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Physiological features of the antioxidant system of the *Equus caballus* organism of different genotypes

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Abstract. In modern physiology, the features of the functional state of the body are increasingly being studied not in terms of breeds, age, and animal husbandry technologies, but in terms of DNA markers. Therefore, in this aspect, it is interesting to compare the functional state of the antioxidant system of the *Equus caballus* organism depending on the breed and DNA markers. The purpose of this study was to conduct a comparative analysis of the functional state of the antioxidant system of *Equus caballus* horses of different breeds and with different genotypes according to the GRM8 gene. The study of the physiological characteristics of the antioxidant system of the body of *Equus caballus* of Russian breeding was carried out from 2005 to 2023. The study involved 70 breeding stallions belonging to 9 breeds. The best superoxide dismutase activity was detected in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550: 11.4 and 2.3% more ($p < 0.05$) than in carriers of the CT/AG and TT/GG genotypes, respectively. Catalase (CAT) activity was at a comparable level but with significant differences. We observed the highest CAT activity in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550, which is 1.8% more in carriers of the CT/AG genotype and 7.9% ($p < 0.05$) more in carriers of the TT/GG genotype. Against this background, the activity of glutathione peroxidase (GPx) was the highest in the blood of stallions carrying the CT/AG genotype according to SNPrs395286150/394524550, which is 9.8% more ($p < 0.05$) in stallions of the CC/AA genotype and 14.8%

more ($p < 0.01$) in stallions of the TT/GG genotype. The amount of malondialdehyde (MDA) was highest in carriers of the GG genotype according to SNPrs1147388106, which is 13.4% more ($p < 0.01$) in carriers of the GA genotype and 2.6% more in carriers of the AA genotype according to SNPrs1147388106. The study of the functional state of the antioxidant system of the horse body, depending on the breed, also shows the presence of significant differences between breeds of different uses.

Keywords: superoxide dismutase, catalase, glutathione peroxidase, stallion, different breeds

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Физиологические особенности антиоксидантной системы организма *Equus caballus* разных генотипов

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Аннотация. В современной физиологии особенности функционального состояния организма все чаще изучают не в разрезе пород, возраста, технологий содержания животных, а ДНК-маркеров. Цель исследования — сравнительный анализ функционального состояния антиоксидантной системы организма лошадей *Equus caballus* разных пород и с различными генотипами по гену GRM8. Исследование физиологических особенностей антиоксидантной системы организма *Equus caballus* российской селекции проводилось с 2005 по 2023 г. В исследовании приняли участие 70 племенных жеребцов, принадлежащих к 9 породам. Наилучшая активность супероксиддисмутазы выявлена в крови жеребцов с генотипом CC/AA по SNPrs395286150/394524550: на 11,4 и 2,3 % больше ($p < 0,05$), чем у носителей генотипов CT/AG и TT/GG соответственно. Активность каталазы (КАТ) была на сопоставимом уровне, но со значительными различиями. Наибольшую активность КАТ мы наблюдали в крови жеребцов с генотипом CC/AA по SNPrs395286150/394524550, что на 1,8 и 7,9 % ($p < 0,05$) выше, чем у носителей генотипов CT/AG и TT/GG. На этом фоне активность глутатионпероксидазы была самой высокой в сперме жеребцов, несущих генотип CT/AG согласно SNPrs395286150/394524550, что на 9,8 ($p < 0,05$) и 14,8 ($p < 0,01$) % больше, чем соответственно у жеребцов генотипов CC/AA и TT/GG. Количество малонового диальдегида (MDA) было самым высоким у носителей генотипа GG согласно SNPrs1147388106, что на 13,4 ($p < 0,01$) 2,6 % больше, чем у носителей генотипов GA AA соответственно согласно SNPrs1147388106. Изучение функционального состояния антиоксидантной системы организма лошади в зависимости от породы также показывает наличие существенных различий между породами различного назначения.

