Lipid profile of stallion seminal plasma and its influence on semen quality and cryoresistance

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Abstract. Semen quality was assessed and lipid spectrum of seminal plasma was determined in 42 stallions of different breeds aged from 5 to 16 years (average 9.8±5.4 years). The concentration of total cholesterol, triglycerides, high density lipoproteins and low-density lipoproteins were determined in semen plasma. We found a negative correlation between the concentration of low- and high-density lipoproteins and progressive sperm motility in both fresh ($p<0.05$) and thawed ($p<0.05$) semen. We also found a negative correlation between the concentration of low-density lipoproteins in sperm plasma with total sperm motility in fresh ($p<0.05$) and frozen ($p<0.01$) semen, and the concentration of high-density lipoproteins in sperm plasma with total sperm motility in fresh and frozen semen ($p<0.05$). We found a significant negative correlation between ejaculate volume and concentration of triglycerides ($p<0.05$), cholesterol ($p<0.01$) and high-density lipoproteins ($p<0.05$). There was a positive correlation between sperm concentration and triglycerides ($p<0.05$), cholesterol ($p<0.001$) and high-density lipoprotein ($p<0.01$) content.

1 Introduction

The quality and cryoresistance of stallion semen depend on many endogenous and exogenous factors. One such factor is the composition of seminal plasma, which includes proteins, free amino acids, enzymes, lipids, monosaccharides, steroid hormones, micro- and macronutrients, prostaglandins, and polyamines [1-2]. Spermoplasm consists of the secretions of the appendicular glands and appendages of the testes and serves as a medium for sperm transport, protection and nutrition after ejaculation [3].

One of the directions of semen quality assessment, which is widely developed nowadays, is the study of biochemical indices of sperm plasma for the selection of markers characterizing the quality and cryoresistance of semen and various pathologies of the reproductive system of stallions. To the indicators used to diagnose various disorders in the spermogram of stallions can be attributed the components of the lipid spectrum of sperm plasma, which directly affects the cryoresistance and fertilising ability of spermatozoa [4].

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The aim of the study was to investigate the relationships between the lipid spectrum of sperm plasma and the parameters of fresh and cryopreserved stallion semen.

2 Material and methods

Experiments were conducted in JSC 'Tersky breeding stud farm No. 169' (Stavropol Territory), JSC 'Horse breeding farm named after the First Cavalry Army' (Rostov region), experimental stables of the All-Russian Research Institute for Horse Breeding (ARRIH, Ryazan Region). Laboratory studies were carried out in the laboratory of cryobiology of Federal State Budgetary Scientific Institution 'ARRIH' (Ryazan region), Department of Biological Chemistry of Ryazan State Medical University (Ryazan) and clinical and diagnostic laboratory 'DiaLab' (Moscow).

Semen was obtained from 42 stallions of different breeds (average age 9.8±5.4 years) during the mating seasons 2021-2023. Semen from stallions was obtained using an artificial vagina per mare in the hunt.

Semen examination.

Freshly obtained semen was filtered using sterile gauze cloth, and general parameters such as ejaculate volume, sperm concentration, total and progressive motility, and survival rate during hypothermic semen storage were recorded.

The volume of ejaculate (in ml) was measured using a measuring cylinder. Concentration was measured using SDM1 photometer (Minitube GmbH, Tiefenbach, Germany). Total and progressive motility were determined using Argus CASA system (ArgusSoft LTD, St. Petersburg, Russia) and Motic BA 410 microscope (Motic, Hong Kong, China) in Mackler chamber. Sperm viability in hours was recorded by determining progressive motility at 24-hour intervals (until it decreased to 5%).

Sperm dilution and cryopreservation

Diluted the semen obtained lactosechelate-citrate-yolk (LCCY) medium at a ratio of 1:3. Freezing was carried out according to standard technology ARRI of Horse Breeding in aluminium tubes (18 ml each). After thawing the cryopreserved semen, total and progressive motility and sperm survival were determined.

Obtaining sperm plasma

Seminal plasma was obtained by centrifugation of a portion of the ejaculate at 3000 rpm for 20 minutes. After microscopic examination of the supernatant, aliquots of seminal plasma free of spermatozoa were frozen at temperature -18 °C and stored at the same temperature until testing.

Biochemical examination of sperm plasma

Biochemical parameters such as triglycerides, total cholesterol, high- and low-density lipoproteins, phospholipids were determined on AU 680 biochemical analyzer (Beckman Coulter, USA) according to unified photometric methods of clinical laboratory tests. The cholesterol/phospholipids ratio was determined by the calculation method.

