eISSN: 09748369

Boar semen cytomorphology features after intramuscular injections of sedimin® and subsequent diet fortification by a zeolite-containing product

Biology and Medicine

Research Article

Volume 6, Issue 1, Article ID: BM-003-14, 2014

Indexed by Scopus (Elsevier)

Co-Publisher: OMICS Group, www.omicsonline.org

www.biolmedonline.com

Boar semen cytomorphology features after intramuscular injections of sedimin® and subsequent diet fortification by a zeolite-containing product

Inessa Yur'evna Arestova*, Vladislav Venjaminovich Alekseev
The Chuvash I. Yakovlev State Pedagogical University, K. Marx Street 38, Cheboksary, Chuvash Republic, 428000, Russia.

***Corresponding author:** The Chuvash I. Yakovlev State Pedagogical University, K. Marx Street 38, Cheboksary, Chuvash Republic, 428000, Russia.

Received: 29th Apr 2014; Accepted: 20th Jun 2014; Published: 1st Jul 2014

Abstract
The aim of this study was the cytomorphology analysis of the semen of Landrace boars maintained on microelement biogenic preparations in view of ecological and geographical conditions. The intergroup differences in sperm and micromorphology of sperm cells have been analyzed. The quantitative and qualitative changes in the semen after using the test biologics have been revealed. Intergroup statistically significant differences have been marked in such morphometric parameters of the semen as head width, tail length, and the head length to its width ratio. It has been revealed that the most usual deviations in sperm morphology are the loop form of the flagellum end, an uneven contour of the middle part, and a cytoplasmic droplet on the flagellum. It was found that with all boars grown with addition of the studied dietary supplements, the number of abnormal sperm cells decreased as they grew older that showed the reducing of teratozoospermic index.

Keywords: Semen; boars; cytomorphology; micro and macronutrients.

Introduction

As a result of the research the methods of stimulating and correcting the boars productivity by using specific hormonal agents, biologically active substances, effective microorganisms, pro-, pre-, and synbiotics as well as natural mineral sorbents are introduced into practice [1-6].

However, carrying out the research we found out that the use of the same bio-products in different soil and climatic conditions may lead to a considerably different morphophysiological effect.

Therefore, at present there is a need for comprehensive studies on the determination of the various biologics influence on the reproductive function of production animals with due regard not only to the breed, sex, age, etc., but also to the ecological and geographical conditions.

The data obtained for a particular agro landscape zone will identify the actual performance of the production animals' reproductive system under the normal conditions and under the conditions of microelementosis, will determine the adaptive capacity of farm livestock breeds, optimize the economic costs, develop new methods, schemes, and approaches to feeding in a particular administrative geographical area.

Among the available literature we haven't found information on the Landrace boars' grown in the southern subzone of the Southeast agro landscape (black earth soil) of the Chuvash Republic, sperm quality and sperm age-specific cytomorphological changes in the period of sexual function formation when administered by Sedimin® and zeolite-containing Permamik added to the diet.

Proceeding from the above, the aim of our research was to study the peculiarities of the Landrace boar's grown in the southern subzone of the Southeast agro landscape (black earth soil) of the Chuvash Republic semen production and semen micromorphology in the period of sexual function formation when administered by Sedimin® and Permamik.

To achieve this goal the following tasks were set:

1. To identify the special characteristics of semen production and sperm cells cytomorphology in the period of sexual function formation of Landrace breed grown in the southern

subzone of the Southeast agro landscape (black earth soil) of the Chuvash Republic.

2. Assess the impact of Sedimin® and Permamik on semen and sperm micromorphology of Landrace boars grown in of the southern subzone of the Southeast agro landscape (black earth soil) of the Chuvash Republic.

Materials and Methods

Digital data have been obtained by processing the biomaterial received from experimental animals kept in the stock breeding complex located in the southern subzone of the Southeast agro landscape (black earth soil) of the Chuvash Republic. The biomaterial processing was carried out at the Department of Bioecology and Geography, as well as at the training laboratory facilities of the Faculty of Sciences of the Federal State Educational Institution of Higher Professional Education (The Chuvash I. Yakovlev State Pedagogical University, Cheboksary, Chuvash Republic, Russia).