Ключевые слова: супероксиддисмутаза, каталаза, глутатионпериксидаза, жеребец, разные породы

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Introduction

In modern physiology, the features of the functional state of the body are increasingly studied not in terms of breeds, age, and animal husbandry technologies, but in terms of DNA markers [1–3]. Therefore, in this aspect, it is interesting to compare the functional state of the antioxidant status of the *Equus caballus* organism depending on the breed and DNA markers [4–6]. Based on this approach, many researchers have successfully studied various physiological functions based on DNA markers in most countries of the world [7, 8]. However, it should be noted that a lot of physiological functions are very poorly transmitted from parents to offspring and therefore the classical approach to studying the breed factor is outdated [9]. Of particular interest to researchers is the study of gene expression during their interaction in different conditions [10], therefore, it is of interest to study the antioxidant system of the body of the same livestock, but in the context of different factors: breed and DNA marker. In our previous studies, we studied the association of the antigenic profile of erythrocytes with the physiological parameters of the reproductive system of horses [2–3]. Therefore, in this study, we want to compare the genetic factor of the breed with the functional state of the antioxidant system of the horse's body and show the advantages and disadvantages of the classical and modern approaches to assessing the influence of the genotype on the physiological functions of the body. In the available literature, we could not find publications on the features of the antioxidant system of the equine body depending on DNA markers.

However, there are a very large number of publications on the effect of DNA markers on a wide variety of physiological functions [4, 5], on animal disease resistance depending on the DNA marker [6], on the functional state of the reproductive system of the body [7, 8], to study the heritability of physiological functions [9], to study the interaction of different genes with each other and their physiological manifestation [10]. The most relevant research in this direction is the study of the association of physiological functions depending on GWAS (Genome-Wide Association Studies) to accelerate the breeding process with breeds [11].

The least studied genetic DNA markers in horses are the genes of the GRM family and their relationship to the physiological functions of the body. In humans and laboratory animals, the GRM8 gene is the most promising for research, according to which it was possible to find certain physiological features of the reproductive system [12], however, this gene has not been studied in horses due to the antioxidant system of the body. Some researchers have proposed using the genes of the GRM family as markers of human reproductive function [13, 14]. Based on this study, we decided to study the issue of the physiological association of this gene with the functional state of the antioxidant system of the *Equus caballus* organism.

The aim of this study was to conduct a comparative analysis of the functional state of the antioxidant system of *Equus caballus* horses of different breeds and with different genotypes according to the GRM8 gene.

Materials and methods

Animals and blood samples. Blood samples of *Equus caballus* were obtained from horses in compliance with the requirements of asepsis and antiseptics from the jugular vein into sterile disposable test tubes. The stallions were clinically healthy between the ages of 3 and 18. The study involved 70 breeding *Equus caballus* belonging to 9 breeds (Arab — 6 heads, thoroughbred horse — 8 heads, Ukrainian horse — 9 heads, Hanoverian — 6 heads, Belgian — 6 heads, Trakehnen — 7 heads, Westphalian — 8 heads, Russian (prize) Trotter — 10 heads and Orlov Trotter — 10 heads).

Evaluation of antioxidant system. After receiving blood samples in the laboratory, the standard parameters of the body's antioxidant system were evaluated, which are most widely used in practice in Russia: glutathione peroxidase activity, catalase activity, superoxide dismutase activity, the level of end products of lipid peroxidation of diene conjugates and malondialdehyde. The activity of antioxidant defense enzymes was measured by spectrophotometric method. Superoxide dismutase (SOD) activity was determined in U/ml; catalase (CAT) activity in U/ml, glutathione peroxidase (GPx) activity in mU/ml. The amount of end products of lipid peroxidation diene conjugates (DC) and malondialdehyde (MDA) was determined in nmol/ml.

DNA research. DNA for genetic analysis was isolated in 2022—2023 in the certified laboratory of genetics Cherkizovo LAB from blood samples of *Equus caballus* using the phenolic-chloroform method using mercaptoethanol [15]. The following primers were used to analyze the genotype of stallions by the GRM8 gene:

F: 5′-GGTCGATCGCATAAAGCATGG-3′

R: 5′-TTCTGCTACAGGGCTGACTTC-3′.