Statistical processing.

Statistical processing was performed using Statistica 13.3 and Microsoft Office Excel 2016 (StatSoft Inc., USA). The normality of distribution of quantitative signs was determined using the Shapiro-Wilk criterion. Nonparametric Mann-Whitney U - test was used to assess statistical significance in the studied groups. The results are presented in the format M±m (M - mean, m - standard error of the mean), min (minimum value) and max (maximum value). Differences were considered statistically significant at $p<0.05$.

3 Results obtained and their discussion

Indicators of quality and cryoresistance of stallion semen are presented in Table 1.

Table 1. Indices of native, diluted and cryopreserved stallion semen, (M±m, min, max), n=42.

Indicator, units of measurement	M±m	Min	Max
Native and diluted semen			
Volume of ejaculate, ml	33.1±15.6	15.0	65.0
Sperm concentration, mln/ml	243.6±95.6	102.0	434.0
Total sperm motility, %	66.2±12.9	30.9	89.0
Progressive sperm motility, %	55.6±13.1	25.1	85.4
Sperm survival rate at the T 2-4 ºC, hour	148.2±62,8	24.0	240.0
Cryopreserved sperm			
Total sperm motility, %	38.7±18.1	8.4	71.2
Progressive sperm motility, %	26.8±14.2	5.0	57.9
Sperm survival rate T 2-4 ºC, hour	81.4±44.3	6.0	156.0

We measured the concentration of lipid spectrum parameters of sperm plasma. The results of measurements of biochemical indices of stallion sperm plasma are presented in Table 2.

Table 2. Biochemical parameters of lipid spectrum of stallion sperm plasma, (M±m, min, max), n=42.

Indicator, units of measurement	M±m	Min	Max
Triglycerides, mmol/l	1.22±0,85	0.21	4.67
Cholesterol, mmol/l	0.14±0,08	0.04	0.42
Phospholipids, mmol/L	0.81±0,53	0.22	2.99
Cholesterol/Phospholipids, conditional units.	0.21±0,12	0.04	0.69
High-density lipoproteins, mmol/l	0.03±0,01	0.01	0.06
Low-density lipoproteins, mmol/l	0.11±0.05	0.01	0.25

We found a significant negative correlation between lipid metabolism parameters with ejaculate volume and a positive correlation with sperm concentration (Table 3). It is possible that lipid components of stallion sperm plasma are of epididymal or testicular origin.

Table 3. Correlation between biochemical indices of lipid metabolism and stallion semen characteristics (Spearman coefficient), r_s).

Sperm counts	Lipid spectrum of sperm plasma	r_s	p-significance
Ejaculate volume	Triglycerides	-0.27	<0.05
Sperm concentration	Triglycerides	0.27	<0.05
Ejaculate volume	Cholesterol	-0.38	<0.01
Sperm concentration	Cholesterol	0.69	<0.001
Ejaculate volume	High-density lipoproteins	-0.32	<0.05
Sperm concentration	High-density lipoproteins	0.62	<0.01
Total sperm motility in native semen	High-density lipoproteins	-0.36	<0.05
Progressive sperm motility in native semen	High-density lipoproteins	-0.27	<0.05
Total sperm motility in cryopreserved semen	High-density lipoproteins	-0.33	<0.05
Progressive sperm motility in cryopreserved semen	High-density lipoproteins	-0.35	<0.05
Sperm concentration	Low-density lipoproteins	0.54	<0.01
Total sperm motility in native semen	Low-density lipoproteins	-0.45	<0.01
Progressive sperm motility in native semen	Low-density lipoproteins	-0.36	<0.05
Total sperm motility in cryopreserved semen	Low-density lipoproteins	-0.27	<0.05
Progressive sperm motility in cryopreserved semen	Low-density lipoproteins	-0.35	<0.05

Triglycerides are one of the main energy substrates for spermatozoa [5]. Low levels of triglycerides in semen may indicate insufficient energy resources, decreased speed of movement, motility and sperm fertilisation ability. According to Halo Jr. M. et al. (2018) the triglyceride concentration in seminal plasma of stallions was as follows 0.48±0.47 mmol/l [6], and the mean values of triglyceride concentrations we obtained were significantly higher (Table 2), which indicates that spermatozoa have sufficient energy supply of lipid substrates. Cevik M. et al (2007) found that triglyceride concentration was significantly higher in bulls with normozoospermia compared to bulls with oligoasthenozoospermia [7]. However, in a study by El-Badry D.A.M. et al. (2013), no significant difference was found between seminal plasma triglyceride levels of stallions with good and poor sperm cryotolerance [8]. In our study, no statistically significant relationship was found between the concentration of triglycerides in sperm plasma and sperm motility and survival rates in native and cryopreserved semen.