A series of experiments was carried out with 40 Landrace boars.

A control and an experimental groups consisting of 20 physiologically mature newborn boars each were formed. Physiological maturity was determined by indicators including their appearance, body weight, the amount of milk teeth, constitution, fleshing, behavior, response to external stimuli, the color of the visible mucosae, skin condition, as well as the time of the pig's standing-up on his feet after birth and the degree of the sucking and defensive reflexes manifestation [7,8].

The investigations were carried out while balanced feeding according to key indicators of the norms and rations [9].

The boars of the control group aged up to 360 days (the experiment duration) were on basic feed (BF).

The second experimental group animals were on BF. All animals were intramuscularly injected with Sedimin®, 2 ml each animal once on the 3rd and 14th day of life, then 10 days before ablactating – 5 ml each animal once (Sedimin® is iodine- and selenium-compounds water mixture on the stabilizing basis of iron dextran complex produced by LLC A-BIO, Pushchino, Russia; the state registration certificate of a drug product for animal use No. ПВР-2-3,6/01651). The animals from the control group were injected intramuscularly with saline solution on the similar dates. Meanwhile the complex powder Permamik was added daily to the BF beginning from 60 to 120 days at a dose of 1.25 g/kg body weight. Permamik consists of zeolite-containing diatomit of Alatyrsky deposit in Chuvashia, cobalt chloride, iron sulfate, sulfuric copper salts, zinc, potassium iodide, and its stabilizer, TU 9317-018-00670433-99.

The boars on 180th and 360th days of life had the ejaculate taken for analysis. The ejaculate was analyzed by macro and microscopic method (determination of the ejaculate volume, sperm concentration, by Goryaev counting cameras, activity assessment under the microscope, and morphology). For micro morphological analysis of native and Blum colored products 200 sperm were counted and measured, the percentage of ill-defined cells was determined, the teratozoospermic index was calculated—the number of defects divided by pathological spermatozoa number (TZI).

Semen smears were examined using light-optical microscope "MIKMED -6" (JSC, LOMO, Saint-Petersburg, Russia) by video visualization. The image was entered and analyzed by the computer with morphometric analysis software "Micro View" (LLC, LOMO- Mikrosystemi, St. Petersburg, Russia).

Statistical analysis was carried out by Microsoft Excel 2007 application packages. The normal-theory test was performed using Shapiro-Wilk statistics, the Kolmogorov-Smirnov. Testing hypotheses about the equality of group means of all quantitative characters was performed using nonparametric Wilcoxon-Mann-Whitney test. Mean values are given as M±S (M: mean; S: standard deviation). The assessment of the significant differences between means was carried out at the critical level of $p = 0.05$ [10].

Results

The data presented in Table 1 show that the volume of semen of the boars in the control group increased from 180 to 360 days age by 70.53 ml, in the second group by 157.17 ml, and in the third group by 154.40 ml.

Sperm motility in boars aged 180 days and at the end of the study of the second group was higher than motility of the control herd mates by an average of 11.0% ($p < 0.05$).

Table 1: Qualitative indicators of boar semen.

Groups	Age (days)	Ejaculate volume (ml)	Motility, score	The number of spermatozoon		
				Total in 1 ml (mln)	Pathologic (%)	Immature (%)
1	180	102.93 ± 1.62	6.73 ± 0.56	112.6 ± 2.50	15.30 ± 0.71	10.60 ± 1.22
2		134.57 ± 19.58*	7.43 ± 0.44*	165.6 ± 6.64*	9.90 ± 0.04*	8.40 ± 0.18*
1	360	173.47 ± 17.29	7.70 ± 0.80	185.0 ± 3.10	9.63 ± 0.49	7.63 ± 0.98
2		288.97 ± 7.78*	8.20 ± 0.33*	236.5 ± 24.71*	6.20 ± 0.40*	5.93 ± 0.22*

*Hereinafter the mark of the significant differences between means in animals of control and experimental.