In the GRM8 gene, SNP, GA and GG, CC/AA, CT/AG, TT/GG were determined. Sanger sequencing was performed in accordance with the "Sanger Sequencing Guidelines at RAMAC".

Statistical analysis. Statistical analysis of the obtained physiological data of *Equus caballus* blood samples was carried out according to the student's t-criterion using a specialized application package SPSS for Windows (IBM, USA) in the context of

SNP rs395286150, SNP rs394524550, SNP rs1147388106. A p-value less than 0.05 is considered significant.

Results and discussion

The obtained results of the analysis of the physiological characteristics of the antioxidant system of the body of *Equus caballus* of Russian breeding according to the preface of the breed are presented in Table 1.

Table 1

Physiological features of the body's antioxidant system *Equus caballus* of different breeds *M ± m*

Breeds (70 heads)	Antioxidant enzymes			End products of lipid peroxidation	
	Superoxide dismutase (SOD), U/ml	Catalase (CAT), U/ml	Glutathione peroxidase (GPx), mU/ml	Diene conjugates (DC), nmol/ml	Malondialdehyde (MDA), nmol/ml
Arab (6 heads)	9.42 ± 0.8	22.02 ± 0.44	1.73 ± 0.2	12.21 ± 0.8	3.51 ± 0.4
Belgian (6 heads)	10.56 ± 0.9*	26.36 ± 0.33	1.95 ± 0.18*	8.85 ± 0.6***	2.15 ± 0.3*
Westphalian (8 heads)	9.48 ± 0.7	33.56 ± 0.19***	2.01 ± 0.21**	9.96 ± 0.7**	1.86 ± 0.2***
Hanoverian (6 heads)	8.81 ± 0.18	25.01 ± 0.25	1.84 ± 0.19	11.77 ± 0.14	3.31 ± 0.35
Orlov Trotter (10 heads)	11.76 ± 0.15**	32.44 ± 0.27*	1.22 ± 0.31	13.47 ± 0.9*	4.01 ± 0.31*
Russian (prize) Trotter (10 heads)	11.28 ± 0.16**	32.6 ± 0.26*	1.05 ± 0.4***	13.19 ± 0.16*	4.3 ± 0.29*
Trakehnen (7 heads)	9.08 ± 0.13	26.96 ± 0.3	1.75 ± 0.13	11.07 ± 0.1	3.49 ± 0.4
Ukrainian horse (9 heads)	9.38 ± 0.16	23.51 ± 0.36	1.65 ± 0.28	10.48 ± 0.15*	2.74 ± 0.76*
Thoroughbred (8 heads)	8.19 ± 0.61*	21.12 ± 0.17	1.61 ± 0.22	13.11 ± 0.1	2.93 ± 0.23

Note. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison with the Arab breed.

Source: completed by P.K. Amulungu, A.V. Tkachev, O.L. Tkacheva.

The analysis of the data in Table 1 allows us to conclude that the classical approach to assessing the physiological state of the body depending on the breed allows us to see the physiological differences in the functional state of the antioxidant system of the equine body. From the data obtained, we see that the activity of superoxide dismutase is at a comparable level in the Arabian breed, Westphalian stallions, Trakehnen breed, and in the Ukrainian riding breed from 9 to 9.5 U/ml. We observed low superoxide dismutase activity in Thoroughbred and Hanoverian breeds from 8 to 9 U/ml. The minimum activity

of this antioxidant protection enzyme was established by us in Thoroughbred stallions, which is 1.19 U/ml less ($p < 0.05$) from Ukrainian horse, 0.89 U/ml less ($p < 0.05$) from Trakehnen breed, 3.09 U/ml less from Russian (prize) Trotter, 3.57 U/mlml less ($p < 0.001$) from Orlov Trotter, 0.62 U/ml less from Hanoverian, 1.29 U/ml less ($p < 0.01$) from Westphalian breed, 2.37 U/ml less ($p < 0.001$) from Belgian breed and 1.23 U/ml less ($p < 0.05$) from the Arab breed. The maximum activity of superoxide dismutase was observed in Orlov Trotter, Russian (prize) Trotter and Belgian breeds — from 10 to 12 U/ml.

