ACTIVITY OF NADH-TETRAZOLIUM REDUCTASE IN RAM SEMEN DURING LIQUID AND CRYOPRESERVATION

ИЗСЛЕДВАНЕ НА НАДН-ТР В СПЕРМАТОЗОИДИ ОТ КОЧ ПРИ КРАТКРОТРАЙНО СЪХРАНЕНИЕ И КРИОКОНСЕРВАЦИЯ

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ABSTRACT

The investigations for the determination of NADH-tetrazolium reductase enzyme system activity in ram semen during liquid and cryopreservation in relation with their viability, were carried out. The semen samples with lower motility of spermatozoa after collection, possess lower values of mean cytochemical coefficient for NADH-tetrazolium reductase activity ($p<0.5$, $p<0.5$) after liquid and cryopreservation.

KEY WORDS: spermatozoa, NADH-tetrazolium reductase, motility, sperm metabolism

РЕЗЮМЕ

Проведени са изследвания за определяне активността на НАДН-тетразолий редуктазна ензимна система в сперматозоиди от коч при краткротрайно съхранение и криоконсервация, във връзка с виталитета им. При сперма с понижен мотилитет на сперматозоидите при получаване, след краткротрайно съхранение и след криоконсервация, са установени достоверно по-ниски стойности на СЦК за НАДН-ТР- активност ($p<0.5$, $p<0.5$).

КЛЮЧОВИ ДУМИ: сперматозоиди, НАДН-тетразолий редуктаза, мотилитет, спермален метаболизъм

ПОДРОБНО РЕЗЮМЕ

Проведени са изследвания за определяне активността на НАДН-тетразолий редуктазна ензимна система в сперматозоиди от коч при краткротрайно съхранение и криоконсервация, във връзка с виталитета им.

В експериментите включихме 14 еякулата, получени по метода на изкуствена вагина от три разплодника изравнени по възраст (2 години), поставени при еднакви условия на хранене, гледане и полово използване, съобразени с нормативните изисквания.

Еякулатите разделяхме на четири части, разредени в съотношение 1+3, като при първите (А и В) използвахме среда за съхраняване при 4°С , а при вторите(С и D) – среда на Нагазе-Нива за криоконсервация. В пробите от групи А иС включихме еякулати с подвижност 65-70%, а в групите В и D такива с подвижност над 70%

Установено е, че при пресни еякулати от коч с понижен витатитет (под 70%), както и след криоконсервацията им, стойности по отношение на мотилитета на сперматозоиди след инкубация при 39°С за 300 мин. са достоверно по-ниски ($p<0.01$, $p<0.01$).

При сперма с понижен мотилитет на сперматозоидите при получаване (под 70%), след краткротрайно съхранение и след криоконсервация, са установени достоверно по-ниски стойности на СЦК за НАДН-ТР- активност ($p<0.5$, $p<0.5$).

Стойностите на СЦК за НАДН-ТР- активност в сперматозоидите могат да се използват като критерий за динамика на метаболитни процеси и виталитета на сперматозоидите от коч.

INTRODUCTION

The sperm metabolism is submitted to the necessity of energy generation which can be transformed into mechanic for their motility maintenance. It was established that the catabolic processes are predominant over the anabolic in the spermatozoa [7]. Essential role on the control of the transformation of energy of male gametes play the ratio between nicotinamid dinucleotid (NAD) and nicotinamid dinucleotid phosphate (NADPH). The group of enzymes referred to this catalyzes oxide-reduction reaction, contributing to the maintenance of intracellular balance and the energy is released for the cell metabolism [6]. The enzyme system NADH- tetrazolium reductase can be attached to them and used as marker for the functional activity of sperm mitochondrion [12, 14].

The aim of this study was to analyze the activity of NADH-tetrazolium reductase enzyme system in ram semen during liquid and cryopreservation in relation to their viability.

MATERIAL AND METHODS

The experiments included 14 ejaculates collected from 3 rams (2 years old), placed on the same condition of food, breeding and sexual use, conformed to the norms. The semen was collected by artificial vagina and the following parameters were evaluated: ejaculate volume (cm^3), sperm motility (%), sperm concentration (nm × 10^6 /cm^3), pH and thermo resistance after incubation at 39°C for 300minutes (%).

Normozoospermic ejaculates were split into 4 parts and diluted in the ratio 1:+3. The groups A and B included the medium 6AG [15] for the comparison of samples after liquid preservation at 4°C and the groups C and D -the medium of Nagase-Niva for cryopreservation [8]. The samples from the groups A and C included the ejaculates with motility 65 -70% and the groups B and D those with motility over 70%.

