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The activity of the antioxidant protection enzymatic system of boars with a decrease in their reproductive capacity under oxidative stress

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Abstract. It was found that enzymatic system activity of the antioxidant protection of the breeding boars organism with a decrease in their reproductive ability under oxidative stress was significantly decreased, which was noted by a decrease in the antioxidant capacity. Males of the experimental group had a decrease in superoxide dismutase activity by 30,2% ($p < 0,01$). At the same time, in the erythrocytes of breeding boars, catalase activity was reduced by 33,9% ($p < 0,001$) compared with the indicators of the control group animals, due to the high sensitivity to nitric oxide, the content of which was significantly increased during oxidative stress; interacting with nitric oxide, a suppressed form of catalase – ferricatalase-NO was formed. A decrease in the activity of these enzymes is characteristic of the oxidative stress development – which is due to the high level of peroxidation processes in the body of breeding boars. With a decrease in the activity of glutathione system components, an imbalance of the prooxidant-antioxidant system was noted. According to the research results, a decrease in the activity of the glutathione redox cycle enzymes – glutathione peroxidase and glutathione reductase was noted, which in boars with a decrease of reproductive ability were also reduced, and this also confirmed the state of oxidative stress. Thus, boars of the experimental group had a pronounced decrease in glutathione peroxidase activity by 28,4% ($p < 0,05$), while the activity of glutathione reductase in erythrocytes was significantly reduced by 20,2% ($p < 0,05$). The lack of physiological activity of antioxidant enzymes will have a negative impact on the fertilizing ability of sperm, because the lack of components of the enzymatic antioxidant defence system affects the activity of enzymes in sperm received from the breeding boars. A balance was observed between the components of the enzymatic antioxidant defense: the ratio of antioxidant enzymes' activity in the male experimental group was lower than the values in the control group, however, the indices of superoxide dismutase/catalase and glutathione peroxidase/glutathione reductase had the same values, indicating that the balance in the enzymatic system of antioxidant defense was maintained. A decrease in enzyme indices in the experimental group indicated a decrease in the antioxidant potential and confirmed a certain state of oxidative stress in the breeding boars organism.

Keywords: prooxidant-antioxidant system; superoxide dismutase; catalase; glutathione peroxidase; glutathione reductase

Активність ензиматичної системи антиоксидантного захисту кнурів зі зниженням репродуктивної здатності за оксидативного стресу

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Анотація. Встановлено, що активність ензиматичної системи антиоксидантного захисту організму кнурів-плідників зі зниженням репродуктивної здатності за оксидативного стресу вірогідно зменшується, що позначається зниженням антиоксидантного потенціалу. У самців дослідної групи виявлено вірогідне зниження супероксиддисмутазної активності на 30,2 % ($p < 0,01$). При цьому в еритроцитах кнурів-плідників каталазна активність була зниженою на 33,9% ($p < 0,001$) порівняно з показниками контрольної групи тварин, що зумовлено високою чутливістю до оксиду нітрогену, вміст якого за оксидативному стресу значно зростає; у взаємодії з оксидом нітрогену утворюється пригнічена форма каталази – ферікаталаза-NO. Зниження активності цих ензимів характерне для розвитку оксидативного стресу – це зумовлено високим рівнем процесів пероксидації в організмі кнурів-плідників. У разі зниження активності компонентів глутатіонової системи відмічають дисбаланс прооксидантно-антиоксидантної системи. За результатами досліджень відмічено зниження активності ензимів глутатіонового редокс-циклу – глутатіонпероксидази і глутатіонредуктази, що у самців зі зниженням репродуктивної здатності також були зниженими – це теж підтверджує стан оксидативного стресу. Так, кнури дослідної групи мали виражене зниження глутатіонпероксидазної активності на 28,4% ($p < 0,05$), тоді як активність глутатіонредуктази в еритроцитах була вірогідно зниженою на 20,2% ($p < 0,05$). Відсутність фізіологічної активності антиоксидантних ензимів буде чинити негативний вплив на запліднювальну здатність сперми, адже нестача компонентів ензиматичної системи антиоксидантного захисту впливає на активність ензимів у спермі, яку отримують від плідників. Спостерігали баланс між компонентами ензиматичного антиоксидантного захисту: співвідношення активності антиоксидантних ензимів у дослідній групі самців була нижчою за показники контролю, проте індекси супероксиддисмутази / каталаза і глутатіонпероксидаза / глутатіонредуктаза мали однакові величини, що свідчить про збере-

ження балансу в ензиматичній системі антиоксидантного захисту. Зменшення індексів ензимів у дослідній групі свідчить про зниження антиоксидантного потенціалу і підтверджує визначений стан оксидативного стресу організму кнурів-плідників.

