Seasonal variation in macronutrient content of stallion spermoplasm

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Abstract. The effects of seasonal factor on the concentration of macronutrients: calcium, potassium, sodium, phosphorus, chlorine and magnesium in the spermoplasm of stallions (n=12) were studied. The average age of animals was 11.2±0.8 years, the animals were located in the central strip of Russia (Ryazan region). We established reliable differences between the content of macronutrients in spermoplasm depending on the season of the year. We obtained data on the dependence on the season of the year of calcium concentration by 30% ($p<0.001$), phosphorus by 15% ($p<0.05$). The maximum values of average concentrations of calcium, potassium and magnesium in spermoplasm were recorded in spring-summer period, sodium and chlorine concentrations in winter. It was also stated that phosphorus concentration had no significant changes in winter, fall and spring, but was lower in summer.

1 Introduction

Numerous factors, such as heredity, age of the animal, individual characteristics of the organism, feeding and housing conditions, affect the quality parameters and resistance of semen to cryopreservation [1-4].

Currently, the results of a large amount of research show that one of the important factors affecting semen quality and cryostability is the elemental status of the organism [5-7], the maintenance of which is considered a prerequisite for its normal functioning [8-10].

Discrepancies in the content of mineral substances in the animal organism, occurring under the influence of exogenous and endogenous factors, have negative consequences on animal husbandry as a whole, which is expressed in the reduction of productivity and fertility of animals, reduced growth, weakening of immunity. Distortion of these values, in particular, can lead animals to pathological conditions, causing, among other things, increased mortality. The cardinal development of technologies of livestock farms and industrial complexes only increases the value of works devoted to studying the elemental status of farm animals and poultry [11- 14].

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It is known that macroelements in the composition of seed plasma play an important role in the reproduction of farm animals [15]. It has been found that element imbalance affects reproductive performance and that elements such as calcium, potassium, magnesium, sodium, chlorine and phosphorus may be essential for reproduction and their deficiency may lead to degenerative changes in spermatogenesis [8, 16-19]. Calcium and phosphorus maintain the functional integrity of spermatozoa [20, 21]. Moreover, the positive effect of calcium on sperm motility was reported by Halo M. et al. (2018) and Marzec-Wroblewska U. et al. (2012) [22, 23]. Magnesium contained in spermoplasm in higher concentration than in blood plasma ensures the activity of seminal enzymes. Low magnesium levels, according to Alloush M. et al. (2006), are associated with premature ejaculation [24].

Some researchers have indicated in their works that the effect of season of the year on seminal plasma composition is more pronounced than on sperm characteristics, and seasonal differences in spermoplasm composition in stallions are probably due to changes in sex steroid levels, which show seasonal variations in both blood and seminal plasma [25].

In males, routine evaluation of seminal plasma includes the analysis of some elements that are associated with semen quality because of their antioxidant properties [25, 26]. However, insufficient research has been conducted on macronutrient content in animal seminal plasma [27]. Few works studying the effect of the season of the year on the content of macronutrients in the spermoplasm of stallions in the scientific literature have been found, although such research makes it possible to significantly expand the knowledge of physiology and reproduction of animals.

The objective of this work was studying the effect of seasonal factor on the content of macronutrients in the spermoplasm of stallions kept in the average strip of Russia.

2 Methods and materials

Studying and analysis were carried out on the basis of the All-Russian Research Institute for Horse Breeding (ARRIH, Ryazan Region) in 2020-2021. All conditions of housing and feeding of the animals under study at the time of testing were in compliance with the established norms and requirements.

Semen obtaining. Semen was obtained from 12 stallions of different breeds (average age 11.2±0.8 years) two times a month (in the middle of each month) during a year with an interval of 48 hours. Semen was obtained per mare in hunting using an artificial vagina (sample of the All-Russian Research Institute of Horse Breeding). A second ejaculate was taken for research of spermoplasm parameters. The first ejaculate, obtained after the period of sexual rest, was not used in this research.

Obtaining spermoplasm. Seminal plasma was obtained by centrifugation of native semen at 3000 rpm for 20 min. After microscopy of the supernatant for the presence of spermatozoa, sperm free aliquots of seminal plasma were frozen in Eppendorf tubes (2.0 ml) at -18 ºC and stored until research at the same temperature.

Biochemical research of spermoplasm. Concentrations in spermoplasm of macronutrients: calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P) were determined using AU 480 biochemical analyzer (Beckman Coulter, USA).

Statistical analysis. For statistical processing of the data obtained, the average value for each indicator was taken for a season of the year. Statistical processing was performed using Microsoft Excel 2010 and Statistica 8.0 programs (“Stat Soft Inc.”, USA). The data were processed using conventional methods of variation statistics and expressed as the arithmetic mean (M) and its standard error (m). Duncan's posterior rank criterion was used to assess differences in averages between paired groups. To reflect the proportion of

variation in the effective feature due to variation in the factorial feature, we calculated the determination coefficient R2 and R2 adjusted. The significance level (p) was used to assess the reliability of the obtained relationship. Differences were considered reliable at $p \leq 0.05$.

