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## PROOXIDANT-ANTIOXIDANT HOMEOSTASIS IN THE SPERM OF FERTILE BOARS DEPENDS ON THE INFLUENCE OF DIFFERENT FACTORS

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The purpose of the study was to establish the peculiarities of the formation of pro-oxidant-antioxidant homeostasis in sperm and seminal plasma of breeding boars depending on the season and the intensity of their use. 10 adult breeding boars of a large white breed aged from 18 to 36 months were selected for the experiment – analogues in terms of the quality of sperm production. Breeding boars were fed according to the norms of the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Agrarian Sciences of Ukraine. It has been established that the best indices of sperm production characterize animals in the spring period. In the summer period, the quality of sperm in breeding boars probably decreases. Such changes are accompanied by a significant decrease in catalase activity by 30.6 % ( $p < 0.01$ ) and a significant accumulation of the TBC-active complexes' content by 46.9 % ( $p < 0.01$ ). The quality of sperm production of breeding boars is significantly dependent on the mode of their use. It was found that increasing the intensity of using boars twice a week does not contribute to a significant acceleration of peroxidation processes.

**Key words:** sperm, boars, sperm production, peroxide oxidation, TBC-active complexes.

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## ПРООКСИДАНТНО-АНТИОКСИДАНТНИЙ ГОМЕОСТАЗ У СПЕРМІ КНУРІВ- ПЛІДНИКІВ ЗАЛЕЖНО ВІД ВПЛИВУ РІЗНИХ ФАКТОРІВ

Метою роботи було встановлення особливостей формування прооксидантно-антиоксидантного гомеостазу в спермі і спермальній плазмі кнурів-плідників залежно від пори року та інтенсивності їх використання. Для дослідження відібрані 10 дорослих кнурів-плідників великої білої породи віком від 18 до 36 місяців – аналогів за якістю спермопродукції. Годівлю кнурів-плідників годували згідно з нормами Інституту свинарства і агропромислового виробництва національної академії аграрних наук України. Встановлено, що найкращими показниками спермопродукції характеризуються тварини у весняний період. У літній період якість сперми у кнурів-плідників вірогідно знижується. Такі зміни супроводжуються істотним зниженням активності каталази на 30.6 % ( $p < 0.01$ ) та суттєвим накопиченням умісту ТБК-активних комплексів на 46.9 % ( $p < 0.01$ ). Якість спермопродукції кнурів-плідників перебуває в істотній залежності від режиму їх використання. Виявлено, що підвищення інтенсивності використання кнурів двічі на тиждень не сприяє суттєвому прискоренню процесів пероксидації.

**Ключові слова:** сперма, кнури, спермопродукція, пероксидне окиснення, ТБК-активні комплекси.

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In the modern pig breeding system, using breeding boars with high breeding value is the most important component of increasing the productivity potential of the herd as a whole [5].

The most intensively highly productive breeding boars are used to produce highly productive offspring [2]. This leads to the search for new approaches in the study of physiological features of the formation of sperm production in these animals [3].

Over the past decades, a significant amount of experimental data has been accumulated on the regulatory action of active forms of Oxygen based on the vital processes of pigs, which are under the dynamic control of pro-oxidant-antioxidant homeostasis [8].

In this connection, there is a need for a comprehensive study of the peculiarities of the formation of pro-oxidant-antioxidant homeostasis in sperm and sperm plasma in breeding boars.

The development of oxidative stress in the body of boars significantly affects their reproductive capacity. It has been proven that a decrease in the antioxidant protection of epidermal cells due to an increased level of active forms of oxygen causes sperm apoptosis and can be among the main factors in reducing their fertilizing ability and impaired ejection function [9, 12], DNA damage – one of the main reasons for the death of zygotes, embryos and offspring.

It was established that the number of TBC-active compounds in human sperm under artificial stimulation of peroxide oxidation increases with a parallel decrease in mobility ( $r=-0.62$ ). Destructive changes in cell membranes cause this. Several researchers believe that motility and pathological changes in spermatozoa are closely related to lipid peroxidation, and the level of TBC-active compounds can become an informative biochemical index of gamete quality [10, 11].

It has been proven that the content of reduced glutathione is in large quantities in the seminal plasma of fertile and infertile animals. At the same time, the level of glutathione and vitamin C is always higher in sperm with a high fertilizing capacity. A significant correlation was established between glutathione content in plasma and sperm motility ( $r=0.5$ ). The introduction of ascorbic acid and glutathione into the composition of bull sperm thinner increases the ability of sperm to fertilize [1, 7]. However, the study of the influence of peroxide oxidation processes on the quantitative and qualitative indicators of sperm production remains relevant.