The physiological features of catalase activity, depending on the breed, make it possible to group breeds into only two groups. The first group includes breeds with catalase activity from 20 to 30 U/ml: Thoroughbred, Ukrainian horse, Trakehnen, Hanoverian, Belgian and Arab breeds. The second group of breeds by catalase activity (more than 30 U/ml) includes Trotter breeds Orlov Trotter, Russian (prize) Trotter and one riding breed Westphalian. We observed the highest catalase activity in the Westphalian breed, which is 2.39 more from the Ukrainian horse, 5.84 U/ml more ($p < 0.05$) from Trakehnen, 11.48 U/ml more ($p < 0.001$) Russian (prize) Trotter, 11.32 U/ml more ($p < 0.001$) from Orlov Trotter, 3.89 U/ml more from Hanoverian, 12.44 U/ml more ($p < 0.001$) from Westphalian, 5.24 U/ml more ($p < 0.05$) from Belgian and 0.9 U/ml more from Arab breed. The data obtained allow us to conclude that the best functional activity of catalase is observed in Westphalian breeds, Russian (prize) Trotter and Orlov Trotter.

The analysis of the functional level of the antioxidant enzyme glutathione peroxidase (GPx) allows us to group the studied rocks into three groups. The first group includes breeds with the lowest glutathione peroxidase activity up to 1.5 mU/ml: Russian (prize) Trotter and Orlov Trotter. The second group includes breeds with average glutathione peroxidase activity from 1.5 to 1.8 mU/ml: Thoroughbred, Ukrainian horse, Trakehnen, Arab. The third group includes breeds with glutathione peroxidase activity of more than 1.8 mU/ml: Hanoverian, Belgian and Westphalian breeds. We observed the highest activity of glutathione peroxidase in Westphalian stallions, which is 0.06 mU/ml more than Belgian stallions, 0.28 mU/ml more than Arab stallions, 0.17 mU/ml more than Hanoverian, 0.79 mU/ml more ($p < 0.01$) Orlov Trotter, 0.96 mU/ml more ($p < 0.01$) Russian (prize) Trotter, 0.26 mU/ml more Trakehnen, 0.36 mU/ml more ($p < 0.05$) Ukrainian horse and 0.4 mU/ml more ($p < 0.05$) Thoroughbred.

The next stage of the study was to study the physiological characteristics of the level of end products of lipid peroxidation diene conjugates and malondialdehyde in *Equus caballus* of different breeds. As a result of the analysis of the data in Table 1, the studied rocks can be conditionally divided into three groups according to the level of diene conjugates. The first group of the studied breeds had blood levels of diene conjugates up to 10 nmol/ml: Arab and Westphalian breeds. In the second group of *Equus caballus* stallions, the concentration of diene conjugates in the blood was from 10 to 13 nmol/ml: Belgian, Hanoverian, Trakehnen, and Ukrainian horse. The third group of *Equus caballus* stallions had the highest concentration of diene conjugates in the blood over 13 nmol/ml: Orlov Trotter, Russian (prize) Trotter and Thoroughbred. The highest concentration of diene conjugates in the blood of stallions was observed in Orlov Trotter breed, which

is 0.28 nmol/ml more than Russian (prize) Trotterstallions, 2.4 nmol/ml more ($p < 0.01$) Trakehnen stallions, 2.99 nmol/ml more ($p < 0.01$) Ukrainian horse, 0.36 nmol/mlml is more Thoroughbred, 1.7 nmol/ml is more Hanoverian, 3.51 nmol/ml is more ($p < 0.001$) Westphalian, 4.62 nmol/ml is more ($p < 0.001$) Belgian and 1.26 nmol/ml is more ($p < 0.05$) Arab breed.

