The effect of serum lipids and spermoplasm on the stallion sperm quality

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Abstract. The quality and cryoresistance of stallion sperm depend on many endogenous and exogenous factors. One of the factors affecting the sperm quality are the parameters of the lipid spectrum of spermoplasm and blood serum. Lipase activity, cholesterol and triglyceride content in blood serum and spermoplasm were studied in 19 stallions of the Arabian breed aged from 5 years to 15 years (on average 10.1±0.3 years). It was found that lipase activity in the spermoplasm (217.0 U/l) is on average 11.4 times higher than in the blood serum (19.1 U/l). The concentration of spermoplasm triglycerides (1.3 mmol/l) is on average 4.3 times higher than in blood serum (0.3 mmol/l). The concentration of cholesterol in the blood serum (2.4 mmol/ l) is on average 12 times higher than the same indicator in the stallion spermoplasm (0.2 mmol/l). The relationship between the parameters of the lipid spectrum of blood serum and spermoplasm with the characteristics of stallion sperm was studied. A significant relationship was found between the activity of lipase in blood serum with progressive mobility (r=0.66; p=0.006) and survival (r=0.67; p=0.005) of spermatozoa in cryopreserved sperm.

1 Introduction

High quality and cryoresistance of stallion sperm are important factors affecting the effectiveness of artificial insemination of mares [1]. The quality of stallion sperm depends on the individual characteristics of the organism, genetic factor [2], age, season of the year, and many other factors [3]. The sperm characteristics are also affected by the state of the somatic and reproductive health of the animal. A close relationship has been established between the sperm parameters and some biochemical parameters of blood serum and spermoplasm. The biochemical composition of spermoplasm and blood serum is diverse and contains proteins, enzymes, carbohydrates, lipids, as well as macro- and microelements, thus, an important aspect of scientific research is to study the effect of spermoplasm and blood serum components on the quality of stallion sperm [4].

The sperm quality and cryoresistance are influenced by the parameters of the protein, enzyme, and elemental spectrum of the spermoplasm [5], [6]. The study of the activity of such semenal plasma enzymes as lactate dehydrogenase, gamma-glutamyltransferase, alkaline phosphatase, allows to assess the integrity of spermatozoa [4]. Indicators of the

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lipid spectrum also affect sperm characteristics [7]. Cryoresistance and fertilizing ability of spermatozoa depend on the parameters of the spermoplasm lipid spectrum [8].

The purpose of this work was to evaluate the activity of lipase, cholesterol, and triglycerides in blood serum and spermoplasm in stallions, to study the relationship between these indicators, as well as to determine their impact on the sperm quality and cryoresistance.

2 Materials and Methods

The research was carried out in JSC "Tersk Stud Farm No. 169" (Stavropol Territory) during the off-season (March-April) 2021, the Laboratory of Cryobiology of the FSBSI "All-Russian Research Institute of Horse Breeding" (Ryazan region) and the Clinical Diagnostic Laboratory "DiaLab" (city of Moscow).

19 stallions of the Arabian pure breed aged from 5 to 15 years (on average 10.1±0.3 years) were used in the studies. During the experimental studies, the conditions of feeding and keeping stallions corresponded to the established standards. Sperm from stallions was received at intervals of 48 hours. 3 ejaculates were received from each stallion.

After receiving sperm, the volume, concentration, and total number of spermatozoa (total number of sperm, TNS) were determined in each ejaculate. Then the ejaculate was divided into two parts, one part was diluted with LCHCY medium in a ratio of 1:3, progressive motility, total number of sperm with progressive motility (TNS PM), morphology, and survival of sperm at a temperature (T) +4°C. were determined. The volume of ejaculate (in ml) after filtration was determined using a measuring cylinder. The sperm concentration was measured with an SDM1 photometer (Minitube GmbH, Tiefenbach, Germany). The assessment of progressive mobility (PM) was carried out using the Argus CASA system (ArgusSoft LTD., St. Petersburg, Russia) and the Motic BA 410 microscope (Motic, Hong Kong, China) in the Makler chamber at a temperature of +37°C. Samples of native sperm were used to study the morphological composition of spermatozoa. The smears were stained with a 2% aqueous solution of eosin. The preparations were examined by light microscopy at 1000× magnification using an Olympus BX41 phase contrast microscope (Olympus Corporation, Japan).

