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Morphological Analysis of the Sperms of Breeding Boars Maintained on Nutritional Supplements

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Abstract: The purpose of the study was to perform morphological analysis of the semen of Landrace boars maintained on nutritional supplements comprising micro- and macronutrients and biologically active compounds of animal and plant origin. The differences between the groups in sperm production and semen biology and morphology were analyzed. Considerable beneficial changes in the amount and quality of sperms were found upon administration of the supplements being tested. Statistically significant differences between the groups were identified in the sperm characteristics such as head width and tail length and the head length-to-width ratio. The most frequently encountered deviations in the sperm morphology were a flagellar coiling, irregular contour of the mid-piece and cytoplasmic droplet on the flagellum. It was found that in all boars maintained on nutritional supplements under study, the numbers of abnormal sperms decreased with age, which was regularly manifested in the decrease in the teratozoospermia index.

Key words: Spermatozoa · Boars · Morphology · Biologically active compounds · Micro- and macronutrients

INTRODUCTION

The morphometric analysis data for boar sperms reported by different authors are variable [1-3]. This may imply that spermatogenesis and spermatozoa are very sensitive to stress factors, toxic impacts, completeness of nutrition and the environmental situation in the region of production [4-8]. This calls for a comprehensive study of all factors that affect the sperm production and sperm morphology, especially during puberty.

Among available publication sources, we were unable to find any data on the sperm production and age-related morphological changes in the sperms of Landrace boars meant for breeding during puberty upon application of Permamic, Calcefit-5 and Sedimin® nutritional supplements.

In view of the foregoing, the goal of our study was to elucidate the details of sperm production and the biology and micromorphology of the spermatozoa of Landrace boars during puberty upon application of Permamic, Calcefit-5 and Sedimin® supplements.

The Following Tasks Were Set to Achieve this Goal:

- To elucidate characteristic features of sperm production and biology and micromorphology of spermatozoa of Landrace boars meant for breeding during puberty.
- To evaluate the effect of Permamic administered together with Calcefit-5 and Permamic administered together with Sedimin® on the sperm production and biology and micromorphology of the spermatozoa of breeding boars.

MATERIALS AND METHODS

The work was performed in the period from 2007 to 2012 at the Chair of Bioecology and Geography of the Federal State Budgetary Educational Institution of Higher Professional Education "I. Ya. Yakovlev Chuvash State Pedagogical University". The experiment with 60 Landrace boar pigs meant for breeding, which were selected according to the analog-group principle, each group comprising 20 animals, were carried out at the pig farm

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Atal located in the northern subzone of the Volga Region of the Chuvash Republic. During the studies, the animals were fed according to the standard rates and rations [9].

The boar pigs of the first group (control) aged from 1 to 360 days (duration of the experiment) were maintained on the basal ration (BR). The animals of the second (test) group were maintained on the OR with daily addition of the complex powder Permamic consisting of zeolite-containing bergmeal from the Alatyrskoe deposit (Chuvash Republic), cobalt chloride, iron sulfate, copper and zinc sulfates, potassium iodide and its stabilizer, TU 9317-018-00670433-99, in the period from 60th to the 120th day of age in the dose of 1.25 g per kg of the body weight. From the 60th to the 180th day of age, the animals were additionally fed with the mineral supplement Calcefit-5 comprising meat-and-bone meal tankage; calcium lactate; calcium glycerophosphate; citrate; magnesium sulfate; sulfur; iron lactate; potassium silicate; potassium iodide; calcium fluoride; freeze-dried extract of the ginseng tincture (Russia, St-Petersburg, Institute of Veterinary Biology, TU 9219-001-50021486-2002) in the dose of 5 g per every 10 kg of the body weight.

The boar pigs of the third (test) group were maintained on the BR with daily addition of Permamic in the periods and doses indicated above; also, they were intramuscularly administered with Sedimin® (aqueous mixture of iodine and selenium compounds on the iron/dextran complex stabilizing base. Russia, Pushchino, Certificate of State Registration of a Veterinary Drug # PVR-2-3,6/01651) on the 3rd and 14th days of life as a single dose (2 ml) in the head; then 7 days before weaning in a 5 ml single dose in the head. On the same days the animals of the control and second groups were administered with the physiological salt solution (i.m.).

The ejaculate was collected from all boars on the 180th day (active physiological maturation and puberty) and the 360th day (sexual maturity), manually before the morning meal. The ejaculate was analyzed by macroscopic (semen volume) and microscopic methods (determination of the concentration of sperms; estimation of activity under a microscope; morphology examination). For micromorphological analysis, 200 sperms were counted and measured in stained samples in two replications; the percentage of abnormal cells was determined; and the teratozoospermia index (TZI = the total number of defects divided by the number of pathological spermatozoa) was calculated. The semen smears were examined using a MIKMED-6 optical microscope equipped with a video camera. The image input and analysis were performed on a computer with a Micro View software for morphometric analysis (LOMO-Microsystems, St.-Petersburg).

The statistical processing of the results was done using the SPSS 12.0.2 program. The normal distribution test was done by the Shapiro-Wilk and Kolmogorov-Smirnov methods. The equality of group means for all quantitative characteristics was tested using the Kruskal-Wallis non-parametric test. The mean values of characteristics are given as Ì±s (Ì is the mean and s is the standard deviation). The statistical significance of the differences between the mean values was estimated at the critical level p=0.05.