3 Results and discussion

The Ryazan region, where the animals are kept, is located in the average strip of the Russian Federation. The climate of the Ryazan region is moderately continental, with relatively cold winters and warm summers. Therefore, animals are not affected by too low temperatures in winter and too high temperatures in summer. We assume that the effect of seasonal factor on the organism of animals from this climatic zone is not as significant as in a sharply continental climate. However, changes in temperature regime and length of daylight hours invariably affect the animal organism, including the reproductive system.

Some researchers point out the differences in the elemental composition of spermoplasm depending on the season of the year. Thus, in the seminal plasma of rams, the concentrations of calcium, sodium, zinc and manganese were higher in the breeding season than in the period of sexual rest, while the concentrations of phosphorus, chromium, molybdenum and boron were lower in the breeding season [28].

According to the results of our research, the content of calcium and phosphorus in the spermoplasm of stallions in summer differed significantly from their levels in other seasons of the year, and potassium - in summer from the concentrations in fall and winter (Table 1).

Table 1. Approximate probabilities for a posteriori criteria for the dynamics of macronutrient content dynamics in stallion spermoplasm depending on the season of the year.

Indicator, units.	Season	Season			
		Spring	Summer	Autumn	Winter
Calcium, mmol/L	Spring		0.010816	0.426047	0.111706
	Summer	0.010816		0.001757	0.000192
	Autumn	0.426047	0.001757		0.366266
	Winter	0.111706	0.000192	0.366266	
Potassium, mmol/L	Spring		0.102955	0.647911	0.313808
	Summer	0.102955		0.049547	0.014258
	Autumn	0.647911	0.049547		0.536145
	Winter	0.313808	0.014258	0.536145	
Sodium, mmol/l	Spring		0.276094	0.959507	0.486712
	Summer	0.276094		0.268732	0.093379
	Autumn	0.959507	0.268732		0.483608
	Winter	0.486712	0.093379	0.483608	
Chlorine, mmol/L	Spring		0.851963	0.976487	0.538717
	Summer	0.851963		0.865101	0.630268
	Autumn	0.976487	0.865101		0.541689
	Winter	0.538717	0.630268	0.541689	
Phosphorus, mmol/L	Spring		0.013949	0.993787	0.767152
	Summer	0.013949		0.011813	0.019123
	Autumn	0.993787	0.011813		0.757874
	Winter	0.767152	0.019123	0.757874	
Magnesium, mmol/l	Spring		0.329035	0.387020	0.290054
	Summer	0.329035		0.084979	0.057187
	Autumn	0.387020	0.084979		0.792841
	Winter	0.290054	0.057187	0.792841	

According to the data in Table 2 - calcium concentration in spermoplasm depended on the season of the year by 30% ($p<0.001$), and phosphorus by 15% ($p<0.05$).

Table 2. Determination coefficients and significance levels of macronutrient content in stallion spermoplasm depending on the season of the year.

Indicator	R^2	R^2 correct.	p
Calcium	0.349919	0.301163	0.000576
Potassium	0.172579	0.110523	0.053349
Sodium	0.082732	0.013937	0.321199
Chlorine	0.014871	-0.059014	0.894894
Phosphorus	0.211051	0.151880	0.022320
Magnesium	0.121941	0.056087	0.153368

Note: R^2 is the determination coefficient, which shows the proportion of variation that is explained by the conjugacy of variation between the signs. Like the original determination coefficient, the adjusted coefficient (R^2) makes it possible to assess the accordance of the regression model with the original data, as well as to compare models with a different number of independent variables. The level of significance (p) reflects the probability of error in the conclusions of the statistical calculation.

Seasonal indices of elemental composition of spermoplasm of the studied stallions are presented in Table 3.

Table 3. Macronutrient content in stallion spermoplasm in different seasons of the year, (M±m).

Indicator, units.	Season			
	Spring	Summer	Autumn	Winter
Calcium, mmol/L	2.78±0.50	5.04±0.93	2.10±0.46	1.33±0.30
Potassium, mmol/L	18.89±0.10	22.21±1.65	17.97±1.83	16.73±0.86
Sodium, mmol/L	83.16±5.04	73.67±6.56	82.75±6.32	88.85±4.33
Chlorine, mmol/L	90.17±3.32	91.25±4.01	90.33±3.88	93.84±3.51
Phosphorus, mmol/L	0.73±0.10	0.41±0.05	0.73±0.09	0.70±0.06
Magnesium, mmol/L	4.29±0.64	5.15±0.53	3.53±0.67	3.30±0.56

Studying the scientific literature, it was found that most of the publications are devoted to the influence of seasonal factor on the concentrations of macronutrients in the blood serum of horses. At the same time, many researchers have observed an increase in the content of the determined indicators in the breeding season. Thus, Dmoch M. et al. (2008) found that in summer there was a higher content of calcium and magnesium in the blood serum of horses [29]. The research of Fedosova O.A. (2017) found that in spring, there were higher concentrations of calcium and phosphorus ($p<0.001$), as well as potassium ($p<0.01$) and chlorine in the blood serum of stallions. Only sodium concentration increased in winter ($p<0.01$), compared to other seasons [30].