Table 2: Micro morphometric parameters of boars' sperm cells.

Groups	1		2	
Age (days)	180	360	180	360
Head length (μm)	9.55 ± 0.61	9.91 ± 0.54	10.20 ± 1.11	9.86 ± 0.90
Body length (μm)	14.69 ± 0.85	13.99 ± 1.08	15.06 ± 1.15	14.78 ± 1.19
Tail length (μm)	39.20 ± 6.30	36.02 ± 5.07	31.09 ± 2.80*	30.78 ± 2.63*
Head width (μm)	4.91 ± 0.90	4.76 ± 0.48	4.41 ± 0.37*	4.32 ± 0.43**
Body width (μm)	0.93 ± 0.18	0.87 ± 0.13	0.88 ± 0.16	0.84 ± 0.14
Sperm head length to body length ratio	0.65 ± 0.05	0.71 ± 0.06	0.68 ± 0.08	0.67 ± 0.08
Sperm head length to its width ratio	2.07 ± 0.89	2.12 ± 0.47	2.33 ± 0.32**	2.30 ± 0.30**

*Hereinafter the mark of the significant differences between means in animals of control and experimental.
**Experimental groups.

The sperm concentration in 1 ml of the ejaculate of the boars in the second group was higher compared to the intact herd mates by 35.4% ($p < 0.05$), while the number of immature cells and cells with pathology–less than by an average of 49.1% ($p < 0.05$).

According to the data presented in Table 2, it was found that at the age of 180 and 360 days the width of sperm heads of boars of the second group was smaller compared with that of the control boars (the difference was up to 10.2%, $p < 0.05$). The tail length of the sperm cell of the animals in the second group was also less than that of the control boars of the same age by an average of 20.7% ($p < 0.05$).

A significant difference in the ratio of the sperm head length to its width was registered in the ejaculate samples of boars from the second group that were 180 and 360 days of age. The difference represents 7.3-11.2% in their favor, as compared with that of the control herd mates ($p < 0.05$).

Table 3: Morphological indicators of boars' sperm cells.

Groups	1		2	
Age (days)	180	360	180	360
Acrosome anomalies (inflated acrosome), % of ill-defined cells	13.64	12.50	–	–
Flagellum middle part deviations and a droplet on the flagellum, % of ill-defined cells	9.09	12.50	13.33	–
The loop form of the flagellum end, % of ill-defined cells	31.82	31.25	26.67	36.36
An uneven contour of the middle part, % of ill-defined cells	18.18	18.75	26.67	27.27
With a cytoplasmic droplet on the flagellum, % of ill-defined cells	31.82	31.25	26.67	36.36
The number of morphologically normal sperms, %	89.00	92.00	92.50	94.50
TZI	1.82	1.75	1.47	1.27

The data in Table 3 indicate that the most encountered morphological violations of boars sperm cells when growing them in the southern subzone of the Chuvash Republic are the loop form of the flagellum end, an uneven contour of the middle part, and a cytoplasmic droplet on the flagellum.

It was noticed that the sperm with acrosome anomaly (inflated acrosome) was not detected in the ejaculate of the second group boars aged 180 and 360 days.

The study revealed that the number of germ cells with such a disorder as a combination of the flagellum middle part anomalies with a droplet on the flagellum in the boars' aged 180 and 360 days of the control group ejaculate samples increased, respectively, by 27.3%, and in samples of the second group animals, it conversely decreased from 13.33% to the total absence.

TZI decreased with animals while aging from 180 to 360 days in the control group by 3.8% and in the second group of boars by 13.6% ($p < 0.05$).

Discussion

Thus, it was determined that in the southern subzone of the Southeast (the black earth) type agro landscape of the Chuvash Republic the use of the biologics under study with boars had a positive impact on their andrological status. Previously, we had obtained similar data for the positive impact of Permamik together with Sedimin® on semen, sperm biology, and micromorphology of Landrace breeding boars in the northern subzone of the Chuvashia Volga region [11].

Numerous factors affect the quality of sperm. By learning how the biogenics used in livestock can affect sperm morphology, we can provide optimal livestock reproduction of production animals.