Cholesterol is the main steroid in the sperm membranes of boars, bulls and stallions, while the main phospholipids include choline, ethanolamine and sphingomyelin. Stallion semen contains prostasome-like particles with a characteristic lipid profile and high levels of cholesterol and sphingomyelin. Cholesterol and phospholipids have been shown to be exchanged between sperm and seminal plasma [9]. Thus, the cholesterol level in seminal

plasma may influence the cholesterol content of the sperm membrane. Although it has been reported that there is no significant correlation between the concentration of cholesterol in seminal plasma and its concentration in the sperm membrane, it has been found that cholesterol-rich media can inhibit the acrosomal response, while phospholipids have the opposite effect [10]. Therefore, an extremely high or low concentration of cholesterol in seminal plasma may affect the exchange of cholesterol between spermatozoa and seminal plasma. When fertility is impaired, there may be a decrease in the amount of phospholipids in spermatozoa and an increase in cholesterol concentration. For the process of capacitation to proceed, sufficient phospholipids must be synthesized in the sperm membrane and excess cholesterol, as well as other steroids and proteins, must be removed. The determining factor controlling the rate of capacitation is the ratio of cholesterol to phospholipids in the semen. In the sperm plasma of subfertile stallions, the Cholesterol/Phospholipids ratio was about 2.5 times higher than in fertile stallions and ranged from 0.97 to 2.6 and 0.61 to 1.2, respectively [11].

On the other hand, increased cholesterol content in sperm membranes increases the cryoresistance of stallion spermatozoa. High cholesterol content may prevent premature capacitation of stallion spermatozoa and actually increase their viability both during hypothermic storage and after sperm cryopreservation. According to El-Badry D.A.M. et al. (2013) the Cholesterol/Phospholipids ratio in the sperm plasma of stallions with good sperm cryotolerance was significantly lower than that of stallions with poor cryotolerance (0.53±0.02 vs 0.78±0.01) [8]. The Cholesterol/Phospholipids ratio obtained by us is 0.21±0.12 (Table 2), which in general may indicate a rather high level of fertility and cryoresistance of semen of the studied animals.

It has been found that in bulls and rabbits in seminal plasma cholesterol is more abundant in the low-density lipoprotein fraction than in the high-density lipoprotein fraction [12]. In the stallions we studied, this pattern was confirmed (0.11±0.05 mmol/l of low-density lipoproteins vs 0.03±0.01 mmol/l of high density lipoproteins) (Table 2). Low density lipoproteins are thought to protect the sperm membrane from damage during sperm thawing. It is also known that the quality of bull semen after freeze-thawing is significantly improved when low density lipoproteins are added to the semen dilution medium [13]. Bergeron A. et al. (2006) showed that a family of lipid-binding proteins present in bull seminal plasma has a negative effect on sperm preservation because these proteins induce the removal of cholesterol and phospholipids from the sperm membrane. Low density lipoproteins interact with lipid-binding proteins, which prevents the removal of lipids from the sperm membrane and has a positive effect on sperm storage in liquid or frozen state [14]. A positive correlation was also found between sperm motility and low-density lipoprotein concentration in seminal plasma of young bulls [12]. In our study, the opposite data were obtained: the concentration of low-density lipoproteins was negatively correlated with the progressive sperm motility in the native ($r=-0.36$; $p<0.05$) and cryopreserved ($r=-0.35$; $p<0.05$) sperm as well as with total motility in native sperm. ($r=-0.45$; $p<0.01$) and frozen-thawed ($r=-0.27$; $p<0.05$) semen (Table 3). The role of low-density lipoproteins in stallion semen requires in-depth study.

In contrast, high-density lipoproteins are cholesterol acceptors and are involved in the initial stages of capacitation. High-density lipoproteins, the only class of lipoproteins present in the oviductal and follicular fluid of cows and women, is a more effective cholesterol acceptor than albumin [15]. In a study by Beer-Ljubic B. et al. (2009), a negative correlation between high density lipoprotein concentration and sperm motility was observed in older bulls [2]. We also found a negative correlation between the concentration of high-density lipoproteins and total sperm motility in native spermatozoa ($r=-0.36$; $p<0.05$) and cryopreserved ($r=-0.33$; $p<0.05$) sperm as well as with progressive motility in native sperm. ($r=-0.27$; $p<0.05$) and cryopreserved ($r=-0.35$; $p<0.05$) semen (Table 3).

