ISSN 1392-0146 (print)
ISSN 2029-0578 (online)
www.lmaleidykla.lt

Biologija

Bendroji, molekulinė ir ląstelės biologija • Biochemija • Biofizika • Biotechnologija • Bendroji, molekulinė ir landšaftų ekologija • Antropogeninis poveikis ekosistemoms • Ekosistemų apsauga • Genetika • Mikrobiologija • Imunologija • Parazitologija • Fiziologija •

General, Molecular and Cell Biology • Biochemistry • Biophysics • Biotechnology • General, Molecular and Landscape Ecology • Anthropogenic Impact on Ecosystems • Ecosystem Protection • Genetics • Microbiology • Immunology • Parasitology • Physiology •

2020 Vol. 66 No. 1

Published: 2018-01-19

Biophysics

- **Event-related synchronization/desynchronization in terms of manual motor program switching in men**

  Olha Korzhyk, Olha Pavlovych, Lyudmyla Shvarts, Tetyana Shevchuk, Olena Dmytrotsa, Andriy Poruchynskiy, Alevtyna Morenko

  - PDF

Physiology

- **Intensity of oxygen consumption by bull sperm due to the action of L-carnitine**

  Viktoriia Koberska, Oleh Yermishev

  - PDF

Ecology

- **Structure of the oribatid mite population (Acariformes, Oribatida) in the topsoil within forest plantations in reclaimed territories (Ukraine)**

  Yuriy Kulbachko, Oleh Didur, Yuliia Ovchynnykova

  - PDF

Genetics

- **Identification of informative markers of agronomic traits in different ecotypes of sand plantain (Plantago psyllium)**

  Mehdi Rahimi, Hamid Hatami Maleki, Mojtaba Mortezavi

  - PDF

Biotechnology

- **Factors affecting Zantedeschia Spreng. dedifferentiation in vitro**

  Vaida Jonytienė, Ramunė Masienė, Natalija Burbulis, Aušra Blinstrubienė

  - PDF

BIOLOGIJA. 2017. Vol. 63. No. 4. P. 306–312

Intensity of oxygen consumption by bull sperm due to the action of L-carnitine

Viktoriia Koberska,

Oleh Yermishev*

Department of Human and Animal Physiology, Vasyl' Stus Donetsk National University, 600-richchya St. 21, Vinnytsia 21000, Ukraine

The research is devoted to the study of peculiarities of oxygen consumption intensity and the activity of enzymes of antioxidant protection of bull sperm due to the action of L-carnitine. We used bull sperm that was mixed with sperm dilution for the "Bioexel" to the ratio 1:1. Diluted semen was divided into two parts – one control and three experimental. L-carnitine was added to the diluent in the amount of 10 mg/100 ml in part 2, 30 mg/100 ml in part 3, and 60 mg/100 ml in part 4; the sperm was dissolved in this liquid to the ratio of 3:1. The intensity of oxygen consumption, the activity of enzymes superoxidedismutase, catalase, glutathioneperoxidase and survival of spermatozoa were thoroughly investigated in the diluted semen. L-carnitine, which was added to the diluent of semen 10 and 30 mg/100 ml, stimulates aerobic glycolysis by 5.9 and 11.9%, and decreases oxidative processes by 5.6% and 11.4%, respectively. It is not related to the synthesis of ATP. It increases the survival of bull spermatozoa. The results of current research show that carnitine promotes a rise in the activity of CAT and GPO, and the activity of SOD decreases. We established that L-carnitine impacts the reduction of the overall consumption of oxygen by increasing the share of mitochondrial oxygen consumption and aerobic glycolysis, along with the decrease in free radical oxidation. It is probably related to the metabolic activation between the semen and the environment. We found that the optimal concentration of L-carnitine in the bull diluted sperm is 30 mg/100 ml.

Keywords: oxygen consumption, antioxidant enzymes, L-carnitine, semen, bulls

INTRODUCTION

Physiological quality of sperm, its survival and fertilization are defined by biochemical processes that occur in the male and female body *in vivo*, and in habitats *in vitro*. However, depending on the components and their ratio in the dilution of ejaculates the use of substrates, survival, and the ability to fertilize gametes changes.