Sperm freezing was carried out in liquid nitrogen vapors in aluminum tubes with a volume of 18 ml according to the standard technology of the All-Russian Research Institute of Horse Breeding and stored in liquid nitrogen at a temperature of -196°C [9]. Thawing of frozen sperm was carried out in a water bath at a temperature of +40°C for 90 seconds, after which the progressive mobility and survival of spermatozoa were also determined at T +4°C.

The other part of the ejaculate was centrifuged at 2000 g for 20 minutes immediately after receiving the sperm using an ELMI CM-6M centrifuge (SIA ELMI, Latvia). After supernatant microscopy, sperm-free aliquots of semenal plasma were frozen in Eppendorf-type test tubes (2.0 ml) and stored at a temperature of -18°C until the studies were carried out.

Blood sampling from each stallion was carried out from the jugular vein once on an empty stomach before morning feeding during the period of sperm production. Blood samples were centrifuged at 400 g for 15 min and the serum was stored at -18°C before the analysis.

Blood serum and spermoplasm parameters (lipase activity, cholesterol and triglyceride concentrations) were studied using an AU 680 automatic biochemical analyzer (Beckman Coulter, USA) according to unified photometric methods of clinical laboratory studies.

Statistical processing was carried out using the program Statistica 10 and Microsoft Office Excell 2016 (StatSoft. Inc., USA). The nonparametric Spearman coefficient (Rs)

was used to determine the relationship between the indicators. The results are presented in the form of median (Me) and quartiles [Q1/Q3], of minimum (min) and maximum (max) values. The differences were considered statistically significant at $p \leq 0.05$.

3 Results and Discussion

During sperm cryopreservation and subsequent thawing, the progressive motility and survival of spermatozoa significantly decreases ($p < 0.01$) (Table 1).

Table 1. Indicators of native, diluted, and cryopreserved stallion sperm, (Me [Q1/Q3], min, max), n=19.

Indicator	Median [Q1/Q3]	min	max
Native and diluted sperm			
Volume, ml	24.2 [21.5/34.5]	18.0	50.0
Concentration, million/ml	245.1 [166.0/338.0]	95.0	436.0
TNS, billion	6.8 [4.5/9.2]	2.1	13.8
TNS PM, billion	4.1 [2.7/6.3]	1.1	9.6
Progressive motility, %	62.0 [54.8/72.0]	52.0	82.0
Pathological spermatozoa, %	26.5 [18.0/30.5]	1.6	45.0
Sperm survival at T +4°C, hour	113.6 [72.0/156.0]	36.0	168.0
Cryopreserved sperm			
Progressive motility, %	21.4 [13.5/38.5]*	4.0	54.0
Sperm survival at T +4°C, hour	42.0 [24.0/72.0]*	55.0	120.0

* Differences between progressive motility and survival of spermatozoa before and after sperm cryopreservation are statistically significant at $p < 0.01$

When studying the lipid spectrum in the spermoplasm and blood serum of stallions, we found that the lipase activity in the spermoplasm is on average 11.4 times higher than in the blood serum (Table 2). The concentration of triglycerides in the blood serum was on average 4 times lower than in the spermoplasm and amounted to 0.3 [0.2/0.4] mmol/l.

Table 2. Values of lipid spectrum parameters in the spermoplasm and blood serum of stallions, (Me [Q1/Q3], min, max), n=19.