The obtained data on the physiological level of malondialdehyde (MDA) allows us to identify 4 groups of stallions of Russian breeding. The first group has a malondialdehyde level of up to 2 nmol/ml and it includes only one breed — the Westphalian. The second group of *Equus caballus* stallions has a malondialdehyde level from 2 to 3 nmol/ml: Thoroughbred, Ukrainian horse and Belgian. The third group of stallions has a physiological level of malondialdehyde from 3 to 4 nmol/ml: Arab, Hanoverian, and Trakehnen breeds. The fourth group of stallions has a high malondialdehyde level of more than 4 nmol/ml: Orlov Trotter, Russian (prize) Trotter. The maximum physiological level of malondialdehyde was established in Russian (prize) Trotterstallions, which is 0.29 nmol/ml more than Orlov Trotter, 0.81 nmol/ml more than Trakehnen breed, 0.99 nmol/ml more than Hanoverian, 2.44 nmol/ml more ($p < 0.001$) Westphalian breed, 2.15 nmol/ml more ($p < 0.001$) Belgian, 0.79 nmol/ml more Arab breed, 1.56 nmol/ml more ($p < 0.01$) Ukrainian horse and 1.37 nmol/ml more ($p < 0.01$) Thoroughbred.

Thus, the classical approach to the analysis of the physiological characteristics of the *Equus caballus* organism in the context of the breed factor has the right to exist for certain tasks and allows you to detect close and more distant breeds by certain characteristics.

However, in modern conditions of scientific development, research is no longer conceivable without determining DNA markers, so we analyzed the physiological characteristics of the antioxidant system of the body of the same stallions but in the context of alleles of the GRM8 gene. The next stage of the study was to analyze the physiological characteristics of blood samples of *Equus caballus* of Russian breeding according to SNPrs1147388106 presented in Table 2.

Table 2

Physiological features of antioxidant capacity of blood samples of Equus caballus SNPrs395286150/394524550 *M ± m*

SNP genotypes 395286150/ 394524550	Antioxidant enzymes			End products of lipid peroxidation	
	Superoxide dismutase (SOD), U/ml	Catalase (CAT), U/ml	Glutathione peroxidase (GPx), mU/ml	Diene conjugates (DC), nmol/ml	Malondialdehyde (MDA), nmol/ml
CC/AA (22 heads)	10.22 ± 0.09	27.26 ± 0.27	1.65 ± 0.02	11.22 ± 0.10	2.93 ± 0.04
CT/AG (25 heads)	9.06 ± 0.06*	26.78 ± 0.21	1.83 ± 0.01*	10.75 ± 0.08*	2.39 ± 0.03**
TT/GG (23 heads)	9.98 ± 0.09	25.08 ± 0.31*	1.56 ± 0.02**	12.33 ± 0.07*	3.78 ± 0.03***

Note. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison with the CC/AA genotype.

Source: completed by P.K. Amulungu, A.V. Tkachev, O.L. Tkacheva.

The analysis of the data presented in Table 2 for the first-time indicates a different level of activity of antioxidant protection enzymes and the number of end products of lipid peroxidation in the blood of stallions of Russian breeding of different genotypes. We observed the best activity of superoxide dismutase (SOD) in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550, which is 11.4% more ($p < 0.05$) in carriers of the CT/AG genotype and 2.3% more in carriers of the TT/GG genotype. Catalase activity (CAT) was at a comparable level but with significant differences. We observed the highest catalase activity in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550, which is 1.8% more in carriers of the CT/AG genotype and 7.9% ($p < 0.05$) more in carriers of the TT/GG genotype. Against this background, the activity of glutathione peroxidase (GPx) was the highest in the blood of stallions carrying the CT/AG genotype according to SNPrs395286150/394524550, which is 9.8 % more ($p < 0.05$) in stallions of the CC/AA genotype and 14.8% more ($p < 0.01$) in stallions of the CT/AG genotype. Russian breeding of TT/GG genotype carriers.