The electrophoretic investigation of NADH-tetrazolium reductase was carried out in water-soluble and Triton X-100-soluble extract of spermatozoa using disc electrophoresis according to the Davis method [3].

The method of Kiernan [5] modified for spermatozoa by Subev and Yonkov [13] was used for cytochemical analysis of NADH-tetrazolium reductase activity. The intensity of the reaction was analyzed microscopically on 500 spermatozoa per sample by using magnification of (400x) according to the 5 grade scale of Hrudka [4]. The mean cytochemical coefficient, informative for the quantitative characteristic of investigated enzyme system was calculated on the base of obtained values according to the method of Astaldi and Verga [2].

The data were processed using the Student's variations-statistical method.

RESULTS AND DISCUSSION

The values of sperm motility, viability and mean cytochemical coefficient for NADPH- tetrazolium reductase in the fresh and frozen ram semen are given at the Tables 1 and 2.

From the presented data it is obvious that in the fresh semen there were some differences related to the sperm motility in the both groups of evaluated ejaculates (A-over 70% and B-under 70%). There were also differences spermatozoa motility before and after incubation for 300 minutes at 39°C (Table 1). In the samples after incubation higher value for the motility was observed in the group B ($p<0.001$).

Values for mean cytochemical coefficient of the spermatozoa from group B were significantly higher than in the ejaculates from group A ($p<0.05$). It was established that the basic functional parameters, i.e.motility and

viability, were positively related to the values of mean cytochemical coefficient for NADPH – tetrazolium reductase.

After the cryopreservation, the sperm motility from the both investigated groups decreased compared to the fresh sperm. From the Table 2 it can be seen that in both cases, before and after incubation, the ejaculates from the group D had higher motility than group C ($p<0.05$ and $p<0.001$ respectively). Significant differences between groups were found also for the activity of tetrazolium reductase ($p<0.05$). Also in cryopreserved sperm the higher viability corresponded to the higher values of mean cytochemical coefficient for NADH-tetrazolium reductase.

The reaction products (formazan granules) of the NADH-tetrazolium reductase reaction were localized in the ram sperm mid-piece (Figure 1). The enzyme reactions of single spermatozoa differed in their intensity.

During the electrophoretic analysis water soluble and Triton soluble extracts of ram sperm a NADH- tetrazolium reductase appeared on the electrophoregram in the form of dark band situated in the anode part of the gel (Figures 2 and 3).

The diformazane granules, stocked in the mitochondria, are indicative for the this organelles of the spermatozoa. For example the reduction of tetrazolium salt is carried out as artificial acceptor of hydrogen atoms in the mitochondria. It plays the role of substrate and determines their ability to oxidize NADH which is the source of electron and protons [9, 10]. We consider that the higher mean cytochemical coefficient which is related to the motility is informative for the functional activity of metabolic processes.

The data from present study indicate that there is a correlation between ram sperm motility and the capacity of mitochondrial enzymes to oxidize the exogenous NADH. Similar results were obtained with human sperm where higher activity of the enzyme system NADH- tetrazolium reductase in the mitochondria also correspond to the

Table 1. Motility of spermatozoa and mean cytochemical coefficient for NADH – tetrazolium reductase in fresh ram sperm (n=12)

Таблица 1 Подвижност на сперматозоиди и среден цитохимичен коефициент за НАДН-ТР при прясна сперма от коч(n=12)

Groups Групи	Motility (%) Подвижност		Mean cytochemical coefficient for NADH–TR Среден цитохимичен коефициент за НАДН-ТР
	Before incubation Преди инкубация	After incubation След инкубация	
A	65.12 ± 4.18	30.25 ± 2.73***	3.05 ± 0.04*
B	77.53 ± 5.31	56.32 ± 3.17***	3.22 ± 0.05*

* $p<0.05$ *** $p<0.001$

Table 2 Motility of spermatozoa and mean cytochemical coefficient for NADH – tetrazolium reductase in ram sperm after cryopreservation (n=16)

Таблица 2 Подвижност на сперматозоиди и среден цитохимичен коефициент за НАДН-ТР при сперма от коч след криоконсервация (n=16)

Groups Групи	Motility(%) Подвижност (%)		Mean cytochemical coefficient for NADH–TR Среден цитохимичен коефициент за НАДН-ТР
	Before incubation Преди инкубация	After incubation След инкубация	
C	34.59 ±1.06*	3.00± 0.70***	3.04 ± 0.04*
D	40.10 ± 2.13*	21.10 ± 1.12***	3.19 ± 0.03*