Indicator	Median [Q1/Q3]	min	max
Spermoplasm			
Lipase, U/l	217.0 [102.2/332.2]	52.2	496.9
Triglycerides, mmol/l	1.3 [0.8/1.9]	0.2	3.5
Total cholesterol, mmol/l	0.2 [0.1/0.2]	0.1	0.4
Blood serum			
Lipase, U/l	19.1 [17.8/20.3]	11.9	30.4
Triglycerides, mmol/l	0.3 [0.2/0.4]	0.1	0.6
Total cholesterol, mmol/l	2.4 [2.3/2.7]	2.1	3.7

The activity of lipase in the blood under physiological conditions is low due to a number of features: most of the enzyme has intra-organ localization (in the liver, pancreas) and, while maintaining the integrity of cell membranes, cannot enter the bloodstream, and the part of the enzyme localized in the bloodstream is located on the luminal surface of the capillary epithelium [10]. In men, there is a higher activity of this enzyme in the spermoplasm, but it is worth noting that in case of normozoospermia, this indicator is 1.3 times higher than hematological, and with oligoastenoteratozoospermia – 1.7 [11]. In one of the conducted studies, it was experimentally established that excessive lipase activity

negatively affects the resistance of spermatozoa to freezing [12]. Nevertheless, in this study we have not established a similar pattern.

According to the results of our studies, the cholesterol concentration in stallion blood serum is on average 12 times higher than the same indicator in the spermoplasm. In the study of Mursky S.I. (2020), a similar dependence was revealed in men, moreover, the higher the concentration of sperm in the ejaculate, the lower the cholesterol content in the blood serum [13].

Table 3. The dependence of sperm parameters on the characteristics of the lipid spectrum of sperm and blood serum (Spearman's rank correlation coefficient (Rs)).

Indicator	Indicator	(Rs)	p
Ejaculate volume	Triglycerides SP	-0.64	0.008
Spermatozoa concentration	Lipase SP	0.64	0.007
Spermatozoa concentration	Cholesterol SP	0.75	0.001
PM of spermatozoa in cryopreserved sperm	Lipase BS	0.66	0.006
Survival of spermatozoa in cryopreserved sperm	Lipase BS	0.67	0.005
Pathological spermatozoa	Cholesterol SP	0.54	0.026
Lipase SP	Cholesterol SP	0.84	0.000
Triglycerides SP	Cholesterol SP	0.54	0.033
Lipase BS	Cholesterol CR	-0.71	0.002

Abbreviations: PM – progressive mobility, SP – in spermoplasm, BS – in blood serum

During the correlation analysis (Table 3) certain patterns were established. A positive correlation was found between the concentration of spermatozoa (r=0.64; p=0.007) in 1 ml of sperm and the activity of lipase in the spermoplasm. A positive correlation was found between serum lipase activity with progressive motility (r=0.66; p=0.006) and survival (r=0.67; p=0.005) of spermatozoa in thawed sperm. At the same time, no reliable relationship has been established between the progressive motility and survival of spermatozoa with lipase activity in the spermoplasm. It should be noted that the physiological state of stallions directly affects the sperm quality [14]. Lipase catalyzes the cleavage of neutral lipids – triglycerides. Free fatty acids formed during the splitting of triglycerides are oxidized to form acetyl residues that are connected to the processes of energy production [15], [16]. We assume that the revealed correlations between blood lipase activity with motility and sperm survival in stallions are mediated by general metabolic effects, which are provided by universal energy sources formed during fatty acid catabolism.

According to Carver D.A. and Ball B.A. (2002), high lipase activity was found in the secretion of bulbourethral glands in goats [12]. In a study by Pellicer-Rubio M.T. et al. (1997), a glycoprotein was found in the spermoplasm, partly homologous to a fragment of pancreatic lipase, which can participate in the synthesis of compounds that have a toxic effect on spermatozoa [17]. Pancreatic lipase ios of particular importance, which in case of diseases of the pancreas is actively released into the blood in large quantities. The addition of pancreatic lipase to sperm decreased sperm motility depending on [12]. That is why the determination of lipase activity in blood serum can allow using this indicator as a marker of sperm cryoresistance (progressive motility and survival of spermatozoa after cryopreservation).