At puberty period, sperm production, and the feeding requirements (usefulness, nutritious, balanced number of micro and macroelements, and vitamins, etc.) have increased.

The bionics under study contribute to increasing the appetite, food digestion, improving the immune, and maintaining the hormonal status due to the balanced macro and micronutrients in the biogenic compounds that, as the researchers note, is important for the reproductive system and the reproductive function formation at the early postnatal ontogenesis [2,3,12-16].

In our opinion, the improvement of the ejaculate quality characteristics and the boars' sperm morphological parameters is related to the zeolite additives sanitizing effect on animal organisms that agree with the data obtained by other researchers [17,18]. Also, the introduction of Sedimin® while feeding with zeolite-containing Permamik enhances the appetite, improves feed efficiency and increases the adaptive capabilities of animals due to the complex macro and micronutrients in the test bionics. Macro and micronutrients in bionics provide restocking of elements (Zn, Fe, Co, I, and Se) extremely necessary for sperm production, increasing the safety of sperm when frozen, maintaining steady organism hormonal environment, and the concentration of testosterone in particular that is important at puberty and maintaining sexual function in general [16,19-22].

Conclusion

The analysis of the experimental boars ejaculate showed that its volume, sperm concentration, and activity were higher on average by 62.6% ($p < 0.05$) in the animals kept on basic feeding with the use of bionics when compared with the control herd mates. The percentage of ill-defined cells and the teratozoospermic index in the control boars' studied biomaterial was higher in all series of the studies than that of the herd mates kept on basic feeding with the use of Permamik with Sedimin®. These parameters superiority reached 37% ($p < 0.05$), which indicates the boars' reproductive function increase.

The agro landscape zoning had been made in Chuvashia in order to start the adaptive-landscape systems of agricultural production, according to which eleven agricultural landscapes were allocated. Prospects for further development of the study are in the necessity for studying the impact of biogenics under study in various combinations and doses on the breeding boars in all agro landscape zones in the Chuvash Republic.

Acknowledgement

This work was financially supported by the Ministry of Education and Science of the Russian Federation within the framework of the State assignment for the provision of services.

References

1. Peshkumov OA (2010) The morphophysiological status of the boars influenced by a new generation of biologics supplementation, Abstract from thesis of Candidate of Biological Sciences, Chuvash I. Yakovlev State Pedagogical University, Cheboksary, p. 22.

2. Kipyegon AN, Mutembei HM, Tsuma VT, Oduma JA (2012) Effects of Ripe Carica Papaya Seed Powder on Testicular Histology of Boars. International Journal of Veterinary Science 1(1): 1-4.

3. López A, Rijsselaere T, Van Soom A, Leroy JL, De Clercq JB, *et al.* (2010) Effect of organic selenium in the diet on sperm quality of boars. Reproduction in Domestic Animals 45(6): e297-e305.

4. Polen T (2011) The Effect of Some Forage Additives Used in Feeding of Weaned Pigs. Scientific Papers: Animal Science and Biotechnologies 44(1): 88-90.

5. Speight SM, Estienne MJ, Harper AF, Barb CR, Pringle TD (2012) Effects of organic selenium supplementation on growth performance, carcass measurements, tissue selenium concentrations, characteristics of reproductive organs, and testis gene expression profiles in boars. Journal of Animal Science 90(2): 533-542. Date Views: 09.01.2014 www.journalofanimalscience.org/content/90/2/533.full.

6. Speight SM, Estienne MJ, Harper AF, Crawford RJ, Knight JW, *et al.* (2012) Effects of dietary supplementation with an organic source of selenium on characteristics of semen quality and *in vitro* fertility in boars. Journal of Animal Science 90(3): 761-770. Date Views: 09.01.2014 www.journalofanimalscience.org/content/90/3/761.full.

7. Kuznetsov AI (1996) Pigs physiological immaturity: factors contributing to its occurrence, the course and manifestations of the organism most important functions, methods of prevention and correction, Abstract from thesis of PhD of Biological Sciences, University of Belgrad, Belgrade, p. 41.

