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Original article

ANALYSIS OF THE INTERCONNECTION OF STALLION SEMEN INDICATORS WITH GENETIC MARKERS OF PROTEINS

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The purpose of the research was to study the associations of genetic polymorphism of proteins and enzymes of blood serum (albumin, transferrin, and esterase) on the quality of freshly obtained and cryopreserved semen of breeding stallions, taking into account their breed characteristics. The authors divided horses into four breed groups, taking into account their characteristics – riding horses, Orlov trotters, prize-winning trotters, and draft horses. A comparative assessment of 154 stallions revealed no significant differences in the quality of semen from different breed groups. For the first time, the influence coefficient (Fisher coefficient) of different genetic variants of proteins and enzymes on the semen parameters of stallions was analyzed and calculated. At the same time, the authors revealed the multidirectional effect of the same alleles of albumin, transferrin, and carboxylesterase on semen activity in different breeds. Thus, the presence of the albumin allele B (ALBB) had a positive effect on the mobility of fresh semen of stallions of riding breeds but a negative effect on stallions of Orlov trotters and draft horses. The heterozygous state of alleles revealed a positive effect on semen parameters. The analysis of variance of all examined stallions showed the highest level of the esterase locus (ESI) on the mobility of fresh semen and the albumin locus (ALBA) – on the mobility of frozen-thawed semen. The reliability of it is close to a statistically significant value. The results obtained suggest that specific gene complexes and associations with productive traits are formed in breeds under the influence of breeding. It must be taken into account as additional criteria for controlling the breeding process.

Keywords: albumins; transferrins; esterase; stallions; semen; breeds

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Научная статья

АНАЛИЗ ВЗАИМОСВЯЗИ ПОКАЗАТЕЛЕЙ СПЕРМЫ ЖЕРЕБЦОВ С ГЕНЕТИЧЕСКИМИ МАРКЕРАМИ БЕЛКОВ

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Целью наших исследований явилось изучение ассоциаций генетического полиморфизма белков и ферментов сыворотки крови (альбумина, трансферрина и эстеразы) с качеством свежеполученной и криоконсервированной спермы жеребцов-производителей с учетом их породной специализации. Лошади были разделены на 4 породные группы с учетом их особенностей: верховые, орловские рысаки, призовые рысаки и тяжеловозы. Сравнительная оценка 154 жеребцов не выявила достоверных различий по качеству спермы разных породных групп. Впервые был проведен анализ и рассчитан коэффициент влияния (коэффициент Фишера) разных генетических вариантов белков и ферментов на показатели спермы жеребцов. При этом выявлено разнонаправленное действие одинаковых аллелей альбумина, трансферрина и карбоксилэстеразы на активность спермы в разных породах. Наличие аллеля альбумина В (ALBв) оказало положительное влияние на подвижность свежей спермы жеребцов верховых пород, но отрицательное – на жеребцов орловских рысаков и тяжеловозов. Положительное влияние на показатели спермы проявилось преимущественно в гетерозиготном состоянии аллелей. Дисперсионный анализ всех обследованных жеребцов показал наиболее высокий показатель локуса эстеразы (*EsI*) на подвижность свежей спермы, и локуса альбумина (*ALBA*) - на подвижность замороженно-оттаянной спермы, достоверность которых приближена к статистически значимой величине. Полученные результаты говорят о том, что под влиянием селекции в породах формируются специфические генные комплексы и ассоциации с продуктивными признаками, что необходимо учитывать в качестве дополнительных критериев для управления селекционным процессом.

Ключевые слова: альбумины; трансферрины; эстераза; жеребцы; сперма; породы

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Introduction

Currently, the fertility of producers is an important economic factor in the development of horse breeding, especially in conditions of a small number of breeds. Researchers have found that many factors, including conditions of keeping, feeding, age, heredity, hormonal regulation, etc., influence the quality of stallions' semen and its suitability for freezing [1; 6; 12].

The search for informative indicators related to the quality of sperm is a topical issue for improving the technology of artificial insemination. Biochemical components, such as proteins, enzymes, sugars, macro- and microelements, are essential for maintaining the viability of spermatozoa.