RESULTS

It follows from the obtained data that the semen volume in the control group boars increased 1.6-fold from the 180th to the 360th day of age; in the second group it increased 2.1-fold ($P<0.05$) and in the third group, it increased 2.2-fold ($P<0.05$) during the same period.

The sperm motility in second- and third-group animals at the age of 180 days was higher than that for the control animals of the same age by 7.9% and 11.3% ($P>0.05$) and at the end of testing it was higher by 3.7% and 5.1% ($P>0.05$).

It was noted that the total number of sperms in 1 ml of ejaculate in all experimental boars increased with respect to the initial data by 30.5% to 40.0%, while the number of pathological and immature germ cells reduced by 20.5% to 37.0%; these changes were more pronounced for second- and especially third-group animals.

Thus, it was found that the ejaculate volume and the concentration of sperms were superior for the boars of the second and third groups over analogs of the intact group by 24.6 – 35.2% ($P<0.05$) at the age of 180 days and by 21.4 – 44.3% ($P<0.05$) at the age of 360 days. Furthermore, at the age of 360 days, the volume of the ejaculate and the concentration of sperms were 7.1 – 9.4% ($P>0.05$) higher in the boars maintained using Permamic together with Sedimin® as compared with the second-group pigs.

The morphometric analysis of the sperms demonstrated that the sperm parameters such as the head and mid-piece lengths, the mid-piece width and the head to mid-piece length ratio did not change significantly during the test either with age or between the groups.

Considerable changes were noted in the head width, tail length and head length-to-width ratio. Thus at the age of 180 and 360 days, the width of the sperm head of the third-group boars was 9.4% and 10.0% smaller as compared with the control group ($P<0.05$). The length of the sperm tail in third-group boars was also smaller than that found for the control boars at the above-mentioned ages by 12.3% and 13.1%, respectively ($P<0.05$). Also, for the age of 360 days, the tail of sperms in second-group boars was found to be significantly shorter compared with the control animals of the same age by 15.3% ($P<0.05$).

A significant difference between the sperm head length-to-width ratio was noted in the ejaculate samples from 180- and 360-day boars of the third group (2.39±0.29 -2.31±0.32 versus 2.12±0.25- 2.09±0.25 and 2.24±0.34- 2.23±0.31 found for the boars of the control and second groups, respectively, at the same ages).

Counting 200 spermatozoa in stained samples for the presence of semen cells with normal and abnormal morphology demonstrated that the number of normal sperms increases with age in all of the tested boars: from 172 to 182 in the first group, from 181 to 188 in the second group and from 184 to 189 in the third group ($P>0.05$).

It was found that the most frequently encountered morphological distortions include abnormal acrosome; flagellar coiling; and cytoplasmic droplet on the flagellum. These distortions account for 71.43% of all abnormal sperms in the samples from 180-day old control boars and for 72.22% for 360-day old boars. For semen smears of the second-group boars, these values were 73.68% and 66.66%; in ejaculate samples of third-group boars, they were 56.25% and 54.54%, respectively.

The number of sperms with abnormal acrosome (knobbed acrosome) decreases from the age of 180 days to 360 days in the case of control and group 2 test animals: by 37.8% and 47.2% ($P<0.05$), while in ejaculate samples of third-group boars this distortion was not detected in the age periods under testing.

Least frequent were the combination of abnormal flagellum in the mid-piece with a flagellum droplet; this accounted, on average, for 10.53% to 12.5% of all abnormal sperms in 180 day old boars and for 8.33% to 18.18% at the age of 360 days.

The TZI was found to decrease from the 180 to 360 days of age by 3.4% in the second group of boars and by 8.0% in the third group ($P<0.05$). Conversely, in the control group, this index was found to increase by 2.4%.

Conclusion: The semen quality can be affected by numerous factors. The understanding of how biologically active supplements can affect the biology and morphology of spermatozoa will help to ensure the optimal reproduction of livestock animals. Often, in the farms, the semen starts to be collected from breeding boars from the age of 180 days. This age period corresponds to intensive physiological development and puberty in boars [10, 11]. The growth and development of both the body as a whole and the reproductive system during this period require complete and balanced nutrition. For sperm production, increased contents of proteins, amino acids, vitamins and macro- and micronutrients are required.

In our opinion, the enhancement of sperm production is related to the sanitizing action of zeolite additives on the animal organism as a whole, which is consistent with data obtained by other researchers [12, 13]. Moreover, the addition of Calcefit-5 and administration of Sedimin® against feeding with Permamic favors enhancement of appetite, increase in the food conversion, replenishment of elements that are vital for sperm production (Zn, Fe, Mn, Mg, Co, I, Ca, F), increase in the spermatozoa preservability upon freezing, maintenance of stable hormonal profile of the whole organism, particularly, testosterone level and, what is important, decrease in the anxiety and increase in fastness of animals by means of balanced formulation of macro- and micronutrients and biologically active compounds contained in the supplements tested [14-18].

CONCLUSIONS

In this study we demonstrated that the supplements we used have no adverse effects (the amounts of iron and selenium in these supplements being fed to animals do not exceed toxic doses).

We found that at a pig farm located in the northern subzone of the Volga Region of Chuvash Republic, the application of Permamic together with Calcefit-5 in boars had less pronounced effect on sperm production and biology and micromorphology of the spermatozoa of Landrace breeding boars than application of Permamic together with Sedimin®.

The experimental results prove that the sperm of 360 day old boars is more promising compared with that of 180 day old boars for the use in breeding.

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