8. Eiby YA, Wright LL, Kalanjati VP, Miller SM, Bjorkman ST, *et al.* (2013) A Pig Model of the Preterm Neonate: Anthropometric and Physiological Characteristics. PLoS One 8(7): e68763. doi:10.1371/journal.pone.0068763

9. Kalashnikov AP, Fisinin VI, Shcheglova VV, Kleimenov NI (2003) Norms and Feeding Diets of Farm Animals: A Handbook. Moscow: Knowledge, p. 456.

10. Berestneva OG, Marukhina OV, Shevelev GE (2012) Applied Mathematical Statistics. Tomsk: Tomsk Polytechnic University publishing house, p. 200.

11. Arestova IYu, Alekseev VV (2013) Morphological analysis of the sperms of breeding boars maintained on nutritional supplements. Global Veterinaria 11(1): 84-87.

12. Dr. Unni Krishnan R (2014) Influence of Nutrition on Livestock Reproduction. Date Views: 30.01.2014 www.en.engormix.com/MA-dairy-cattle/genetic/articles/influence-nutrition-livestock-reproduction-t3101/103-p0.htm.

13. Karvelis G, Nuevo SA (2013) Feeding boars for optimal reproductive results. Date Views: 30.01.2014www.wattagnet.com/Feeding_boars_for_optimal_reproductive_results.html.

14. Kolodziej A, Jacyno E (2005) Effect of selenium and vitamin E supplementation on reproductive performance of young boars. Archiv Tierzucht 48: 68-75.

15. Kozink DM, Estienne MJ, Harper AF, Knight JW (2004) Effects of dietary L-carnitine supplementation on semen characteristics in boars. Theriogenology 61(7-8): 1247-1258.

16. Marzec-Wróblewska U, Kamiński P, Łakota P (2012) Influence of chemical elements on mammalian spermatozoa. Folia Biologica (Praha) 58(1): 7-15.

17. Tiwari J (2007) Zeolite as natural feed additives to reduce environmental impacts of swine manure. Department of Bioresource Engineering McGill University, Montreal. Date Views: 30.04.2013 www.webpages.mcgill.ca/staff/deptshare/FAES/066-Bioresource/Theses/theses/363JagannathTiwari2007/363JagannathTiwari2007.pdf

18. Yannakopoulos A, Tserveni-Gousi A, Kassoli-Fournaraki A, Tsirambides A, Michailidis K, *et al.* (2000) Effects of dietary clinoptilolite-rich tuff on the performance of growing-finishing pigs. In: Natural Zeolites for the third Millennium, Colella C, Mumpton FA (Eds.). Napoli, Italy: De Frede Editore, pp: 471-481.

19. Akinloye O, Arowojolu AO, Shittu OB, Adejuwon CA, Osotimehin B (2005) Selenium status of idiopathic infertile Nigerian males. Biological Trace Element Research 104(1): 9-18.

20. Dimitrov SG, Atanasov VK, Surai PF, Denev SA (2007) Effect of organic selenium on Turkey semen quality during liquid storage. Animal Reproduction Science 100(3-4): 311-317.

21. Wilson ME, Rozeboom KJ, Crenshaw TD (2004) Boar Nutrition for Optimum Sperm Production. Advances in Pork Production 15: 295-306.

22. Mogielnicka-Brzozowska M, Wysocki P, Strzežek J, Kordan W (2011) Zinc-binding proteins from boar seminal plasma – isolation, biochemical characteristics and influence on spermatozoa stored at 4°C. Acta Biochimica Polonica 58(2): 171-177. Date Views: 20.06.2013 www.actabp.pl.

23. Ramamoorthi RV, Rossano MG, Paneth N, Gardiner JC, Diamond MP, *et al.* (2008) An application of multivariate ranks to assess effects from combining factors: metal exposures and semen analysis outcomes. Statistics in Medicine 27(18): 3503-3514.

Citation: Arestova IY, Alekseev VV (2014) Boar semen cytomorphology features after intramuscular injections of sedimin® and subsequent diet fortification by a zeolite-containing product. Biol Med 6(1): BM-003-14.