Protein spectrum and blood biochemical parameters in stallions with different sperm motility

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Abstract. The analysis of the protein spectrum of blood was carried out by electrophoresis in agarose gel in combination with the determination of biochemical parameters in 30 stallions of the Arabian breed, divided into groups according to the indicator of progressive sperm mobility. Group 1 included stallions with progressive mobility (PM) up to the 25th percentile (PM, % <53.35), group 2 - stallions with progressive mobility within the 25-75th percentile (53.35 <PM, % <65.18), and group 3 - stallions with progressive mobility above the 75th percentile (PM, % >65.18). In the 1st group of stallions, the highest concentrations of α₂-globulins and γ-globulins of blood were found. The 3rd group of stallions showed the lowest concentration of glucose and urea.

1. Introduction

Semen quality indicators of stallions can be influenced by both the health status of the producer and the individual characteristics of the organism. Semen quality and cryostability indicators are not the same for different stallions. Low rates of mobility, survival and structural integrity of sperm in fresh and thawed semen can lead to a decrease in its fertility ability [1,2].

Among the numerous, and most importantly, available criteria that must be paid attention to when selecting stallions for reproduction and for cryopreservation of semen, an important place is occupied by indicators of general and biochemical blood tests. One of the relevant directions of scientific research in horse reproduction is the study of the influence of the characteristics of the protein spectrum of whey on the quality of stallions semen.

Total blood protein is understood as a set of numerous proteins belonging to the fraction of albumin and globulins. Measurement of the concentration of total protein in blood whey is used to diagnose and monitor various physiological and pathological conditions; it is one of the most frequent routine tests performed to detect electrolyte abnormalities, inflammatory or infectious diseases in horses. Determination of total blood protein is carried out in conjunction with the separation of proteins into fractions and their quantitative determination [3]. Most often, for these purposes in the clinic, the method of electrophoresis in agarose gel is carried out [4]. If the results are correctly interpreted, then gel electrophoresis can be considered one of the most useful diagnostic tools available to the clinician [5]. Evaluation of whey protein fractions by electrophoresis is carried out in horses with diseases of the gastrointestinal tract and musculoskeletal system [6], is widely used to
identify various dysproteinemias, liver diseases, nephrotic syndrome, autoimmune diseases [7], as well as to identify the effect of age on protein spectrum [8].

In healthy horses, electrophoresis does not detect prealbumin; six zones are easily identified: albumin, α1-globulin, α2-globulin, β-globulin and γ-globulins [9]. Blood proteins perform many different functions, such as participation in blood coagulation (fibrinogen), protection against pathogens (immunoglobulins), maintenance of osmotic pressure and preservation of the reserve of amino acids (albumin), transport of metabolites and various biologically active compounds (albumins, transferrin, haptoglobin), inhibition of proteolysis (α1-antitrypsin) [4]. The study of protein fractions in inflammatory diseases is of particular interest. They are accompanied by increased synthesis of positive proteins of acute phase. Among them α-globulins occupy a special position [6]. According to Muñoz A. et al. (2010) albumin is a negative protein of acute phase due to its decrease in plasma during inflammatory conditions; α1-globulins increase during fasting, α2-globulins respond to acute inflammation, while β-globulins increase in inflammatory conditions and liver disease, while γ-globulins increase in chronic infection in horses [10].

Thus, the study of the protein spectrum of blood is widely used in veterinary medicine for the diagnosis of various pathological and physiological conditions, but very little is known about the relationship of the protein spectrum of blood with the process of spermatogenesis and indicators of the quality of horse sperm. In addition, despite all the advantages of the electrophoresis method, it is rarely possible to make a final diagnosis without a more extensive biochemical analysis, reflecting the slightest changes in the metabolism of the animal. In this regard, the purpose of this study was to study the protein spectrum and some biochemical parameters of the blood of stallions with different progressive sperm mobility.

2. Material and methods
All procedures were carried out in accordance with the “European Convention for the protection of vertebrates used for experimental and other scientific purposes” ETS No. 123 (18 March 1986) and the Law of the Russia Federation on Veterinary Medicine No. 4979-1 (14 May 1993). The protocol of the present investigation was approved by the Local Ethics Committee of the All-Russian Research Institute for Horse Breeding (ARRIH), Ryazan Oblast, Russia.

