sperm with a “pseudo-droplet”, the greatest number of such sperm was found in the ejaculates produced by boars from 24 to 36 months of age.

In this respect our findings are similar to the research results of other authors [Gączarzewicz *et al.* 2000, Udała *et al.* 2005].

CONCLUSIONS

Thanks to a morphological analysis of sperm taken from boars of different origin, some significant differences in the number of primary defects may be found. The number of these defects depends on the breed of the examined boars.

The best quality semen of purebred boars, characterised by the smallest number of sperm with secondary changes, came from Polish White Lop-eared boars. The percentage of normal sperm in the semen of boars of this breed was 95.6%. The greatest number of spermatozoa with morphological defects of spermatozoa was found in the semen of Pietrain boars.

As regards the occurrence of primary and secondary changes in the ejaculates of breeding boars of different ages, the best quality semen characterized young boars – not older than 24 months.

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Streszczenie. Celem pracy było dokonanie analizy i oceny wpływu rasy i wieku knurów stacyjnych na morfologię plemników. Przeanalizowano 90 ejakulatów pobranych od knurów rasy wielkiej białej polskiej, polskiej białej zwisłouchej, pietrain i knurów mieszańców: pietrain × duroc, duroc × pietrain, hampshire × pietrain. Knury podzielono na trzy grupy wiekowe: I – do 24 miesięcy, II – do 36 i III – powyżej 36 miesięcy życia. Ocena plemników obejmowała: plemniki normalnie wykształcone, plemniki zmienione wtórnie (z kroplą protoplazmatyczną, z zawiniętą wtką lub wstawką, luźne główki), plemniki zmienione pierwotnie (z niedorozwiniętą główką i wstawką). Stwierdzono istotny wpływ rasy/wariantu krzyżowania na odsetek występowania wad głównych w nasieniu knurów. Zaobserwowano tendencję występowania mniejszej ilości plemników z pętlą na końcu wtki, główką luźną i niby-kroplą w ejakulatach knurów młodszych. Natomiast liczba plemników z wadami łącznie w nasieniu knurów z różnych grup wiekowych różniła się minimalnie.

Słowa kluczowe: rasa, wiek, knur, nasienie, plemniki, morfologia plemników, wady plemników