The level of cholesterol in the spermoplasm positively correlates with the concentration of spermatozoa (r=0.75; p=0.001). Cholesterol and phospholipids occupy about 90% of the lipids of the stallion spermoplasm [18]. In comparison with the semenal plasma of wild boar and bull, stallion cholesterol levels are lower with a slight increase in the amount of

phospholipids [18], despite the fact that it is the main steroid in sperm membranes [19]. According to Antonov M.P. and Zhigulina V.V. (2012), in men with impaired fertility, cholesterol levels may be increased and the concentration of phospholipids in the spermoplasm may be reduced [20]. This is explained by the fact that for capacitation, the sperm membrane must synthesize phospholipids and remove cholesterol.

This assumption can be confirmed by the results obtained during the implementation of this study. We have established a positive correlation between the number of pathological spermatozoa in the ejaculate ($r=0.54$; $p=0.026$) and the concentration of cholesterol in the spermoplasm.

A highly reliable positive correlation was established between lipase activity and cholesterol concentration in spermoplasm ($r=0.84$; $p=0.00005$) and a negative correlation between lipase activity and serum cholesterol concentration ($r=-0.71$; $p=0.002$). A positive correlation can be noted between the level of triglycerides and the cholesterol concentration in the sperm ($r=0.54$; $p=0.033$). The revealed pattern may be related to the fact that acetyl residues formed during the degradation of triglycerides under the action of lipase in the sperm are substrates for the cholesterol synthesis. The higher the lipase activity, the more intense the cleavage of fatty acids and their subsequent oxidation, which results in the formation of substrates for the cholesterol synthesis. There is no such pattern in the blood, since the total cholesterol level in the blood depends on many factors: the level of low and high density lipoproteins, the activity of the key enzyme of cholesterol synthesis – HMG-CoA reductase, the concentration of insulin and glucagon. Even in the presence of a large number of substrates, the process of cholesterol synthesis can be inhibited by a number of regulatory factors [21], [22].

Triglycerides are one of the main energy substrates for spermatozoa [23]. Triglycerides are present in small amounts in the stallion spermoplasm [19]. In goats, only 1% of neutral lipids are triglycerides [17]. Halo Jr. M. et al. (2018) found that the concentration of triglycerides in the semenal plasma of stallions was 0.481± 0.469 mmol/l [14]. We found that the level of triglycerides in the sperm of stallions averaged 1.3 [0.8/1.9] mmol/l. Such a level of triglycerides in the spermoplasm indicates a high energy supply of stallion spermatozoa with lipid substrates.

According to the results of the conducted studies, the relationship between the parameters of the lipid spectrum of blood serum and spermoplasm with the indicators of the quality of stallion sperm was studied. It was found that such indicators of blood serum and spermoplasm as lipase activity, total cholesterol and triglycerides have a prognostic value in assessing the quality of stallion sperm.

4 Conclusions

Lipase activity in spermoplasm (217.0 [102.2/332.2] U/l) is on average 11.4 times higher than in blood serum (19.1 [17.8/20.3] U/l). The concentration of triglycerides of spermoplasm (1.3 [0.8/1.9] mmol/l) is on average 4.3 times higher than in blood serum (0.3 [0.2/0.4] mmol/l). The concentration of cholesterol in the blood serum (2,4 [2,3/2,7] mmol/l) is on average 12 times higher than the same indicator in the stallion spermoplasm (0,2 [0,1/0,2] mmol/l). The relationship between the parameters of the lipid spectrum of blood serum and spermoplasm with the characteristics of stallion sperm was established. A significant relationship was found between the activity of lipase in blood serum with progressive mobility ($r=0.66$; $p=0.006$) and survival ($r=0.67$; $p=0.005$) of spermatozoa in cryopreserved sperm. We propose to use the assessment of lipase activity in blood serum as an indicator characterizing the cryoresistance of stallion sperm.

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