Sperm plasma proteins are represented by both the main blood proteins and specific ones. Albumin is the basic protein of blood serum and seminal plasma and plays an important role in the transfer of various chemical compounds, and is also a reserve of amino acids [19, 21]. It was found that this protein is synthesized not only in the liver, but also in the epididymis and plays an important role in fertilization [16]. The study of the biochemical composition of the sperm plasma of stallions showed that the high quality of freshly obtained and cryopreserved sperm is associated with a high level of albumin [2].

Transferrin belongs to the group of β -globulins and is the main carrier of iron in the bloodstream, and also takes part in the body's defense against infections [8]. In human studies, a positive correlation was established between the level of transferrin in spermatozoa and the concentration of spermatozoa [18]. According to A.M. Atroshchenko et al. [17], stallions with good sperm quality had significantly higher levels of transferrin in seminal plasma.

The quality of mammalian sperm production is regulated by a number of enzymes, including dehydrogenases, maltase, acid and alkaline phosphatases, alpha-glycosidase, and a group of esterases. Carboxylesterase accounts for 90% of the activity of all seminal plasma esterases and belongs to the class of hydrolases that break down esters of carboxylic acids and neutralize toxins in the liver and testicles. In addition, in the testes, carboxylesterase is involved in the biosynthesis of testosterone in the testes and protects Leydig cells from the effects of damaging and toxic agents [7]. The effect of esterases on the quality of stallion sperm and their fertility has not yet been studied.

Immunogenetic factors also play an important role in the processes of fertilization, implantation and gestation of the fetus [4]. It has been established that the degree of differences between stallions and queens in terms of blood group antigens affects reproduction [3]. There is evidence that the genotypes of stallions in terms of blood group antigens are associated with the quality indicators of their sperm production [11; 15].

Materials and Methods

The purpose of the research was to study the effect of genetic polymorphism of blood serum proteins on the quality of semen of breeding stallions, taking into account their breed specialization. The task was to assess the degree of influence of the albumin protein allele, transferrin, and carboxylesterase enzyme alleles on the indicators of fresh and canned semen of stallions of four breed groups.

The material for the research was a database of the quality of fresh and cryopreserved semen of breeding stallions (n=154) of different breeds, obtained from the Bioresource collection “Cryobank of Genetic Resources” of the “All-Russian Research Institute of Horse Breeding” (Ryazan region, Russia). According to a generally accepted method, the semen of stallions was received on an artificial vagina, and its qualitative indicators were evaluated. They include the activity of fresh and thawed semen in points, the survival rate of thawed semen in hours (at t 4 °C). The resulting ejaculates were diluted with LCHCY medium and frozen, in accordance with the recommendations [9]. Immunogenetic testing of horses was carried out in the genetics laboratory of the Institute of Horse Breeding by types of albumin [ALB], transferrin [Tf], esterase [Es] [13]. The Horse Comparison Test, conducted by the International Society of Animal Genetics [ISAG], confirmed the reagent serum.

The authors divided the breeding stallions into four groups, taking into account the breed characteristics: (1) the first group included stallions of riding breeds (Arab, Akhal-Teke, Budennovskaya, Hanover, Trakenenskaya, Thoroughbred); (2) the second – Orlov trotters; (3) the third – prize-winning trotters (American standardbred, Russian and French trotters); (4) the fourth – representatives of draft-horse breeds (Russian and Soviet draft-horses).

The authors carried out biometric data processing using the Statistica 12 program [5]. They calculated the average values, their standard errors, Fischer’s F-criterion, its reliability level. When processing the data, the authors used a general linear model of variance analysis, based on the Statistica 12 program [5]. The research was carried out using the equipment of the Center for Collective Use [CCU] of the All-Russian Research Institute of Horse Breeding (Ryazan region, Russia).

Results

When calculating the average seed quality indicators of stallions, some differences between breeds were revealed. However, they were not statistically reliable since large individual fluctuations occur in each breed, affecting the average indicators.

The results of testing stallions by albumin locus revealed a high level of polymorphism in representatives of all breeds, with the exception of prize-winning

breeds, in which the ALB^A allele clearly dominated (Table 1). In the genotypes of riding stallions, the allele ALB^B was somewhat more common (51.72%). It is associated with higher quality indicators of fresh and cryopreserved semen. At the same time, stallions, heterozygous for the albumin locus, demonstrated the best activity indicators of fresh and thawed semen (4.53 and 1.92, respectively).