* $p<0.05$ *** $p<0.001$

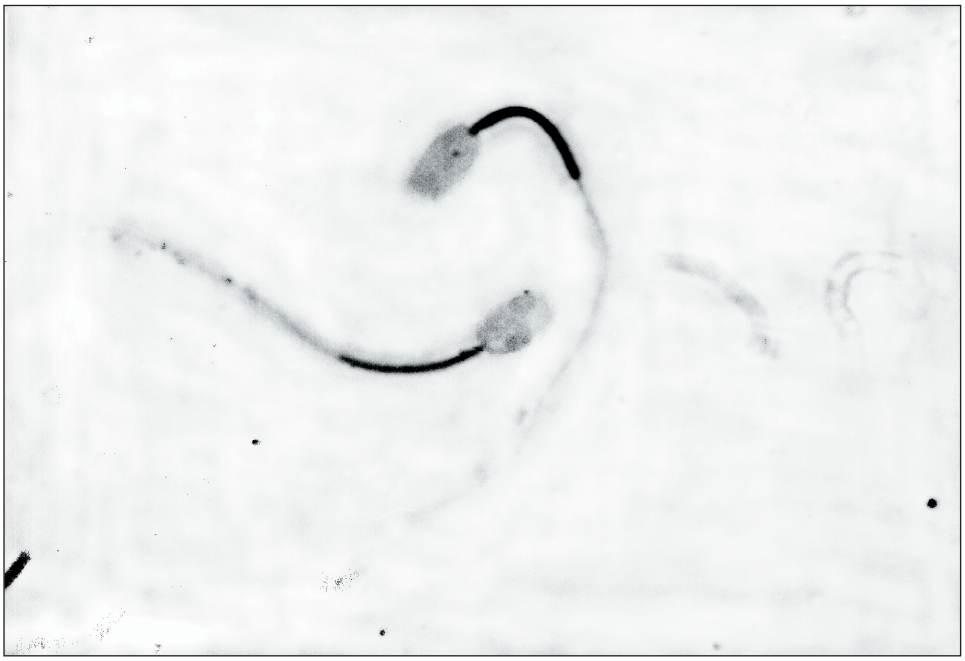

Fig.1 NADH-tetrazolium reductase activity in ram sperm with different intensity of enzyme reaction-1- strong reaction; 2- weak reaction (Magnification: × 400)

Фиг. 1 НАДН-ТР- активност при сперма от коч с разлечна по интензитет цитохимична реакоция – 1 – силна реакция, 2- слаба реакция (увеличение: × 400)

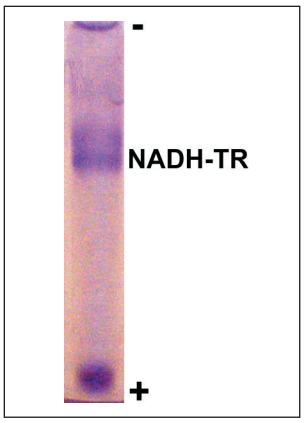

Fig.2 Electro-phoregram of NADH-tetrazolium reductase (water-soluble extract) from fresh ram sperm

Фиг. 2. Електро-фореграма на НАДН-ТР (воден екстракт) при прясна сперма от коч

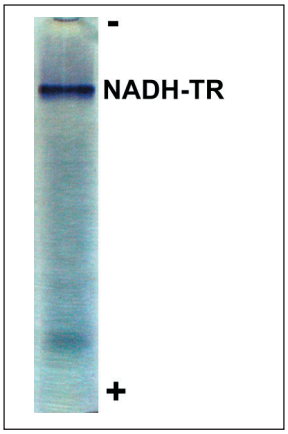

Fig.3 Electro-phoregram of NADH-tetrazolium reductase (Triton X -100-soluble extract) from fresh ram sperm

Фиг. 2. Електро-фореграма на НАДН-ТР (тритон Х -100 екстракт) при прясна сперма от коч

higher motility and viability of male gametes [1,11]. This allow us to accept that the values of mean cytochemical coefficient of NADH- tetrazolium reductase can be used as marker of dynamics of metabolite processes which are related to the ram sperm motility.

CONCLUSIONS

It was established that the fresh ram ejaculates with lower motility (below 70%) as well as after cryopreservation have values referred to the motility after incubation at 30°C for 300min that are significantly lower ($p<0.01$, $p<0.01$)

The spermatozoa with lower motility during collection of semen (below 70%) after liquid and cryopreservation possess lower values of for NADH- tetrazolium reductase activity($p<0.5$, $p<0.5$)

The values of for NADH- tetrazolium reductase activity of spermatozoa can be used as criterion for the dynamic of metabolic processes and the viability of ram sperm.

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