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PROTECTIVE EFFECT OF THE ANTIOXIDANT ENZYME ON SPERM FROM *Bubalus bubalis* TYPE AFTER CRYOCONSERVATION

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Abstract: Studies were carried out in order to determine the presence of damaged intracellular proteins in sperm from water buffalo as a result of toxic action of free radicals and their attitude towards the vitality of the gametes. Experiments were performed with thawed semen samples as the trial has been added antioxidant enzyme superoxide dismutase. It was found that added to the test sample exogenous antioxidant makes inoffensive much of the available free radicals in sperm, respectively reduces the amount of damaged intracellular proteins and helps protect the vitality of sperm.

Key words: water buffalo, sperm, superoxide dusmutase, proteins

Introduction

In terms of in vitro storage of semen as a result of on going metabolic processes in the sperm, increases the amount of carbonyl groups in protein molecules of the germ cells. A major factor being the direct effects of superoxide anion *(Davies, 1987)*. The quantitative changes are the reason for the occurrence of membrane dysfunction, morphological damage and decreased sperm motility in environments with abnormal ratio between the presence of oxidants and antioxidants *(Aitken et al., 1989; De Lamirande and Gagnon, 1992; Aitken, 1995; Stefanov et al., 2004)*. These disabilities are a cause of decreased fertility in varying degrees of germ cells.

In this context the purpose of this study is exploring the impact of the added antioxidant enzyme on the content of carbonyl groups stored in the in vitro conditions sperm.

Materials and Methods

Studies were conducted with 8 normozoospermic ejaculates receipt and 13 - diluted and stored at ultra-low temperature (-196 ° C) obtained from 3 clinically healthy animals of the species Bubalus Bubalis. Dose added to the semen samples examined enzyme superoxide dismutase (SOD, EC 1.15.1.1) was determined based on results from preliminary tests on optimizing the effect of antioxidants on the vitality of the sperm from other sires *(Stefanov et al., 2004)*. The experimental setup consists of two groups of samples - test and control. To test sample prior to freezing by adding 30UI (Union Internationale) SOD, EC 1.15.1.1 obtained from the strain Humicola lutea 103, belonging to the Mycological Collection of Institute of Microbiology, BAS, biosynthesis and purified by *Angelova et al. (2001)*. Dilution and cryopreservation of sperm has been made through the yolk-glycerol protective environment include extra to it, trisodium citrate-lactose. After equilibration the semen at +40 ° C for 4 hours was applied technology in sequins cryopreservation method *Cassou (1964)*, followed by programmed freezing with the help of biofreez (Minucool -40PC).

Sperm motility before and after freeze-thaw is setting the light microscope (Biolar L, Poland) in ten degrees scale presence of gametes with straight-offensive moves. Experimental and control samples are further processed by means of centrifugation was separated from sperm plasma cells, sperm cells are then destroyed by several freeze-thaw, followed by centrifugation. When so treated plasma and sperm gametes before and after incubation for 300 minutes at 39 ° C is the determination of carbonyl groups using the method of *Levine (2000)*. As a reagent was used 2,4-dinitrophenylhydrazine, causing red coloration with carbonyl groups. The amount of carbonyl groups was calculated in nmol carbonyl /nmol protein.

The results are processed variational-statistical.

Results and Discussion

The results in terms of sperm motility determination and content of carbonyl groups of buffalo ejaculates receipt are reflected in Table1.

Table1. Sperm motility and content of carbonyl groups in intracellular proteins ejaculates of buffalo receipt (n = 8)

Motility %	Content of carbonyl groups [nmol carbonyl /nmol protein]
63.12 ± 1.75	1.96 ± 0.04

Established values are informative for the content of carbonyl groups in intracellular proteins normozoospermic ejaculates of buffalo receipt.

Table 2 presents data on sperm motility after freezing-thawing and their relationship with values for the content of carbonyl groups.

Table 2. Sperm motility and content of carbonyl groups in intracellular proteins of the buffalo semen after freezing (n=13)

Variants	Motility %	Content of carbonyl groups [nmol carbonyl/nmol protein]
Without SOD, EC 1.15.1.1	30.50 ± 1.80	4.38 ± 0.07
With SOD, EC 1.15.1.1	48.75 ± 1.46	1.40 ± 0.09
	p<0,01	p<0,01

These results indicate that a relationship exists between sperm motility and content of carbonyl groups in the sample. It was reliably lower levels of carbonyl groups in the samples with the presence of SOD, EC 1.15.1.1 compared with controls. More over, total sperm motility was higher in samples with added SOD, EC 1.15.1.1. It is evident that the content of carbonyl groups reflects on sperm viability.