4 Conclusion

The conducted research allowed us to study the relationship between the components of the lipid spectrum of sperm plasma and stallion semen parameters.

They found a negative correlation between low- and high-density lipoprotein concentrations with progressive sperm motility both in fresh ($p<0.05$), as well as in the thawed ($p<0.05$) semen. They also found a negative correlation between the concentration of low-density lipoproteins in sperm plasma with total sperm motility in fresh semen ($p<0.05$) and frozen ($p<0.01$) semen and high-density lipoprotein concentration in sperm plasma with total sperm motility in fresh and frozen semen ($p<0.05$).

They found a significant negative correlation between ejaculate volume and triglyceride concentration ($p<0.05$), cholesterol ($p<0.01$) and high-density lipoproteins ($p<0.05$). We found a positive correlation between sperm concentration and triglyceride content ($p<0.05$), cholesterol ($p<0.001$) and high-density lipoproteins ($p<0.01$). We assume that the obtained direct and inverse correlations between sperm plasma lipid spectrum parameters and ejaculate volume and sperm concentration parameters may indicate the testicular or epididymal origin of these parameters.

5 Acknowledgements

The research was carried out at the expense of the grant of the Russian Science Foundation No. 20-16-00101-P.

The research was carried out using the equipment of the Core Centrum of the FSBSI "All-Russian Research Institute of Horse Breeding".

References

1. M. M. Atroshchenko, M. G. Engalycheva, A. M. Kudlayeva, E. Yu. Borodkina, Horse Breeding and Equestrian Sport **1**, 4-8 (2021) doi: 10.25727/HS.2021.1.62626
2. M. M. Atroshchenko, A. M. Kudlaeva, M. A. Fomina, V. V. Kalashnikov, et al., IOP Conf. Series: Earth and Environmental Science **341**, 012162 (2019) doi:10.1088/1755-1315/341/1/012162
3. T. Katila, M. Kareskoski, E. Akcay, et al., Havemeyer Foundation Monopgraph Series **18**, 3-5 92005)
4. D. M. Niu, J. J. Wang, Zhonghua Nan Ke Xue **15(7)**, 651-655 (2009)
5. F. Lahnsteiner, R. A. Patzne, T. Weismann, Reproduction Nutrition Development **33**, 349-360 (1993) doi: 10.1051/rnd:19930404
6. M. Halo Jr., F. Tirpák, A. Kováčik, P. Lípová, A. Greń, P. Massányi, Journal of Microbiology, Biotechnology and Food Sciences **7(5)**, 516-518 (2018) doi: 10.15414/jmbfs.2018.7.5.516-518
7. M. Cevik, P.B. Tuncer, U. Tafidemir, T. Ozgurtafi, Turkish Journal of Veterinary and Animal Sciences **31(6)**, 381-387 (2007)
8. D.A.M. El-Badry, F.I. Gabr, The Journal of the Egyptian Medical Association **73(4)**, 749-768 (2013)
9. L. Huacuja, N. M. Delgado, L. Calzada, A. Wens, R. Reyes, N. Pedrón, A. Rosado, Archives of Andrology **7(4)**, 343-349 (1981) doi: 10.3109/01485018108999329
10. N. L. Cross, Biology of Reproduction **54**, 138-145 (1996) doi: 10.1095/biolreprod54.1.138

11. S. P. Brinsko, C. C. Love, J. E. Bauer, M. L. Macpherson, D. D. Varner, Animal Reproduction Science **99**, 65-71 (2007) doi: 10.1016/j.anireprosci.2006.03.018
12. B. Beer-Ljubić, J. Aladrović, T.S. Marenjak, R. Laškaj, I. Majić-Balić, S. Milinković-Tur, Theriogenology 72(8), 1132-1140 (2009) doi: 10.1016/j.theriogenology.2009.07.009
13. M. Moussa, V. Martinet, A. Trimeche, D. Tainturier, M. Anton, Theriogenology **57**, 1695-1706 (2002) doi: 10.1016/s0093-691x(02)00682-9
14. A. Bergeron, P. Manjunath, Molecular Reproduction and Development **73(10)**, 1338-1344 (2006) doi: 10.1002/mrd.20565
15. J. Langlais, F. W. K. Kan, L. Granger, L. Raymond, G. Bleau, K. D. Roberts, Gamete Research **20**, 185-201 (1988) doi: 10.1002/mrd.1120200209