Ключові слова: прооксидантно-антиоксидантна система; супероксиддисмутаза; каталаза; глутатіонпероксидаза; глутатіонредуктаза

Introduction

The main cause of hypofertility in males is oxidative stress (OS), which occurs as a result of increased synthesis of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Ko et al., 2014; Agarwal et al., 2018; Barik et al., 2019; Ritchie & Co., 2020). The problem of OS in the development of various pathological conditions has not lost its relevance.

It was found that in breeding boars a decrease in reproductive capacity (RC) is characterized by deterioration of sperm quality especially sperm motility and the motile sperm count in the ejaculate and is accompanied by the development of OS due to accumulation of lipid peroxidation (LPO) products and increased content of stable metabolites (Koshevoy & Naumenko, 2020).

In animals toxic products of peroxidation are opposed by the antioxidant defence system (ADS). This complex biochemical system consists of non-enzymatic and enzymatic units, each of which is multicomponent and has a variety of biological effects. The main enzymes of ADS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-Rd) (Mukherjee et al., 2014; Owoade et al., 2019).

Total antioxidant capacity is one of the indicators of the body's ability to resist the action of ROS and is used as a diagnostic criterion in the diagnosis of male hypofertility (Sharma et al., 1999; Rouchoudhury et al., 2016). However, to assess the state of ADS it is necessary to determine the content of its individual components. ADS enzymes exert their action through various biochemical mechanisms: SOD catalyzes the neutralization reactions of superoxide radicals, CAT detoxifies hydrogen peroxide, and GSH-Px destroys and inactivates hydrogen peroxide and peroxide radicals – the main toxic ROS; GSH-Rd catalyzes the reversible redox reaction of oxidized glutathione, which is one of the main components of the non-enzymatic system of ADS. Decreased activity of these enzymes leads to the impossibility of an adequate response of the body to the action of ROS and RNS (Bachawat & Yadav, 2018).

Boars and their sperm are vulnerable to biological and technological factors (Jezek et al., 2011; Gadella, 2017). Sperm is damaged by the use of reproductive technologies, which is manifested by an imbalance in the antioxidant system (Subramanian et al., 2018). There is data concerning the activity of the antioxidant system of boars in the literature (Koziorowska-Gilun et al., 2011; Shostya et al., 2020), but a comprehensive study that reflects the activity of antioxidant enzymes in breeding boars with decreased RC under the influence of OS was not carried out.

The aim of the research was to find out the superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activity as components of the enzymatic system of antioxidant protection of the organism of boars with decreased reproductive capacity under the influence of oxidative stress.

Material and methods

The research was conducted on boars in Vlada agricultural farm (Yuriyivsky district, Dnipropetrovsk region). They were divided into two groups – control (n = 5) and experimental (n = 5) one and were selected taking into account sperm quality indicators and the content of OS markers in blood serum. The animals of both groups were 4-6 years old, with a live weight 180-200 kg and kept on a standard diet and had free access to water. In boars of the control group, all the determined parameters of the ejaculate quality were in

reference range. In the experimental group of animals (experiment) sperm quality was reduced, especially in terms of sperm motility and the motile sperm count in the ejaculate with a significantly higher content of OS markers in males blood serum (Koshevoy & Naumenko, 2020). Blood samples for biochemical studies were taken after ejaculate was obtained.

In boars' red blood cell hemolysate the following parameters were spectrophotometrically determined: SOD (EC 1.15.1.1) activity by the degree of inhibition of the reaction by the enzyme to reduce nitrobluetetrazolium in the presence of NADH and phenazinemethosulfate (Dubinina et al., 1990); CAT (EC 1.11.1.6) activity on the ability of hydrogen peroxide to form a stable complex with ammonium molybdate, the colour intensity of which was measured at $\lambda = 410$ nm (Korolyuk et al., 1988); GSH-Px (EC 1.11.1.9) activity in the rate of oxidation of the reduced glutathione in the presence of tertiary butyl hydroperoxide in the colour reaction with 5,5-dithiobis-2-nitrobenzoic acid and measurement at a wavelength of 412 nm (Moin, 1986); GSH-Rd activity (EC 1.6.4.2) by reducing the content of NADFN at 37 °C for 1 min at $\lambda = 340$ nm (Carlberg & Mannervik, 1985).