We also obtained for the first-time data on the number of end products of lipid peroxidation for diene conjugates (DC) and malondialdehyde (MDA) in stallions of Russian breeding for carriers of different genotypes according to SNPrs395286150/394524550. The number of end products of lipid peroxidation of diene conjugates (DC) in the blood of stallions of Russian breeding was the best (lowest) in carriers of the CT/AG genotype according to SNPrs395286150/394524550, which is 4.4% less ($p < 0.05$) in stallions with the CC/AA genotype and 14.7% less ($p < 0.01$) compared to the TT/GG genotype. At the same time, the amount of malondialdehyde (MDA) in the blood samples of stallions of Russian breeding was also the best (lowest) in carriers of the CT/AG genotype according to SNPrs395286150/394524550, which is 22.6% less ($p < 0.01$) in carriers of the CC/AA genotype and 58.2% less ($p < 0.001$) in carriers of the TT/GG genotype.

For the first time, the data obtained on the level of antioxidant enzymes and the level of end products of lipid peroxidation in the blood samples of *Equus caballus* of Russian breeding according to the genotype SNPrs1147388106 are presented in Table 3.

Table 3

Physiological features of antioxidant capacity of blood samples of Equus caballus SNPrs1147388106 (*M*± *m*)

SNP genotypes	Antioxidant enzymes			End products of lipid peroxidation	
	Superoxide dismutase (SOD), U/ml	Catalase (CAT), U/ml	Glutathione peroxidase (GPx), mU/ml	Diene conjugates, nmol/ml	Malondialdehyde (MDA), nmol/ml
AA (23 heads)	9.54 ± 0.07	24.84 ± 0.21	1.71 ± 0.01	11.54 ± 0.07	2.98 ± 0.03
GA (24 heads)	9.55 ± 0.12	27.93 ± 0.28**	1.73 ± 0.02	11.12 ± 0.12	2.65 ± 0.05*
GG (23 heads)	10.04 ± 0.09*	28.78 ± 0.27***	1.65 ± 0.02*	11.01 ± 0.09*	3.06 ± 0.05

Note. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ in comparison with the AA genotype.

Source: completed by P.K. Amulungu, A.V. Tkachev, O.L. Tkacheva.

The analysis of the data obtained in Table 3 makes it possible for the first time to identify the genotype according to SNPrs1147388106, which has the best superoxide dismutase (SOD) activity in the blood samples of *Equus caballus* of Russian breeding; this genotype turned out to be GG, in which SOD activity was 4.9 % higher ($p < 0.05$) from the AA and GA genotypes. The activity of the catalase enzyme (CAT) was the worst in carriers of the AA genotype, which is 12.4% less ($p < 0.01$) in carriers of GA genotype and 15.9% less ($p < 0.001$) in carriers of the GG genotype according to SNPrs1147388106. At the same time, the activity of glutathione peroxidase (GPx) was worse in carriers of the GG genotype, which is 4.8% less ($p < 0.05$) compared with the AA and GA genotypes. At the same time, the number of end products of lipid peroxidation of diene conjugates was approximately at a comparable level in all three genotypes according to SNPrs1147388106. The amount of malondialdehyde (MDA) was highest in carriers of the GG genotype according to SNPrs1147388106, which is 13.4% more ($p < 0.01$) in carriers of the GA genotype and 2.6% more in carriers of the AA genotype according to SNPrs1147388106.

The correlation coefficient of sperm motility after defrosting with the amount of direct current was minus 0.43 ($p < 0.01$), with the amount of malondialdehyde — minus 0.47 ($p < 0.01$). At the same time, the correlation coefficient of sperm survival after thawing with the amount of MDA was stronger and amounted to minus 0.5 ($P < 0.01$), and with the amount of malondialdehyde — minus 0.57 ($P < 0.01$).