It also established an increased number of male gametes with violations in their structure, compared with control samples (Figure 1).

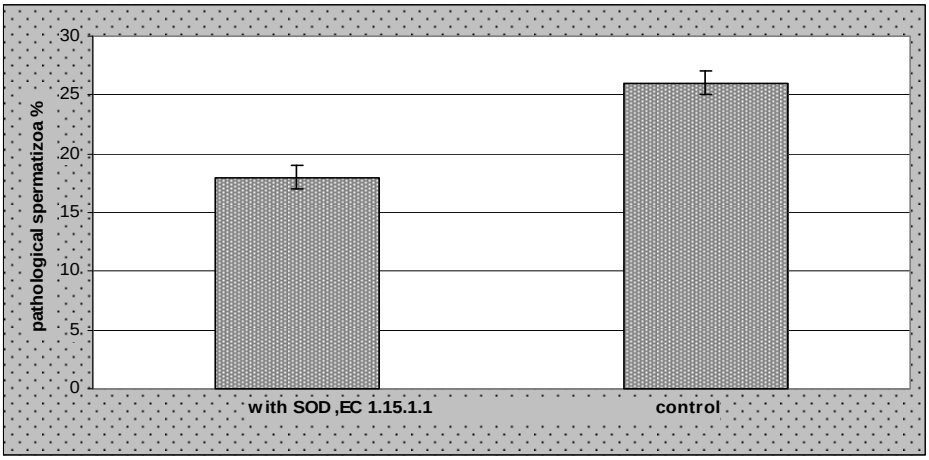

Figure 1. Percent pathological spermatozoa from ejaculates with SOD, EC 1.15.1.1 and from ejaculates without SOD, EC 1.15.1.1

The injuries were mainly in the acrosomal and tail. (Figure 2 and 3).

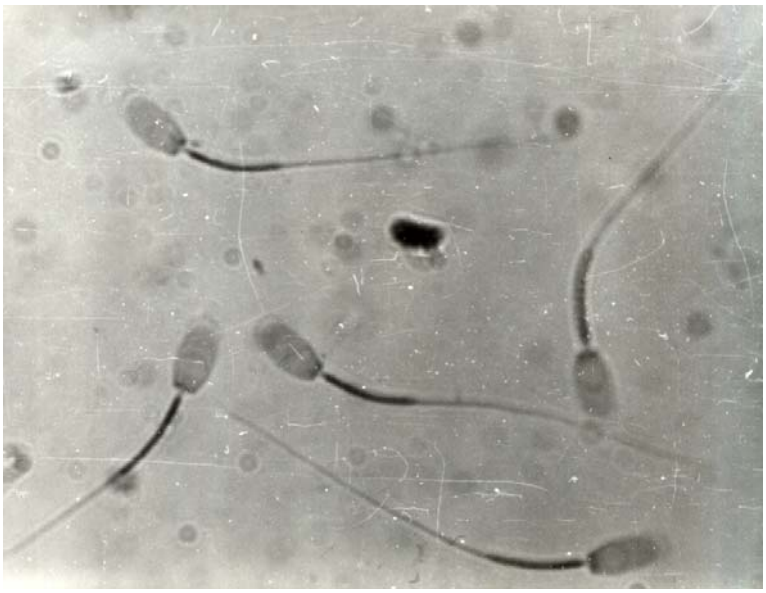

Figure 2. Spermatozoa with disabilities in the acrosomal

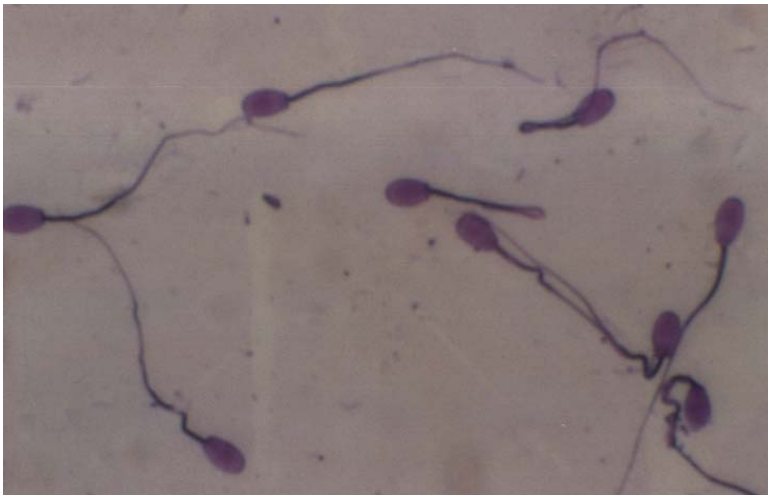

Figure 3. Spermatozoa with disabilities in middle and tail

The results show that in the process of in vitro storage of male gametes in terms of ultra-low temperatures, increases the amount of carbonyl groups in protein molecules, as a possible result of the direct effects of superoxide anion. Most