Among the Orlov trotting stallions, carriers of the ALB^A allele had the best indicators of the activity of freshly obtained and survival of cryopreserved semen. However, due to the large individual variability, the differences between the alternative groups were not statistically significant ($P < 0.95$).

Table 1.

Indicators of semen quality of stallions, depending on albumin and transferrin alleles

Breed Group	Locus /allele	Number of Stallions	Activity of fresh semen	Activity of thawed semen	Survival rate of thawed semen
1 Riding	ALB ^A	42	4.22±0.171	1.81±0.122	64.98±4.894
	ALB ^B	45	4.54±0.150	1.88±0.099	74.43±4.326
	Tf ^D	38	4.36±0.202	1.94±0.124	69.11±4.046
	Tf ^F	67	4.44±0.122	1.84±0.086	67.52±3.562
	Tf ^H	12	4.44±0.285	1.84±0.086	72.00±11.764
	Tf ^D	25	4.19±0.266	1.67±0.153	67.84±6.616
	Tf ^R	10	3.99±0.183	1.58±0.165	70.00±10.553
	average	87	4.44±0.121	1.91±0.082	72.31±3.59
2 Orlov trotters	ALB ^A	22	4.60±0.134	1.71±0.091	72.82±6.483
	ALB ^B	10	4.37±0.234	1.75±0.321	62.99±6.482
	Tf^D	9	4.73±0.191	1.67±0.312	76.67±14.621
	Tf ^F	19	4.46±0.149	1.62±0.124	68.44±7.203
	Tf ^H	10	4.51±0.263	1.88±0.282	65.55±8.637
	Tf ^D	0	-	-	-
	Tf ^R	20	4.46±0.155	1.73±0.111	68.58±6.443
	average	32	4.51±0.123	1.67±0.106	70.00±5.78
3 Prize- winning trotters	ALB ^A	21	4.68±0.150	1.85±0.126	75.19±0.536
	ALB ^B	1	5.00	2.00	96.00
	Tf ^D	13	4.64±0.204	1.94±1.165	77.85±7.209
	Tf ^F	18	4.66±0.168	1.81±0.086	74.61±5.785
	Tf ^H	1	5.00	2.00	72.00
	Tf ^D	2	4.25±0.250	2.10±0.100	78.00±18.000
	Tf ^R	1	5.00	2.00	80.00
	average	22	4.51±0.123	1.67±0.106	70.00±5.78

4 Draft- horses	ALB ^A	6	4.62±0.295	2.12±0.270	70.00±11.764
	ALB ^B	7	4.51±0.464	2.29±0.224	78.86±7.340
	Tf ^D	2	3.90±0.100	2.25±0.250	72.00±24.000
	Tf ^F	8	4.59±0.454	2.25±0.250	78.00±8.485
	Tf ^H	7	5.04±0.257	2.43±0.127	78.86±7.340
	Tf ^O	1	2.00	1.00	48.00
	Tf ^R	1	4.70	2.20	96.00
	average	13	4.57±0.305	2.24±0.179	78.08±6.71

Source: Compiled by the authors.

The final evaluation of the semen quality of 154 stallions of different breeds did not reveal essential and statistically significant differences between carriers of different albumin alleles. Breeding stallions with the allele ALB^A, on average, in all groups, characterized by slightly reduced activity of freshly obtained and cryopreserved semen (4.45±0.123 and 2.81±0.069, respectively). However, they remained active for a longer time after thawing (73.44±3.586). The highest value of Fischer's F-criterion of 1.934 (P=0.068) was observed in stallions in terms of the activity of frozen-thawed semen (Table 2).

Table 2.

The effect of albumin, transferrin, and esterase alleles on stallion semen parameters

Systems and alleles of proteins	Activity of fresh semen		Activity of thawed semen		Survival rate of thawed semen	
	F	P	F	P	F	P
ALB ^A	0.944	0.474	1.934	0.068	0.847	0.549
ALB ^B	1.036	0.408	1.273	0.473	0.865	0.536
Tf ^D	1.087	0.375	0.753	0.627	1.920	0.070
Tf ^F	1.172	0.322	0.606	0.751	0.307	0.755
Tf ^H	0.912	0.499	1.068	0.387	0.859	0.541
Tf ^O	1.898	0.085	0.981	0.440	0.743	0.616
Tf ^R	1.234	0.390	1.418	0.202	0.972	0.454
Es ^F	1.257	0.276	1.069	0.386	1.079	0.380
Es ^G	1.078	0.381	0.465	0.859	0.731	0.646
Es ^I	1.972	0.073	0.399	0.879	1.402	0.218

Note*: F – the Fisher criterion, P – the validity of the influence of the F-criterion.
Source: Compiled by the authors.