It is known (Agarval et al., 2005) that after ejaculation spermatozoa have weak endoplasmic protection and are the most sensitive to oxidative stress. This is due to the change of environment, aeration, and the access to the secret ejaculate of active forms of oxygen (AFO), which are involved in the regulation of the basic functions of male

* Corresponding author. Email: o.yermishev@donnu.edu.ua

germ cells (Aitken et al., 1996). The determining factor in this process is the amount of absorbed oxygen (Noland et al., 2009). Thus, any damage to the inner mitochondrial membrane activity violates the chain of the electron transport, contributing to the generation of the AFO (Calò et al., 2006; Vlizlo et al., 2012). Therefore the respiratory activity of cells, except for the processes that provide energy, characterizes oxidative processes which are associated with the formation of AFO.

An important role in cell energy processes is performed by L-carnitine, which regulates the intensity of energy metabolism in mitochondria. The association has acylic radical release of CoA and connects the bound intermediates of the oxidative processes (Di Paolo et al., 2006). L-carnitine is found in high concentrations in the epididymis (Hu et al., 2010), where it plays an important role in sperm maturation, affects its mobility, and serves as an antioxidant (Eskenazi et al., 2005).

MATERIALS AND METHODS

We studied the intensity of oxygen consumption and the activity of enzymes of the antioxidant system in the semen of bulls. The aim of this study was to investigate the impact of L-carnitine on the intensity of redox processes in semen and to identify links between biochemical and physiological parameters of the ejaculates.

The study was done at the Institute of Animal Biology of the National Academy of Agricultural Sciences of Ukraine. The material for laboratory research was semen of 6-year-old Holstein bulls. Ten samples of their sperm diluted 1:1 were used for the experiment. They were divided into one control and three experimental parts (Table 1). The proportion of the diluent to the diluted semen carnitine was 3 : 1.

The intensity of oxygen consumption is polarographic (ng-atom O/min × 0.1 ml of semen) at the temperature of 38.5°C, the activity of enzymes superoxidedismutase (SOD), catalase (KAT), and glutathioneperoxidase (GPO) were investigated (Vlizlo et al., 2012) in the diluted semen. Determination of oxygen consumption was carried out polarographically (ng-atom O/0.1 ml of sperm suspension per minute) in a thermostated cell (+38.5°C) with automatic registration of the process. The method is based on the ability of sperm to "absorb" oxygen and to change the oxidation-reducing potential, with what changes the voltage on the electrodes, which is recorded by the polarograph and potentiometer. In this case, we used inhibitors: glycolysis – NaF; NADH-dependent part of the respiratory chain – amital; terminal – NaN_3. The activity of SOD was determined by the level of inhibition by the enzyme of the reduction of nitrosine tetrazolium using a spectrophotometer at a wavelength of 540 nm. The activity of KAT was determined by a method based on the ability of the hydrogen peroxide to form a coloured complex with molybdenum salts, which was measured on a spectrophotometer at a wavelength of 410 nm. The activity of GPO was determined by the rate of oxidation of glutathione using a spectrophotometer at a wavelength of 412 nm. We also determined the survival of sperm at the temperature of 2–4°C until they stopped their movement. Statistical analysis of the results was carried out using the program Microsoft Office Excel.

It is known that sperm produces ATP during glycolysis and respiration. In order to establish the amount of the oxygen that cells used in

Table 1. **The scheme of the experiment**

Parts of sperm	Diluent	The added quantity of L-carnitine
1 – control	Bioexel	–
2 – experimental	Bioexel and added L-carnitine	10 mg/100 ml
3 – experimental		30 mg/100 ml
4 – experimental		60 mg/100 ml

these processes, we added the following inhibitors to the semen: aerobic glycolysis – sodium fluoride (NaF; 10^{-3} M), NAD-dependent electron transport chain parts – amital (5×10^{-3}M) and terminal parts (cytochrome) – sodium azide (NaN_3; 5×10^{-2} M). To determine the proportion of the oxygen consumed during the oxidation of free fatty acids, we used Na_2EDTA (0.6×10^{-3} M). The research on the intensity of breath spermatozoa was provided in phosphate-saline buffer (NaCl – 0.8 g, KCl – 0.02 g, Na_2HPO_4 – 0.11 g, KH_2PO_4 – 0.02 g, $MgCl_2$ – 0.01 g, H_2O – 100 ml).