Statistical processing of experimental results to determine biometric indicators (average values and their errors, comparison of average values by Student's criterion) was performed using *Microsoft Excel*.

After statistical processing of research mathematical data processing to determine the balance of the ADS enzyme system activity and to derive the ratios of indicators and indexes of enzyme activity was carried out. The ratios for SOD, CAT and GSH-Px were calculated by rounding numbers, the obtained values were divided by 10. To express the ratio of GSH-Rd the data obtained by rounding numbers to tenths was used. SOD/CAT indexes were counted by summing the data by groups, dividing by 10 and rounding to integers. GSH-Px/GSH-Rd indexes were determined by summing the rounded data by groups and dividing by 3. The results of the calculation of indexes were expressed in relative units (rel. units).

Results

The conducted studies of ADS enzyme activity in boars with decreased reproductive capacity under the influence of oxidative stress indicate the presence of significant changes in antioxidant protection, which confirms the development of OS.

In males a decrease in the activity of antioxidant enzymes that counteract radical cell damage during the first stage of ADS was determined. Data on the activity of SOD and CAT in groups of boars are shown in Fig. 1.

In males of the experimental group a decrease in superoxide dismutase activity to $8,75 \pm 0,4$ rel.units/mgHb (by 30,2%, $p < 0,01$) was found. In erythrocytes of breeding boars the catalase activity was reduced to $19,84 \pm 0,44$ $\mu\text{mol}/\text{H}_2\text{O}_2/\text{l-min}$ (by 33,9%, $p < 0,001$) compared to the control group of animals. The decrease in the activity of these enzymes is characteristic for the development of OS – this is due to the high level of peroxidation processes in the body of breeding boars.

Indicators of the enzyme activity of glutathione link of ADS enzyme activity in groups of males are shown in Fig. 2.

In case of the decrease in the activity of components of glutathione system the imbalance in prooxidant-antioxidant system is indicated. The activity of glutathione enzymes – GSH-Px and GSH-Rd in males with decreased RC under the influence of OS were also reduced. Thus, the boars of the experimental group

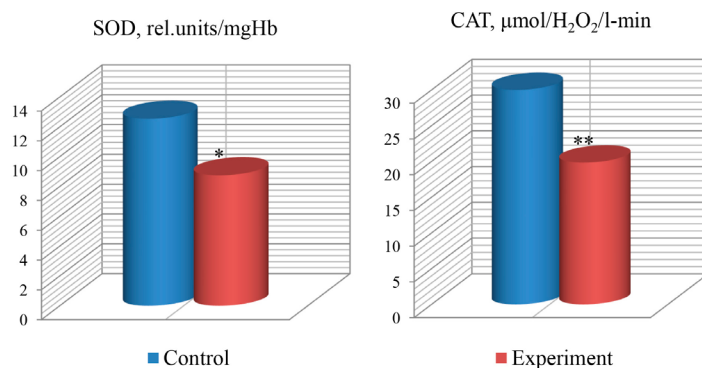


Fig. 1. Superoxide dismutase and catalase activity in breeding boars with decreased RC under the influence of OS (M ± m, n = 5); Notes. In fig. 1 * – p < 0,01; ** – p < 0,001 statistically reliable data compared to the control

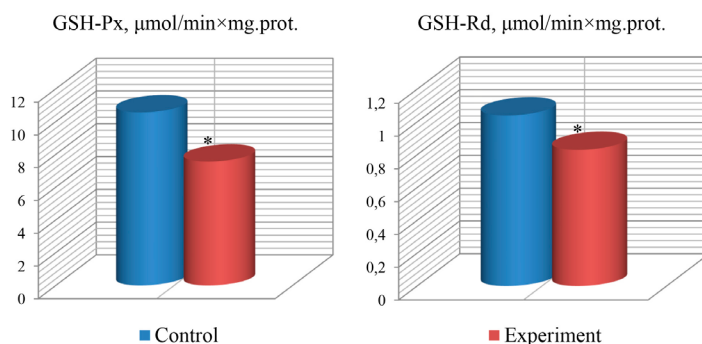


Fig. 2. Glutathione peroxidase and glutathione reductase activity in the body of boars with decreased RC under the influence of OS (M ± m, n = 5); Note: In fig. 2 * – p < 0,05 statistically reliable data compared to the control

had a pronounced decrease in glutathione peroxidase activity to $7,56 \pm 0,58 \mu\text{mol}/\text{min} \times \text{mg.prot.}$ (by 28,4%, $p < 0,05$), while the activity of glutathione reductase in erythrocytes was reduced to $0,83 \pm 0,04 \mu\text{mol}/\text{min} \times \text{mg.prot.}$ (by 20,2%, $p < 0,05$) compared to the control group.