**The purpose** of the study was to establish the peculiarities of formation of pro-oxidant-antioxidant homeostasis in sperm and sperm plasma of breeding boars depending on the season and the intensity of their use.

**Materials and methods.** The work was carried out in the conditions of the reproductive physiology laboratory of the Institute of Pig Breeding and Agro-Industrial Production of the National Academy of Sciences of Ukraine, the breeding plant for breeding large white pigs of the State Enterprise “Stepne” IS and APV of the National Academy of Sciences in 2020. For the experiment, 10 adult breeding boars of the large white breed aged from 18 to 36 months were selected – analogues in terms of the quality of sperm production, the experiment was carried out 8 times. Breeding boars were fed in accordance with IS and APV norms of the National Academy of Sciences. The main indicators of sperm quality: ejaculate mass, sperm concentration in 1 cm<sup>3</sup> and the total number of sperm in ejaculate, sperm motility and heat-resistant test were determined according to generally accepted methods, according to the Instructions for artificial insemination of pigs.

To assess the level of peroxidation, the following were determined: the concentration of primary products of peroxidation – diene conjugates spectrophotometrically (Gavrilov VB, 1983); secondary products of peroxide oxidation – TBK-active compounds (aldehydes and ketones) – photoelectrocolorimetrically (Kogan VE, 1986).

To assess the level of antioxidant protection in blood and tissue samples, the following were determined: superoxide dismutase activity – photometrically (Brusov OS, 1986); activity of catalase was carried out according to the method using the vanadium-molybdate reaction [1, 6], the content of the reduced form of glutathione – photoelectrocolorimetrically with Elman's reagent (Elman GL, 1959); concentration of ascorbic and dehydroascorbic acids according to the amount of ozone by a modified method (Patent of Ukraine for a utility model No. 67054A).

The obtained digital material was statistically processed using the Statistisa program for Windows XP. After comparing the studied indicators and their intergroup differences, the Student's t-test was used, and the result was considered probable after  $p<0.05$ .

**Results of the study and their discussion.** The obtained data indicate that qualitative and quantitative indicators of sperm production in breeding boars significantly depend on the season (Table 1). The studied animals were characterized by the maximum ejaculate mass in the spring. It is important to note that in the summer and autumn periods, the ejaculate mass was lower by 11.5 % ( $p<0.05$ ) and 10.3 %, respectively, compared to spring. At the same time, with the onset of the winter period, this indicator increases relative to the minimum level in the summer period by 6.2 %.

**The influence of the season on the physiological indices of the quality of sperm production in breeding boars, (M+m), n=80**

Season	Mass of ejaculate, g	Sperm concentration, million / cm <sup>3</sup>	Total number of sperm, billion	Sperm motility, %	Sperm survival, %
Spring	285.28±10.63	210.43±7.29	50.82±1.64	85.06±1.07	75.06±0.98
Summer	252.28±11.38*	153.32±3.53**	40.70±1.92***	78.0±1.58***	65.0±1.85***
Autumn	255.87±11.86	162.56±4.93**	41.66±2.17***	79.93±1.11***	70.75±1.05*
Winter	268.82±12.23	168.06±8.4**	55.51±8.11	83.06±1.01	73.0±1.1

Note \* – p<0.05; – \*\* p<0.01; – \*\*\* p<0.001, compared to the data of the spring period.

The maximum concentration of sperm in the ejaculates of boars is established in the spring. However, we emphasize that in summer and autumn, sperm concentration was lower than in spring, by 1.37 (p<0.001) and 1.3 times (p<0.01), respectively. With the onset of winter, this index increases by 8.7 % compared to the minimum level in the summer period. The maximum number of sperm in ejaculates was characterized by boars in the winter period. This index was lower in the summer and autumn periods compared to the winter period, respectively by 26.67 % (p<0.001) and 24.95 % (p<0.001). With the onset of spring, it increased by 19.9 % compared to the minimum level in the summer period. In the ejaculates of boars, the maximum sperm motility was recorded in spring and winter. However, in summer and autumn, sperm motility decreased by 8.3 and 6.03 % compared to spring. Accordingly, mobility in winter increased by 6.09 % relative to the minimum level in summer.

One of the important indices of sperm quality, which is determined using a heat-resistant test, is the survival of sperm outside the boar's body. It was established that it was highest in boars in the spring. In summer and autumn, sperm survival was lower by 13.4 % (p<0.001) and 5.74 % (p<0.05). With the onset of winter, this index increases compared to the summer period (minimum level) by 11 %.