RESULTS

The results showed that the presence of L-carnitine in bull sperm affects the physiological parameters and changes in the respiratory activity of sperm (Table 2).

Our research showed that the addition of L-carnitine to the semen affects the respiratory activity of spermatozoa. We established a probable decrease of oxygen consumption that was within 10.17 and 13.88 ng-atom O/min × 0.1 ml of semen. It should be noted that the intensity of oxygen consumption by sperm using the electron transport links to the acceptor (oxygen) is also different in experimental parts of sperm if compared to the control one. Particularly, the dilution of ejaculates with carnitine at the concentration of 10 mg/100 ml, the quantity of oxygen consumed by aerobic glycolysis was 2.9 ng-atom O/min × 0.1 ml of semen (23.2% of the oxygen used by semen), at the concentration of 30 mg/100 ml – 2.97 ng-atom O/min × 0.1 ml of semen (29.2%), and at 60 mg/100 ml – 2.8 ng-atom O/min × 0.1 ml of semen (27.4%). But in the control part the respiratory activity level was 2.4 ng-atom O/min × 0.1 ml of semen that corresponded to 17.3% of the total consumption of oxygen.

We also found a difference in the intensity of respiration by NAD-dependent mitochondrial respiratory links in the chain. With the dilution of ejaculates in parts 2, 3, and 4, the amount of the activity of the respiratory electron transport system was 1.94; 2.43; 2.28 ng-atom O/min × 0.1 ml of semen. It was 15.7%, 23.9%, and 22.4% of the total consumption of oxygen, respectively. In the control part, the consumption amounted to 1.55 ng-atom O/min × 0.1 ml of semen (11.2%).

Thus, the semen with L-carnitine had a higher level of electron transport through NAD-dependent mitochondrial respiration link of spermatozoa, while the highest oxygen consumption was in part 3.

The oxygen consumption by the terminal link of airway in the second part of sperm was 20.2%, in part 3 – 17.7%, and in part 4 – 19.6% of the oxygen consumed by sperm, while in the control part this index was 15.1%. Consequently, the use of carnitine improves electron transport through a terminal respiratory chain of spermatozoa to the acceptor – oxygen.

Table 2. **Intensity of oxygen consumption by bull sperm that was diluted after adding L-carnitine (M ± m, *n* = 10)**

Oxygen consumption, ng-atom O/min × 0.1 ml of semen	Parts of sperm			
	1 – control	2 – 10 mg/100 ml L-carnitine	3 – 30 mg/100 ml L-carnitine	4 – 60 mg/100 ml L-carnitine
Sperm	13.88 ± 0.55	12.39 ± 0.61	10.17 ± 0.67***	10.18 ± 0.44***
under the action of inhibitors: NaF	11.48 ± 0.59	9.51 ± 0.70	7.20 ± 0.36***	7.39 ± 0.28***
Amital	9.93 ± 0.32	7.57 ± 0.54**	4.77 ± 0.37***	5.11 ± 0.30***
NaN_3	7.83 ± 0.32	5.08 ± 0.29***	3.02 ± 0.20***	3.13 ± 0.29***
Na_2 EDTA	5.50 ± 0.35	3.69 ± 0.18***	2.47 ± 0.24***	2.51 ± 0.19***

* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$, the results are probably related to the values in the control part.

The use of Na_2EDTA shows the inhibition of free radical oxidation of unsaturated fatty acids in semen which was diluted with the addition of carnitine. In the control part of sperm oxygen consumption in these processes were the largest and constituted 16.8% of the oxygen consumed at the a time when in parts 2, 3 and 4 this ratio was 11.2%, 5.4%, and 6.1%, respectively. Thus the diluted semen of bulls with the addition of L-carnitine along with the normalization of links in the chain of breath spermatozoa. Free radical oxidation of unsaturated fatty acids is inhibited.