The calculated data concerning the activity balance of ADS enzyme system in males is given in Table.

According to the table, the ratio of ADS enzyme activity in the experimental group of males was lower than the control indicators, however SOD/CAT and GSH-Px/GSH-Rd indexes had the same values. This indicates the preservation of balance in ADS enzyme system. The decrease in the indexes of enzymes in the experimental group indicates a decrease in the AO potential and confirms a determined state of oxidative stress in breeding boars.

Discussion

The decrease in the activity of ADS enzyme system differs from the control group that indicates the lack of LPO compensation. The

Table. The ratio of the ADS enzyme activity and their indexes in breeding boars with decreased RC under OS

Indicators	Groups of animals	
	Control	Experiment
The ratio of SOD activity	1,3 : 0,9	
The ratio of CAT activity	3 : 2	
SOD/CAT Index, rel. units	4	3
The ratio of GSH-Px activity	1,1 : 0,8	
The ratio of GSH-Rd activity	1 : 0,8	
Index GSH-Px/GSH-Rd, rel.units	4	3

obtained data complies with the data of other researchers. Thus, the decrease in the activity of antioxidant enzymes occurs under the influence of heat stress (Ali, 2016). This is marked in the body of boars by a decrease in the activity of SOD and CAT (Shostya et al., 2020). The decrease in CAT activity occurs due to the high sensitivity to NO, the content of which increases significantly under the influence of OS (El-Baky & Hafez, 2017). It is known that SOD provides catalase with a substrate – hydrogen peroxide, however the activity of both enzymes was significantly reduced. Interacting with NO a suppressed form of CAT-ferricatalase-NO is formed (Kim & Han, 2000). Anti-peroxide and anti-radical protection of cells is facilitated by complex glutathione system, which includes GP and GR enzymes, as well as reduced glutathione. They are inextricably linked and form a complex inhibiting effect on free radical oxidation (Honchar & Mankovska, 2007; Bachhawat & Yada). According to the results of research, the decrease in the activity of enzymes of the glutathione redox cycle was indicated that also confirmed the state of oxidative stress. Our results coincide with the use of antioxidant enzymes in the liver of wild boars under the influences of OS against the background of selenium deficiency occurred during the winter (Jankowiak et al., 2015). Similar results of the research of a decrease in the activity of antioxidant enzymes under OS in boars in the testes and epididymis and increasing their values after correction (Tang et al., 2019). As a result of calculating the ratios of enzymes in groups of animals the decompensatory nature of lipoperoxidation was determined. The SOD/CAT and GSH-Px/GSH-Rd indexes prove the preservation of balance in the antioxidant system. In case of decreased reproductive capacity under the influence of oxidative stress deterioration of sperm quality, in particular sperm motility and the number of motile sperm in ejaculate, as a consequence of toxic effects of peroxidation products and increased content of stable metabolites of the nitrogen oxide cycle (Koshevoy & Naumenko, 2020) were observed. In this case, the lack of physiological activity of antioxidant enzymes will have a negative impact on the fertility

of sperm, as the lack of components of ADS enzyme system affects the activity of enzymes in sperm received from the breeding boars. Therefore, the obtained data states the impossibility of an adequate response of the male body to the action of toxic LPO products and the need to correct this condition.

Conclusions

The activity of the enzymatic system of antioxidant protection in breeding boars with decreased reproductive capacity under the influence of oxidative stress significantly reduces. This is marked by a decrease in antioxidant potential, in particular, a decrease in superoxide dismutase activity by 30,2%, catalase activity by 33,9%, glutathione peroxidase activity by 28,4% and glutathione reductase activity by 20,2%, while the indexes of enzyme activity indicate a balance between the components of the enzymatic ADS. The prospect for further research is the development of means for the correction of decreased reproductive capacity under the influence of oxidative stress, taking into account the peculiarities of reducing the activity of antioxidant enzymes.

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