The hereditary variability of transferrin alleles in the genotypes of stallions of different groups was represented quite widely and generally corresponded

to the genetic structure of breeds. Stallions with the Tf^F , Tf^H allele had the best semen indicators among riding and draft-horse breeds representatives. Whereas among trotting breeds, producers with the Tf^D allele ($P>0.05$) stood out with high activity and cryostability of semen. The rare Tf^O allele in trotting and draft-horse breeds corresponded to low activity of fresh semen and riding breeds – to low fresh and frozen semen activity.

Stallions of all the compared breed groups had genetic variants of carboxylesterase, with the Es^I allele dominating in the frequency of occurrence. The presence of this allele in the genotype of stallions is associated with higher average indicators of fresh semen activity (5.7%) and the activity and survival of cryopreserved semen (25.6% and 22.1%, respectively). However, these differences were not statistically significant ($P>0.05$). The F-criterion of the influence of the Es^I allele on the activity of fresh semen was 1.97. It was close to a reliable value ($P=0.073$). A positive effect of the Es^F allele on semen quality indicators found in American trotters (a group of prize-winning breeds of trotters), and mainly in a heterozygous state. And, among the stallions of groups two and four, belonging to “cold-blooded” breeds, heterozygous carriers of the Es^G allele turned out to be the best, in terms of semen quality.

Discussion

The data obtained indicate a multidirectional effect of the same alleles of albumin, transferrin, and carboxylesterase on semen parameters in different breeds. Differences in the genetic structure of horse breeds in the frequency of occurrence of biochemical markers are due to both their origin and breeding by productive qualities [3; 14]. It indicates the need to consider breed characteristics when studying various interconnections [15].

The Es^I esterase allele ($F - 1.972$) showed the greatest relationship with the motility of fresh sperm of stallions, and the allele of albumin ALBA ($F - 1.934$) with the motility of frozen-thawed sperm.

Most often, the heterozygous state of alleles pronounces the positive effect on semen indicators. It is also noted by M. Serdyuk [10] in the research of the interconnection of semen production of boars with blood group antigens. R. Dubrovskaya, I. Starodumov [3], and T. Leeb [20] found that the degree of differences between stallions and queens by blood group antigens affects reproduction rates.

A positive association of the Tf^D transferrin allele with sperm resistance to freezing was noted ($F-1.920$). The presence of a rare allele of transferrin Tf^O in the genotype of stallions as a whole showed a certain negative relationship with the motility of fresh sperm ($F - 1.898$). However, the differences in seed

quality between breeds were not statistically significant due to the large individual variability. It should be noted that the Tf^D allele is more common in horses of riding breeds (28.7%) and, in isolated cases (0.05%), in representatives of trotting and draft-horse breeds [14].

Given the important biological role, played by proteins and enzymes in the metabolic processes of the organism and mammalian seminal plasma, the authors assumed that the mechanism of balanced polymorphism support the genetic variability of these loci. Wherein, certain associations of structural genes with selected traits, form in populations. This indicates the breed specificity of complex interconnections of structural and regulatory genes.

Conclusion

A multidirectional relationship of the same alleles of albumin, transferrin and carboxyesterase with sperm parameters in different breeds was established. A trend of a positive effect of the heterozygous state of the studied loci on the quality of fresh and frozen sperm was revealed.

The EsI esterase allele (F - 1.972) showed the greatest association with fresh sperm motility in stallions; with frozen-thawed sperm motility - albumin allele ALBA (F - 1.934). A positive association of the Tf^D transferrin allele with sperm resistance to freezing was noted (F-1.920). A rare allele of transferrin Tf^D, found mainly in saddle breeds, had a pronounced negative effect on fresh sperm motility (F - 1.898).

Differences in seed quality between varieties were not significant and statistically significant due to high individual variability.

Specific gene complexes and associations with productive traits are formed in breeds of different directions. This must be taken into account in the methodology of scientific research.

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