According to other authors on the contrary, the maximum values of macronutrients in blood plasma were observed in the fall-winter period. For instance, Górski K. et al. (2017) observed a significant ($p<0.05$) increase in serum calcium concentration in fall in both mares (end of May - 3.06±0.07 and end of November - 3.19±0.09 μmol/L) and geldings (end of May - 3.02±0.13 and end of November - 3.12±0.12 μmol/L) [31]. In the research of Gündüz H. et al. (2000), results were obtained in which it was found that the calcium and phosphorus contents in the blood of stallions were higher in winter (calcium: winter - 11.02±0.43, spring - 8.24±0.49, summer - 10.16±0.36 % mg; phosphorus: winter - 3.14±0.15, spring - 2.4±0.21, summer - 2.48±0.22 % mg) [32]. At the same time, plasma

calcium values were significantly different in winter from spring ($p<0.001$), spring from summer ($p<0.05$), and phosphorus values were significantly different in winter from spring ($p<0.05$) [32].

In several other works, heterogeneous data were obtained. Thus, according to Vranković L. et al. (2015) the highest plasma calcium concentrations in stallions were found in fall and were significantly higher than values measured in spring ($p=0.042$) and summer ($p=0.003$). However, inorganic phosphorus and magnesium concentrations were significantly lower in fall and winter compared to concentrations measured in spring and summer ($p=0.002$, $p<0.001$ and $p<0.001$, $p=0.002$, respectively) [33]. In research on Noma breed horses, some blood parameters were found to be higher in winter: calcium was 12.71±0.58 in winter and 12.08±0.53 mg/dL in summer ($p<0. 0001$); inorganic phosphorus - 2.67±0.51 in winter, 2.51±0.51 mg/dL in summer; chlorides - 104.7±1.6 in winter, 101.8±2.7 mEq/l in summer ($p<0.0001$); sodium - 141.0±2.2 in winter, 137.6±2.4 mEq/l in summer ($p<0.0001$). But the plasma concentration of other macronutrients, on the contrary, increased in summer. For instance, magnesium concentration was 2.00±0.17 mg/dL in winter and 2.14±0.17 mg/dL in summer; potassium concentration was 4.20±0.60 in winter and 4.70±0.70 mEq/l in summer [34]. Such different data obtained due to the influence of both different climatic conditions and diet, physical loads, physiological state of animals in different seasons of the year.

Research aimed at studying the influence of the season of the year on the macroelement composition of stallion spermoplasm is quite few, so our work can also contribute to studying the influence of the seasonal factor on the reproductive status of stallions.

We found that the maximum concentrations of calcium in stallion spermoplasm were observed in spring and summer (2.78±0.5 and 5.04±0.93 mmol/l, respectively), and the phosphorus content was minimal in summer 0.41±0.05 mmol/l (Table 3). Similar data were obtained by other authors. According to Gündüz H. et al. (2000) in stallion spermoplasm, calcium was higher in summer (13.22±2.54% mg) than in winter (12.66±2.81% mg); phosphorus, on the contrary, was higher in winter (9.75±1.65% mg) than in summer (8.83±1.29% mg) [32]. According to Pesch S.K. (2005) the concentration of ionized calcium in stallion spermoplasm was higher in April-May and July-August (1.6 and 1.5 mmol/L, respectively) than in November-December (1.2 mmol/L). Phosphorus concentration increased in November-December (2.6 mmol/L) than in spring and summer (2.0 and 2.5 mmol/L, respectively). Thus, according to Pesch S.K. (2005), the increase in calcium concentration may be related to the increased activity of the accessory sex glands during the breeding season, which are probably the main source of this macronutrient [35].

Studying the influence of the seasonal factor on semen and spermoplasm parameters will make it possible to identify optimal seasons for cryopreservation of stallion semen, studying how spermoplasm composition changes depending on the season of the year. Thus, the conducted research made it possible to obtain new information on the influence of the seasonal factor on the macroelement composition of spermoplasm and to identify reliable differences in the concentration of a number of elements in seminal plasma depending on the season of the year.

4 Conclusions

The conducted research made it possible to study the influence of seasonal factor on the content of macronutrients in stallion spermoplasm. We found significant differences between the content of macronutrients depending on the season of the year. The concentration of calcium in spermoplasm depended on the season by 30% ($p<0.001$), and phosphorus by 15% ($p<0.05$). The maximum values of average concentrations of calcium, potassium and magnesium in spermoplasm were in the breeding season (spring-summer

period), sodium and chlorine - in winter, and phosphorus concentration had no significant fluctuations in winter, fall and spring, but was lower in summer.

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