Experimental data indicate that the quality of sperm production of breeding boars depends on the influence of the season and the intensity of their load. Thus, the regime of one cage of a boar per stuffed animal per week contributes to an increase in the mass of ejaculate. Collecting semen from a boar twice a week reduces ejaculate by 9.75 %, and the three-time regimen by 16.5 % compared to the one-time regimen.

A comparative analysis of sperm concentration data in ejaculates confirms that, relative to the two-time mode of taking sperm from boars, this index is by 10.23 % higher than the one-time mode, and the sperm was more rarefied by 20.93 % in the three-time mode.

A one-time mode of obtaining sperm helps to increase the total number of sperm in the ejaculate. The use of two-fold and three-fold methods of obtaining sperm reduces this index was by 1.2 and 1.5 times, respectively.

A significant influence of the mode of use of boars on sperm motility was also established; the highest activity was observed during a one-time, and probably the lowest – during a three-time collection of sperm. However, sperm survival was recorded as the maximum in the case of sexual activity twice a week, which is 15.9 % (p<0.05) more compared to the intensity of use: once and 22.5 % (p<0.001) than three times a week.

With the change of physiological indices of sperm production during the year, the course of peroxide oxidation processes was carried out differently. The content of beta-pre-beta lipoproteins in boar serum was maximal in summer, and in spring, autumn and winter it decreased by 18.1 %, 42.4 %, and 49.3 %, respectively.

It was established that the highest rate of beta-pre-beta lipoproteins in the sperm plasma was registered in the spring. In the subsequent study periods, the content of these substances significantly decreased by 1.5 times in autumn and 1.7 times in winter compared to the spring period.

The level of superoxide dismutase was minimal in summer, however, with a decrease in temperature, its activity increased by 48.8 % (autumn), 66.1 % (winter), and 68.6 % (spring). In the sperm plasma during the experimental period, the following dynamics were established: maximum activity in the spring with a further decrease in the summer by 5.7 times and in the autumn by 2.09 times, as well as an increase in the level in the winter period. Thus, catalase activity in boar sperm was maximal in spring. It should be noted that the activity of this enzyme was lower in summer by 1.4 times (p<0.001), in autumn by 1.2 times (p<0.001) and in winter by 1.5 times (p<0.001), compared to the spring period. This tendency is also observed in the sperm plasma (Table 2).

Table 2

## System of antioxidant protection in the sperm of breeding boars, (M+m), n=80

Season	Content of beta-pre-beta lipoproteins	Activity of super oxide dismutase	Activity of catalase mM/min of protein	Content of $\mu\text{mol/l}$ of reduced glutathione	Content of ascorbic acid	The content of hydroascorbic acid
<b>Semen</b>						
Spring	6.71±0.34	1.34±0.13	13.72±0.52	0.26±0.03	9.81±0.79	6.32±0.58
Summer	8.2±0.16	0.42±0.05	9.52±0.53	0.25±0.02	5.62±0.26	12.42±0.27
Autumn	4.72±0.18	0.86±0.1	11.34±0.48	0.2±0.05	10.61±0.4	8.05±0.27
Winter	4.15±0.05	1.24±0.1	9.11±0.13	0.40±0.08	8.14±0.55	11.15±0.63
<b>Seminal plasma</b>						
Spring	6.39±0.35	1.29±0.13	10.13±0.07	0.31±0.01	7.75±0.68	6.15±0.68
Summer	5.96±0.27	0.22±0.01	7.4±0.06	0.23±0.003	6.22±0.27	10.94±0.39
Autumn	4.23±0.18	0.62±0.06	8.38±0.08	0.27±0.01	11.32±0.4	7.02±0.38*
Winter	3.8±0.07	1.02±0.01	8.55±0.07	0.44±0.002	10.46±0.5	10.11±0.61

The reduced glutathione content in the examined tissue was the lowest in summer and autumn, and reached the maximum values in winter. The ratio of concentrations of ascorbic acids changed significantly during the year. In the summer and autumn seasons, a predominance of the amount of the oxidized form of ascorbic acid with the reduced form was found.

The content of diene conjugates in sperm during the experimental period fluctuated within insignificant limits, reaching maximum values in winter and spring, and minimum values in autumn.

The content of TBK-active complexes was minimal in spring, being lower than the level established in summer – 46.9 %, in autumn – 15.6 % and in winter – 31.2 %. The intensity of the formation of TBK-active complexes in the sperm of wild boars in the conditions of iron-ascorbate buffer in the summer was higher than in the spring period, by 55.8 %, which indicates the exhaustion of the antioxidant defense system in this tissue.

