Scientific Papers. Series D. Animal Science. Vol. LVIII, 2015
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

THE PROTECTIVE EFFECT OF L-CARNITINE DURING THE HYPOTHERMIC STORAGE OF BOAR SEMEN

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Abstract

The effectiveness of livestock farming in great extent depends on the efficiency of animal reproduction. Therefore, researches in this direction acquire theoretical and practical significance. Given the fact that in the practice of reproduction of pigs widely was used the method of artificial insemination of sows' chilled sperm, the purpose of the conducted research was to improve the synthetic mediums through introducing into their composition of the biologically active compounds. As such substance was used L-carnitine. By method of consecutive rows was determined dose-dependent effect of investigated substance included in the base medium on the basis of electrolytes and non-electrolytes. It was found that after dilution of boar sperm the mobility of spermatozoa and their absolute indicator of survival after 12 hours of storage at hypothermic temperatures (16-18˚C) is increased by a statistically significant amount. It is concluded about protective and stimulating activity of L-carnitine at the dilution and storage of boar semen.

Key words*: synthetic mediums, hypothermic storage, sperm, L-carnitine, boar semen.*

INTRODUCTION

The possibility of dynamic growth in livestock production is determined, among other factors, the intensification of reproduction of the herd using artificial insemination. Zoo veterinarian benefits of this biotechnological method of reproduction in comparison with natural reproduction determine its leading role in the technology of production of pigs in farms with different production and economic structure. However, the potential of artificial insemination of pigs is not realized in full. And the reason for this is the fact that the freezing and long-term preservation of boar semen not yet found wide industrial application in connection with cryo technological difficulties and insufficient knowledge of species-specific, morpho–physiological, biochemical and physico–chemical characteristics of reproductive cells of these species (Федина, 2007). In addition, there are significant age, breed and individual differences in cryo sustainability of seed material, and the preparation of sperm for long-term storage also leads to serious disturbances of lipid metabolism, manifested in the change of the content of glycolipids, phospholipids and fatty acids. Progressive method of embryo transfer, due to the complex and costly mediums, tools and equipment also are not used in practice. In this regard, in production conditions generally is accepted the insemination of sows with diluted boar semen stored in hypothermal conditions at 16–18 ºC. However, in this case too more intensively metabolic processes occur. As a result, there is accumulation of toxic products of metabolism and the decrease of the functional parameters of spermatozoa.

Given the fact that under the influence of drugs containing carnitine was noted normalization of acrosomal reaction of spermatozoa (Zhou et al., 2007), was detected a correlation between the concentration of carnitine in sperm and the integrity of the nuclear DNA of the gametes (De Rosa et al., 2005), osmotic resistance of spermatozoa (Yeste et al., 2010), as well as the positive impact of the use of carnitine on the levels of reduced glutathione and 8-hydroxydeoxyguanosine in the testes (Abd-Allah et al., 2009), but the molecular mechanisms of antioxidative action of carnitine

remain unclear until the end (Wang et al., 2010), which served as a prerequisite for its introduction in the composition of the synthetic medium for boar semen.

Based on the above, the purpose of the conducted research was to study the qualitative indicators of reproductive cells of the boar stored after dilution with synthetic medium containing biologically active substance L-carnitine.

MATERIALS AND METHODS

As experimental material used semen of the boars breeders of Landrace breed which contained in the conditions of the breeding enterprise "Moldsuinhibrid", the relevant veterinary requirements. The sperm was received by fractional method using an artificial vagina, the temperature of which was in the range 38-40 °C. For dilution it was used the synthetic medium consisting of glucose, EDTA and sodium citrate. Medium components were dissolved in bidistilled water. In our experiments we used pharmacological 2% L–carnitine and ferric sulfate chemically pure quality. Dilution of semen was performed 1:1 in compliance with the rules of asepsis when working with the experimental material. After that, it was kept in hypothermal conditions at room temperature. The optimal concentration of the test substances was determined by the method of consecutive rows of Milovanov V. K. (Милованов, 1962). Qualitative indicators of the diluted semen were determined using a light microscope "AMPLIVAL" of company Carl Zeiss (Jena) at 200 × magnification. Sperm motility was determined in points on a ten-point grading scale, and the absolute survival rate (ASR) is in conventional units, which is the sum of survival indices multiplied by the number of hours their survival.

Statistical processing of the results of research were conducted using the criterion of Student's t-test.

RESULTS AND DISCUSSIONS

The synthetic mediums for dilution and storage of semen of farm animals at 16–18ºC, as a rule, are not complex. They are designed to maintain osmotic pressure, pH and the viability of spermatozoa. However, the problem of improving of the functional status and increasing of life expectancy of the cells continues to be relevant for practitioners involved in the swine reproduction. Therefore, we consider it expedient to enter into the composition of mediums the components which contribute to homeostasis of metabolic processes. For this purpose, we have studied the protective properties of L-carnitine in the composition of the basic medium for dilution and storage of semen of the boar. The results of conducted researches are presented in table 1.