The study was carried out on the basis of the Tersk stud No. 169 (Stavropol Territory). Laboratory research was carried out in the cryobiology laboratory of the All-Russian Research Institute of Horse Breeding (Ryazan Region) and the Department of Biological Chemistry of the Ryazan State Medical University (Ryazan).

30 Arabian pure bred stallions aged 4 to 21 years were used in the experiments. The conditions of feeding and keeping the stallions corresponded to the established norms during the experimental studies.

Semen from stallions was obtained during the breeding season (March-May) at intervals of 48 hours using an artificial vagina for a mare in the hunt. After a long period of sexual rest (10 days or more), three ejaculates were taken from stallions with an interval of 48 hours, the first two ejaculates were not used in the studies; the third ejaculate was taken for the studies.

Immediately after obtaining the semen, the gel was removed, then the semen was filtered using a sterile gauze filter. Next, the progressive mobility (PM) was assessed using the Argus CASA system (ArgusSoft LTD, St. Petersburg, Russia) and a Motic BA 410 microscope (Motic, Hong Kong).

A blood sample from each stallion from the jugular vein was taken once before morning feeding during the breeding season (March-May). Blood samples were centrifuged at 400 g for 20 min and plasma stored at -18 °C until analysis.

In blood whey, the concentration of total protein, glucose, urea, as well as the activity of α-amylose was determined on biochemical analyzer AU 680 (Beckman Coulter, USA), the concentration of phospholipids was determined on biochemical analyzer AU 480 (Beckman Coulter, USA) according to unified photometric methods of clinical laboratory research. To separate blood proteins into fractions (albumin, α1-, α2-, β- and γ-globulins), electrophoresis in agarose gel was used with the help
of the SAS-1 Plus / SAS-2 system (Helena Biosciences Europe, Great Britain). The value of the protein coefficient "albumin / globulins" was determined by the calculation method.

Statistical processing was performed using Statistica 10 and Microsoft Office Excel 2016 (StatSoft Inc., USA). To assess the statistical significance in the study groups, the nonparametric U-Mann-Whitney test was used. The results were presented as the median Me of upper and lower quartiles [Q1; Q3]. Differences were considered statistically significant at p < 0.05.

3. Results and discussion
The division of stallions into groups was carried out depending on the indicator of progressive semen mobility. Group 1 included stallions with progressive mobility up to the 25th percentile (PM, % < 53.35), group 2 - stallions with progressive mobility within the 25-75th percentile (53.35 < PM, % < 65.18), and 3 group - stallions with progressive mobility above the 75th percentile (PM, % > 65.18). The studied biochemical parameters, as well as the content of protein fractions in the studied groups, are shown in table 1.

Table 1. The content of protein fractions and biochemical parameters of the stallions blood, Me [Q1; Q3].

| Indicator                        | Group 1 (n=8)          | Group 2 (n=14)         | Group 3 (n=8)          |
|----------------------------------|------------------------|------------------------|------------------------|
| Albumin, g/l                    | 66.0 [63.90;69.08]     | 63.6 [62.40;64.43]     | 65.4 [61.78;68.10]     |
| α1-globulins, %                  | 48.1 [44.18;52.66]     | 51.9 [48.63;54.65]     | 50.8 [48.63;52.85]     |
| α2- globulins, %                 | 2.3 [2.03;2.41]        | 2.1 [1.96;2.40]        | 2.3 [2.13;2.50]        |
| β - globulins, %                 | 10.7* [10.38;11.52]    | 9.4 [8.89;9.79]        | 9.2 [9.05;9.59]        |
| γ- globulins, %                  | 10.2 [9.54;11.12]      | 10.3 [9.64;11.47]      | 10.9 [9.76;13.57]      |
| «Albumin/globulins», conventional units | 32.5* [27.91;34.89]   | 24.8 [22.56;29.01]     | 25.8 [24.77;27.11]     |
| α-amylose, U/l                   | 9.0 [7.97;10.03]       | 1.1 [0.95;1.21]        | 1.0 [0.96;1.12]        |
| Glucose, mmol/l                  | 3.7 [3.13;4.18]        | 4.2 [4.00;4.73]        | 4.0 [2.83;4.93]        |
| Urea, mmol/l                     | 4.6 [4.22;4.81]        | 4.4 [4.09;4.67]        | 3.8* [3.25;3.94]       |
| Phospholipids, mmol/l            | 5.0 [4.38;5.90]        | 4.8 [4.23;5.25]        | 4.0* [3.73;4.30]       |

* - statistically significant differences from group 1 (p < 0.05);
▲ - statistically significant differences from group 2 (p < 0.05);
● - statistically significant differences from group 3 (p < 0.05).