It was found that the mode of use of breeding boars significantly affects the processes of peroxide oxidation in sperm (Table 2). The content of beta-pre-beta lipoproteins in the serum of boars was maximal when used once a week. The activity of lipoproteins was 1.36 and 1.97 times lower with the two-fold and three-fold mode of use compared to the single mode. It was established that in the sperm plasma the highest indices were with a single mode of use and accordingly fell down. During the experiment, the activity of superoxide dismutase showed the highest values with single use, which was by 36 % more than with two times and by 63 % more than with three times. In the seminal plasma, there was a corresponding decrease in indices, as in the semen. Thus, catalase activity was relatively mean when boar sperm were collected twice a week, but by 1.1 times higher ( $p<0.001$ ), when it was collected once, and by 1.28 times lower ( $p<0.001$ ) when collected three times a week.

The two-time mode of obtaining sperm was accompanied by the maximum saturation of this tannin with the amount of reduced glutathione and ascorbic acids.

Diene conjugates in semen are the highest when used once a week, which is by 1.09 and 1.24 times higher than when used twice and three times. During the experiment, it was established that in the sperm plasma, their number is the largest with a single mode of use, and the smallest with a three-fold mode of use, which indicates exhaustion of the body.

The content of TBK-active complexes was maximum in the mode of use of boars once a week. Semen sampling in two and three times led to a decrease in the content of TBK-active complexes, which is probably due to the dilution of the boars' sperm. Let's emphasize that under the conditions of incubation of this tissue in an iron-ascorbate buffer against the background of an increased mode of sexual use, a decrease in the intensity of accumulation of the TBC-active complexes' content from 29.32 to 20.42  $\mu\text{mol/l}$  is observed. Under the one- and three-time mode of obtaining boar semen, the content of TBK-active complexes increased by 36–38 %.

In order to establish the relationship between the indices of the quality of sperm production and the components of pro-oxidant-antioxidant homeostasis in boar semen, the values of correlation coefficients ( $r$ ) were calculated and compared, depending on the season and mode of their use.

The data of the performed statistical analysis of the correlation between the physiological indices of the sperm production quality in boars of the Large White breed indicate the existence of significant positive relationships in the spring season, that is, between the weight of the ejaculate and the number of sperm ( $r=0.56$ ;  $p<0.001$ ), motility and survival ( $r=0.88$ ;  $p<0.001$ ).

A significant correlation was established between the content of TBC-active complexes and the mass of ejaculate, which was  $r=0.46$  under the condition of a one-time sperm collection's mode. With an increase in the sexual load of breeding boars, the concentration of TBK-active complexes significantly correlated with the number of sperm ( $r=0.30$ ) and their motility ( $r=0.87$ ). This proves that the physiological and biochemical indices of the sperm production's quality are interconnected, and their level of connection mostly depends on the season and the intensity of the load on breeding boars [3, 6].

Therefore, the maintenance of breeding boars in conditions of elevated temperature contributes to the acceleration of peroxidation processes and the depletion of antioxidant protection in sperm plasma and semen. This is accompanied by a decrease in the quantitative and qualitative indices of sperm production. In addition, biochemical and physiological processes of reproduction with wines are noted by Zasiadczyk L, et al. [4, 11], which is additionally confirmed by a violation of the functional under the influence of elevated temperatures.

### Conclusions

1. It was established that, depending on the season of the year, the qualitative and quantitative indicators of sperm production of breeding boars change. Animals in the spring period are characterized by the highest values of sperm production. In the summer, sperm quality in breeding boars probably decreases: ejaculate weight by 11.5 %, sperm concentration by 27.1 %, total sperm count by 26.7 %, sperm motility by 8.3 %, and their survival rate by 13.4 %. Such changes are accompanied by a significant decrease in the activity of catalase, and a significant accumulation of the content of TBC-active complexes on. However, with the onset of the winter period, the biological quality of ejaculates obtained from breeding boars increases.

2. The quality of sperm production of breeding boars is significantly dependent on the mode of their use. It was found that increasing the intensity of use of boars – (two and three times a week) leads to a decrease in the concentration of sperm in the ejaculate, as well as their mobility. However, the survival rate of sperm under the two-time regime of using boars is significantly higher than the one-time and three-time collection of sperm, which is obviously due to the higher capacity of the antioxidant protection system.

Further research will be aimed at developing effective methods of improving the quality of sperm production in breeding boars.

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