vulnerable to these effects are cell nucleus, respectively sperm DNA *(Donelly et al., 1998; Aitken et al., 2003)*.

This fact explains the positive impact of further additions to the media of the dilution and freezing of semen antioxidant enzyme superoxide dismutase.

The results of the studies conducted to establish the influence of SOD, EC 1.15.1.1 on the content of carbonyl groups in sperm after thawing and incubation of samples at +39°C for 300 minutes are shown in the graph in Figure 4.

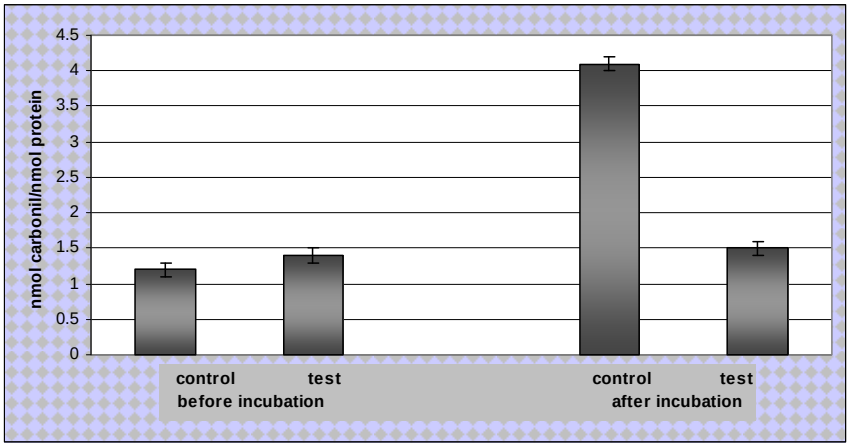

Figure 4. Effect of SOD, EC 1.15.1.1 on the content of carbonyl groups of buffalo bull semen after thawing and incubation at +39 ° C for 300 min (n = 13)

As with experienced and in control samples before incubation values reflecting the amount of carbonyl groups are very close. During the incubation period, showing an increase in the amount of reliable carbonyl groups in the control samples, compared with their presence in ejaculates with added SOD, EC 1.15.1.1. Based on these results it can be assumed that oxidative damage to proteins in sperm after cryopreservation is dependent on the time of their incubation.

Increased oxidation of cellular proteins related causes abnormal sperm vitality in terms of in vitro storage *(Julian et al.,1998)*. Under normal physiological conditions, the protective role of SOD, EC 1.15.1.1 is determined by place of formation of oxygen radicals in mitochondria, which are mainly structure of germ cell *(De Lamirande et al., 1997; Aitken and Baker, 2004)*. In this regard, the action of the antioxidant during the different stages of storage of semen has a protective effect on sperm viability *(Griveau and Lannou, 1997)*.

Conclusion

Oxidative damage to proteins in sperm after cryopreservation is dependent on the time of their incubation.

Increasedoxidationof cellular proteins associated causeof impaired sperm vi tality in terms of in vitro storage.

The antioxidant's action, during the different stages of storage of semen, has a protective effect on sperm vitality

Acknowledgment

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Zaštitno dejstvo antioksidans enzima na spermu *Bubalus bubalis* (domaćeg bivola) nakon kriokonzervacije

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Rezime

Studije su sprovedene kako bi se utvrdilo prisustvo oštećenih intracelularnih proteina u spermi bivola kao posledica otrovnog dejstva slobodnih radikala i njihov uticaj na vitalnost gameta. Ogledi su izvedeni sa otopljenim uzorcima semena a kao proba je dodat antioksidansni enzim superoksid dismutaza. Utvrđeno je da dodat egzogeni antioksidans u zorak čini neškodljivim mnogo dostupnih slobodnih radikala u spermi, odnosno smanjuje količinu oštećenih intracelularnih proteina i doprinosi vitalnosti sperme.

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