Table 1

Dose-dependent effect of L-carnitine in the hypothermal storage of boar semen

The experimental variant	Concentration of L-carnitine, mg/ml	Motility of spermatozoa after dilution, points	ASR, c.u.	
			After 12 hours	After 24 hours
1	Control	6.2 ± 0.42	74.7 ± 5.02	132.0 ± 18.00
2	0.02	7.6 ± 0.27	88.8 ± 5.37	165.7 ± 10.73
3	0.04	7.6 ± 0.27*	91.2 ± 3.29*	177.6 ± 6.57
4	0.08	7.6 ± 0.27*	91.2 ± 3.29*	177.7 ± 6.57
5	0.16	7.0 ± 0.35	84.0 ± 4.24	160.8 ± 9.10
6	0.32	6.9 ± 0.27	81.6 ± 4.03	160.8 ± 9.10

*The difference is statistically authentic

The data of table 1 demonstrates that L-carnitine has a dose-dependent effect. Its use in the composition of the medium for dilution of boar semen at a concentration of 0.04-0.08 mg/ml has a positive effect on the functional indices of reproductive cells. In the best experimental variants the motility of spermatozoa after dilution and absolute survival rate after 12 hours of storage of the sperm at 16-18ºC amounted respectively 7.6 ± 0.27 and 91.2 ± 3.29, which indicates an increase of the studied parameters on 22.6 and 22.1% compared to the control variant. L-carnitine (3-Hydroxy-4-(trimethylazaniumyl) butanoate) refers to indispensable substances because they perform the basic role in the transport of fatty acids across the mitochondrial membrane (Спасов et al., 2005). However, L-carnitine is synthesized also in the animal body in the liver and kidneys where through the blood stream is transported to other tissues and organs. Great interest to L-carnitine is due to its role in metabolic processes. Among them we

should mention: transport of long-chain fatty acids into the mitochondrial matrix, where they are included in the process of formation of acetyl coenzyme A; stabilization of the content of the acetyl coenzyme A and the deletion of short-chain fatty acids from mitochondria; regulation of the contents of the CoASH, which is required for detoxification of metabolic products; the maintenance of the optimal ratio of acetyl CoA/CoASH for stimulation of anabolic processes; the maintenance of cell activity through the involvement of L-Carnitine in energy metabolism with the participation of phospholipids (Копылевич, 2005). It is suggested that the increased of mitochondrial energy metabolism may indirectly prevent the formation of free radicals (Abd-Allah et al., 2009; Lombardo et al., 2011). In this regard, it is recommended to use as the most widely utilized antioxidant for regulation of metabolic processes of spermatozoa (De Rosa et al., 2005; Божедемов et al., 2012).

From the analysis of the submitted information it follows that the protective effect of carnitine in the composition of the medium for dilution and hypothermal storage of boar semen, mainly may be due to the regulation of energy metabolism and the detoxification of the products of this process. At the same time, it is obvious that the normalization of the antioxidant characteristics of seminal fluid is a mandatory prerequisite for the recovery of the fertilizing ability of ejaculate, especially in conditions of oxidative stress in hypothermal storage of sperm. The data presented in table 1 prove conclusively that vitamins can perform a protective function, protecting the spermatozoa of the boar from the harmful effects of internal and external factors.

Minerals, along with vitamins and other biologically active substances, are mandatory elements providing cell viability. Therefore, in the next series of experiments it was investigated efficacy of ferric sulfate in hypothermal storage of boar semen and determined its optimal concentration (table 2).

The study results which are presented in table 2 show that the optimal concentration of ferric sulfate in the composition of the medium is 0.6 mg/ml. In this experimental variant, the motility of spermatozoa was 6.9±0.11 points and the absolute survival rate after 12 hours of storage has reached 82.8 ± 1.34 c.u., which respectively is more with 13.4 and 13.1% in comparison with the control variant.

Table 2

The influence of ferric sulfate on the functional indices of boar semen

The experimental variant	Concentration of ferric sulfate, mg/ml	Motility of spermatozoa after dilution, points	ASR, c.u.	
			After 12 hours	After 24 hours
1	Control	6.1 ± 0.17	73.2 ± 1.34	72.0 ± 12.01
2	1.0	6.0 ± 0.01	72.0 ± 0.01	0
3	0.8	6.1 ± 0.27	73.2 ± 6.57	19.2 ± 2.15
4	0.6	6.9 ± 0.11*	82.8 ± 1.34*	96.0 ± 4.24
5	0.4	6.7 ± 0.22	80.4 ± 2.68	96.0 ± 4.24
6	0.2	6.1 ± 0.17	73.2 ± 2.68	86.4 ± 6.57

*The difference is statistically authentic

The positive effect of the use of ferric sulfate may be due to the fact that this element not only maintains the water-salt metabolism, but also participates in the composition of cytochromes in a number of redox reactions (Овчинников, 1987). As a result, may be the inclusion of certain processes that lead to an increase of the functional activity of spermatozoa.

CONCLUSIONS

The researches allow making the following conclusions:

1. For maintenance of the functional status of boar semen after it is received the significant role acquires the regulation of metabolic processes at different technological stages.
2. Improving the quality of boar semen stored at hypothermal conditions can be realized through including in the composition of mediums of L-carnitine and ferric sulfate.
3. For hypothermal storage of boar semen is preferable to use a dense fraction, the volume of which will be increased after the first and repeated dilution.
4. If necessary the prolonged storage of boar sperm after its receipt should be diluted one-for-one with medium containing L-carnitine, and before the actual research of artificial insemination of sows re-diluted one to 0,5 with a similar medium containing ferric sulfate.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Physiology and Sanocreatology and was financed from Project "Nutrition in accordance with constitution types. The impact of nutrition on the sanogenity of male gametes".

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