We found changes in the respiratory activity of bull sperm due to the actions of carnitine, which agrees with the activity of antioxidant enzymes (Figure).

The results of the current study show that carnitine promotes the increasing activity of CAT and GPO, and the activity of SOD decreases.

Thus, the increase of carnitine in the composition of the diluent to 10 and 30 mg/100 ml helped to reduce the activity of SOD by 34.5% ($p < 0.001$) and 27.6% ($p < 0.001$), respectively, compared with the control part. However, the activity of this enzyme in part 4 of the ejaculate (60 mg/100 ml) was higher than in parts 2 and 3, and it was close to the control part. The activity of CAT and GPO in diluted semen with concentration of carnitine 10 mg/100 ml was higher by 25.4 ($p < 0.05$) and 32.7% ($p < 0.001$), respectively, and in part 3 of ejaculate (30 mg/100 ml) it was higher by 22.4% and 38.7% ($p < 0.001$), compared with the control part.

The dynamics of the activity of antioxidant enzymes in part 4 of the ejaculate was somewhat different. Thus, in the sperm with the highest concentration of carnitine (60 mg/100 ml) the catalase activity dropped by 12.9% compared to the dilution of 30 mg/100 ml. The activity of GPO in part 4 of the ejaculate was prevailed five times ($p < 0.001$) than the indicator in the control sperm and was the highest.

It was established that the quantity of carnitine was an important aspect that could regulate oxidative processes in the sperm. The results confirmed that the biochemical effect of the investigated substance depended on its concentration in sperm. Thus, L-carnitine served as the effector (activator or inhibitor) of the enzymes.

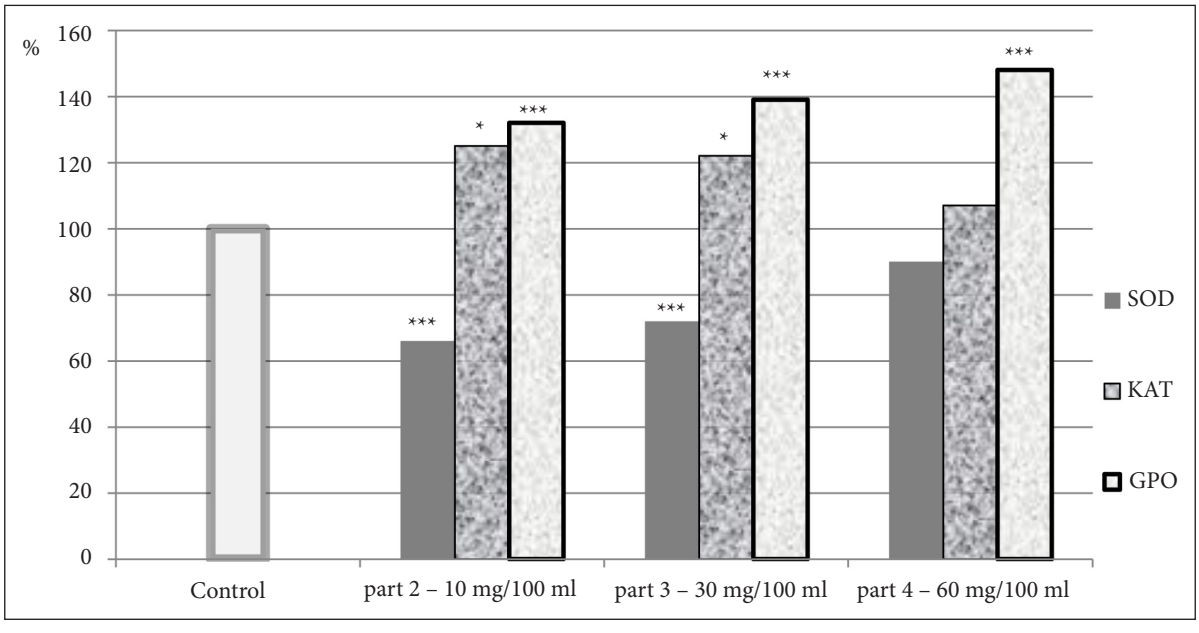

Figure. The activity of antioxidant enzymes of diluted bull sperm after adding L-carnitine (M ± m, $n = 10$)

* – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$, the results are probably related to values in control part

Therefore, to establish the efficiency of a different L-carnitine quantity in the diluent composition, the survival of spermatozoa at the temperature of 2–4°C was defined. It had the highest rates in parts 2 and 3 – 13% ($p < 0.001$) and 21% ($p < 0.001$), respectively – compared to the survival in the control part. However, the survival of spermatozoa in part 4 was lower by 9% than in the control part.