In the blood of stallions from the first group with the lowest progressive mobility, the highest concentration of α2-globulins was observed; statistically significant differences were obtained in comparison with the second group (p = 0.004) and with the third group (p = 0.031). It is known that the fraction of blood α-globulins includes acute phase proteins, the concentration of which increases in response to the inflammatory process and traumatic conditions [6]. Inflammatory processes, as we know, negatively affect the quality of semen, including leading to a decrease in sperm mobility [11].

The highest level of γ-globulins was also found in the first group of stallions; statistically significant differences were obtained in comparison with the second group (p = 0.044). It should be noted that the content of γ-globulins in the blood of animals of group 1 exceeds the reference values of healthy horses, which are 18-24% [12]. The γ-globulin fraction of the blood of most mammals includes 5 classes of immunoglobulins - IgG, IgA, IgM, IgD and IgE, which differ in molecular size, charge, amino acid composition, and carbohydrate content [13]. An increase in the content of γ-globulins is characteristic of chronic inflammation, cancer, AIDS, and autoimmune diseases [14]. Normally, the blood-testis barrier prevents the contact of the immune system with the spermatogenic epithelium, however, if it is damaged in some inflammatory diseases, injuries, congenital anomalies [15,16], antisem恩 antibodies can be produced, which reduce sperm mobility and their fertility ability [17].
In addition, in the third group of stallions with the highest progressive mobility, the lowest blood glucose concentration was found. Statistically significant differences were obtained in comparison with the first group (p = 0.014) and with the second group (p = 0.013). Glucose is an essential energy substrate for all cells in the body. The ATP level and comparable percentages of mobility in mammals sperm are maintained not only by monosaccharides, but also by other substrates, since both glycolysis and oxidative phosphorylation are active in sperm [18]. However, it is glucose that stimulates sperm capacitation [19]. It is known that glucose metabolism in the testes plays an important role in maintaining spermatogenesis [20]. The transport of glucose and other metabolites from blood to germ cells is strictly controlled due to the presence of the blood-testis barrier. The passive transport of glucose across the blood-testis barrier by glucose transporters (GLUT) is an essential event in spermatogenesis. Such glucose carrier proteins as GLUT 1, GLUT 3, GLUT 8 were found in Sertoli cells [21, 22]. The lowest concentration of glucose in the blood of stallions of the third group can be explained by the intensive utilization of the monosaccharide, also for maintaining the energy balance of sperm and a high level of their progressive mobility.

We also found that the urea concentration in the blood of stallions of the third group was statistically significantly lower than in stallions from the first (p = 0.037) and second groups (p = 0.046). In earlier studies, we had obtained a negative correlation between the concentration of urea in the semen plasma of stallions and sperm motility [23]. Urea is the end product of protein catabolism; the accumulation of this metabolite indicates the predominance of catabolic processes and is observed in patients with pathospermia [24]. In our study, the blood of stallions from the group with the highest sperm mobility has the lowest urea content; the determination of this indicator can be predictive in the selection of stallions with the best characteristics of the semen quality.

4. Conclusions
The first group of stallions with the lowest progressive sperm mobility showed the highest concentrations of α2-globulins and γ-globulins in the blood, which, in all likelihood, is associated with the course of inflammatory processes and their negative effect on the semen production process.

In the third group of stallions with the highest progressive sperm mobility, the lowest glucose concentration was observed, which can be explained by the high level of glucose utilization in cells, including sperm, to maintain a high level of progressive mobility. The third group of stallions is characterized by the lowest blood urea content, which may indicate the predominance of anabolic processes in the body of these animals.

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