The correlation coefficient of sperm motility after thawing with the activity of superoxide dismutase (SOD) was 0.08, with catalase (CAT) 0.21 ($p < 0.01$), with glutathione peroxidase (GPx) 0.57 ($p < 0.01$). The correlation coefficient of sperm survival after thawing with the activity of superoxide dismutase (SOD) was 0.04, with catalase (CAT) 0.17 ($p < 0.01$), with glutathione peroxidase (GPx) 0.37 ($p < 0.01$).

At the same time, the degree of influence of the studied genotypes on the activity of antioxidant enzymes and the number of end products of lipid peroxidation was low — from 2 to 9%, but the confidence level was high, $p < 0.001$.

After several researchers showed the possibility of using the GRM8 gene as a DNA marker of physiological functions in humans [13, 14], studies of the associative relationship of various physiological functions of the animal body with the same and other targeted genes began to expand. In practical veterinary medicine, establishing the connection of a DNA marker with any function of the body will make it possible to carry out breeding and treatment of sick animals more effectively. Therefore, the data we have obtained on the physiological differences of stallions in the functional state of the antioxidant system of the human body will allow us to better understand the physiological processes under normal and pathological conditions. For example, there is a study of genetic markers in mares in connection with the functional state of their uterus [16], which may increase the effectiveness of endometritis treatment in mares, but this author does not study the antioxidant system of the equine body. The practical application of data on the state of the antioxidant system can be used to improve the cryopreservation of horse semen [13, 14].

Our data on the relationship between the activity of antioxidant protection enzymes and the number of end products of lipid peroxidation in *Equus caballus* sperm are

generally consistent with the results of many other studies [17–19], which describe that with an increase in enzyme activity and a decrease in the number of end products of lipid peroxidation in stallion body, the results of many other studies are generally consistent [17–19]. Due to the products of lipid peroxidation, the physiological characteristics of spermatozoa deteriorate, especially after freezing-thawing. At the same time, in the available literature, we could not find studies on the level of antioxidant enzymes and end products of lipid peroxidation in stallions of Russian breeding, depending on the genotypes according to SNPrs395286150/394524550 and SNPrs1147388106. Molecular identification methods are used in many studies [20–23] but there is not enough research on horses of Russian breeding. Most molecular genetic studies in horse breeding are devoted to the diagnosis of various diseases [24].

The results of the analysis of the physiological characteristics of the functional state of the *Equus caballus* body's antioxidant system of Russian breeding are discussed as following. These stallions turned out to be carriers of the GA genotype according to SNPrs1147388106 and CT/AG according to SNPrs395286150/394524550, which can already significantly simplify breeding work in Russia.

Conclusion

Thus, for the first time, physiological differences between blood samples of *Equus caballus* of Russian breeding with different genotypes according to the GRM8 gene are shown. The best activity of superoxide dismutase (SOD) in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550, which is 11.4% more ($p < 0.05$) in carriers of the CT/AG genotype and 2.3% more in carriers of the TT/GG genotype. Catalase activity (CAT) was at a comparable level but with significant differences. We observed the highest catalase activity in the blood of stallions with the CC/AA genotype according to SNPrs395286150/394524550, which is 1.8% more in carriers of the CT/AG genotype and 7.9% ($p < 0.05$) more in carriers of the TT/GG genotype. Against this background, the activity of glutathione peroxidase (GPx) was the highest in the blood of stallions carrying the CT/AG genotype according to SNPrs395286150/394524550, which is 9.8% more ($p < 0.05$) in stallions of the CC/AA genotype and 14.8% more ($p < 0.01$) in stallions of the CT/AG genotype. Russian breeding of TT/GG genotype carriers. The amount of malondialdehyde (MDA) was highest in carriers of the GG genotype according to SNPrs1147388106, which is 13.4% more ($p < 0.01$) in carriers of the GA genotype and 2.6% more in carriers of the AA genotype according to SNPrs1147388106. The study of the functional state of the antioxidant system of the horse body, depending on the breed, also shows the presence of significant differences between breeds of different uses.

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