The research results established that the concentrations of L-carnitine in the proportion of 10 and 30 mg/100 ml revealed a protective effect after adding of the sperm diluent and improved the parameters of semen.

DISCUSSION

L-carnitine reduces the overall consumption of oxygen by increasing the share of mitochondrial oxygen consumption and aerobic glycolysis, while free radical oxidation decreases. Probably it is related to a metabolic activation between the semen and the environment. It is possible that under the influence of L-carnitine, spermatozoa use more exogenous energy substrates for the resynthesis of ATP (Hu et al., 2010). Thus, L-carnitine realizes hydration properties and interacts well with the components of environment and cell membranes.

We believe that the reduction of the concentration of free fatty acids, which are the main substrate for the formation of free radicals, is one of the main mechanisms of efficacy of L-carnitine. It is possible that the action of L-carnitine is activated using lipids in the energetic metabolism of spermatozoa. However, the normalization and maintainance of the energetic metabolism of cells is implemented by providing the formation of acetyl-CoA in the mitochondrias, the delivery of substrates oxidation in the Krebs cycle, and the activation mechanisms of fatty acids β-oxidation.

A possible reason for increasing the survival of spermatozoa is the ability of carnitine to support the mitochondrial membrane potential, its influence in the use and utilizationof substrates, and its antioxidant properties (Di Paolo et al., 2006).

Thus, L-carnitine affects the intensity of oxygen consumption in sperm. Its action provides the different ability to use and utilize the energy substrates of the environment. This is probably due to the activation of the oxidative energy metabolism with the participation of L-carnitine and effective disposal of toxic metabolites (Eskenazi et al., 2005). In this case, antioxidant enzymes are transforming AFO in semen with the formation of endogenous oxygen that, as a result, provides the maintenance of oxygen homeostasis of spermatozoa. Such conclusions are consistent with changes in activity of catalase and glutathione peroxidase that are directly involved in the formation of endogenous oxygen. Thus, there are some changes in the use of acceptors, from intracellular to extracellular.

It is obvious that the reduction of SOD activity is caused by the decreasing of substrate in the environment – superoxide anion radical, produced in smaller quantities in redox reactions in sperm. It is likely that the reduction of the SOD activity occurs because of the effect of carnitine in the multi-redox system cells. In this way it induces the synthesis of the enzyme by increasing concentrations of electron donors, or inhibits it in the case of accumulation in cells acceptors (Aitken et al., 1996). One can say that the CAT destroys the inhibitor of SOD – hydrogen peroxide, thus supporting the SOD activity on a certain level and, in our opinion, the work of these two enzymes is synchronized optimally. When catalase (CAT) functions as an oxidant, the process of reduction of hydrogen peroxide takes place to form molecular oxygen. This compensation increases the useful life of exogenous oxygen in sperm during energy transformation. Thus, in the metabolic processes of oxidative phosphorylation the share of molecular oxygen is returned to the body that is restored to one-electron way. It is likely that carnitine affects the intensity of aerobic oxidation restored equivalents and the catalase prevents the accumulation of hydrogen peroxide.

The main and universal physiological regulator of AFO is a GPO, which regulates small

physiological concentrations of H_2O_2 and restore shydroperoxides of polyunsaturated fatty acids, phospholipids of membranes, and other peroxide compounds (Agarval et al., 2005; Eskenazi et al., 2005). The increase in experimental semen activity of GPO is the evidence of the reduction of the lipid peroxidation intensity and antioxidant properties of carnitine, which are consistent with a decrease in oxygen consumption using Na_2EDTA inhibitor.

We found the optimal concentration of L-carnitine in diluted bull sperm, which is 30 mg/100 ml. This dose inhibits the intensity of reactive oxygen bulls' sperm production, normalizes the activity of antioxidant enzymes and promotes the growth of the metabolic activity of gametes, which increase the survival of spermatozoa.

CONCLUSIONS

L-carnitine is involved in the normalization of cellular metabolism in the conditions of development of oxidative stress, because it improves the use of substrates environment, reduces oxygen consumption by cells, and provides a protective effect. The use of L-carnitine as a part of a diluent for sperm provides a regulatory effect to the activity of enzymes of the antioxidant protection system, mitochondrial respiratory chain, and the intensity of oxygen consumption. It leads to the improvement of physiological parameters of semen. L-carnitine, which was added to the diluent of semen in proportions of 10 and 30 mg/100 ml, stimulates aerobic glycolysis by 5.9% and 11.9%. It decreases oxidative processes that are not related with the synthesis of ATP by 6% and 11.4%, which increases the survival of bull spermatozoa. For the improvement of the biochemical parameters and the quality of sperm, it is recommended to add L-carnitine in the diluent for the sperm in the amount of 30 mg/100 ml.

Received 20 September 2017
Accepted 3 Novembr 2017

References

1. Agarval A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. Journal of Andrology. 2005; 26(6): 654–60.
2. Aitken RJ, Buckingham DW, Carreras A. Superoxide dismutase in human sperm suspensions: relationship with cellular composition, oxidative stress, and sperm function. Free Radic Biol Med. 1996; 21: 495–504.
3. Calò L., Pagnin E., Davis P, Semplicini A, Nicolai R, Calvani M, Pessina AC. Antioxidant effect of L-carnitine and its short chain esters. Relevance for the protection from oxidative stress related cardiovascular damage. International Journal of Cardiology. 2006; 107(1): 54–60.
4. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature. 2006; 12: 651–7.
5. Eskenazi B, Kidd SA, Marks R, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Hum Reprod. 2005; 20(4): 1006–12.
6. Hu JH, Tian WQ, Zhao XL, Zan LS, Wang H, Li QW, Xin YP. The cryoprotective effects of ascorbic acid supplementation on bovine semen quality. Anim Reprod Sci. 2010; 121(1–2): 72–7.
7. Noland RC, Koves TR, Seiler SE, Lum H, Lust RM, Ilkayeva O, Stevens RD, Hegardt FG, Muoio DM. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. J Biol Chem. 2009; 284(34): 22840–52.
8. Vlizlo VV, Fedoruk RS, Ratych IB. Laboratorni metody doslidzhen u biolohii, tvarynnytstvi ta veterynarnii medytsyni. Lviv: SPOLOM, 2012. 762 pp. Ukrainian.

Viktoriia Koberska, Oleh Yermishev

L-KARNITINO POVEIKIS DEGUONIES SUNAUDOJIMO INTENSYVUMUI BULIŲ SPERMOJE

Santrauka

Tyrimas skirtas išstudijuoti L-karnitino poveikį bulių spermos deguonies sunaudojimo intensyvumui ir fermentų antioksidacinei apsaugai. Švieži buliaus spermatozoidai buvo sumaišyti su spermos skiedikliu „Bioexel“ santykiu 1:1. Spermos skiedinys buvo padalytas į 4 dalis (kontrolinė ir 3 eksperimentinės). Praskiestas tirpalas papildytas L-karnitinu (10 mg/100 ml; 30 mg/100 ml; 60 mg/100 ml) ir šiuo paruoštu tirpalu sperma atskiesta santykiu 3:1. Tyrimo rezultatai rodo, kad optimalus L-karnitino koncentracijos kiekis bulių spermos skiedinyje yra 30 mg/100 ml. Ši dozė slopina reaktyviojo deguonies gamybos intensyvumą bulių spermoje, normalizuoja antioksidantinių fermentų aktyvumą, skatina gametų metabolinį aktyvumą, o tai padidina spermatozoidų išlikimo galimybę.

Raktažodžiai: deguonies sunaudojimas, antioksidaciniai fermentai, L-karnitinas, sperma, buliai