

INFLUENCE OF CRYOPRESERVATION ON THE ACTIVITY OF ANTIOXIDANT ENZYMES SUPEROXIDE DISMUTASE (SOD) AND CATALASE (CAT) IN EJACULATES OF RAMS OF INDIGENOUS BULGARIAN SHEEP BREED

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The aim of the experiment is to examine the influence of cryopreservation on the activity of enzymes of antioxidant protection - SOD and CAT in ejaculates of rams of breed - Sofia (Elin-Pelin). The breed is insufficiently studied in terms of its reproductive qualities. It is endangered of extinction and it is important to explore the possibilities for its protection.

Material and methods

Animals

For the experiment, 10 ejaculates from 5 rams were examined. The rams are placed under the same conditions of feeding, breeding and sexual use. The animals are 2 to 4 years old, all with previous sexual experience. The ejaculates were obtained by the method of the artificial vagina and were diluted 1:12 with medium 6AG. Each ejaculate was tested for sperm motility and SOD and CAT enzyme activity before freezing and after thawing of ejaculate.

Techniques for cryopreservation of spermatozoa

The semen is frozen in straws (length 133mm, diameter 2mm and volume 0.25cm³). Once filled with semen, the straws are loaded into special tripods. Cryopreservation of semen is performed by rapid cooling, up to -80°C for 5 minutes and the temperature was monitored with a minus thermometer. The straws were then transferred from liquid nitrogen to liquid nitrogen at -196°C and placed in a canister for long-term storage at ultra-low temperatures. Thawing is done on a water bath at 37°C for 10 s.

Sperm Analysis

Sperm motility was examined with a computer sperm analyzer (CASA). The activity of enzymes SOD and CAT was examined spectrophotometrically with Spekol 11 (Carl Zeiss, Jena).

Results

Sperm motility after cryopreservation decreased by 26.66% ($P \leq 0.001$) (Fig.1).

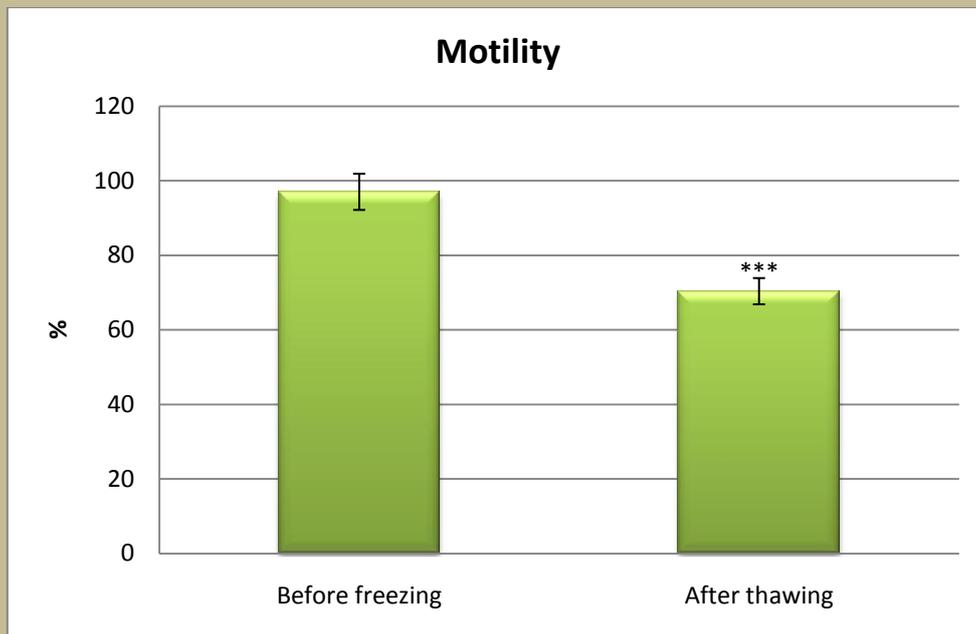


Fig.1 Total sperm motility

The SOD activity after cryopreservation was reduced by about 30% ($P \leq 0.01$) and CAT activity was reduced by 50% ($P \leq 0.01$) (Fig. 2 and Fig.3).

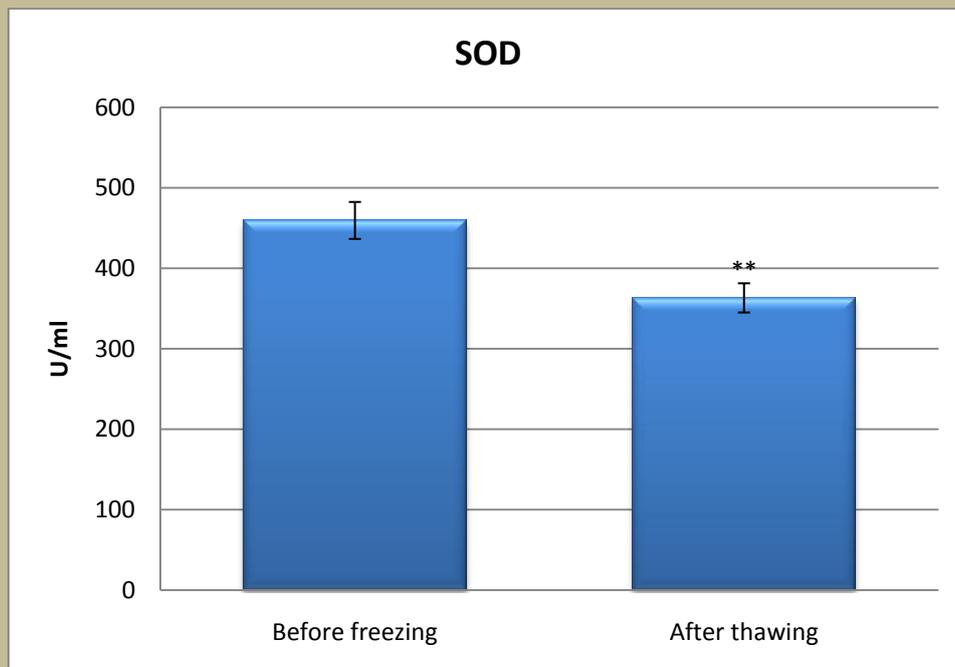


Fig.2 SOD enzyme activity

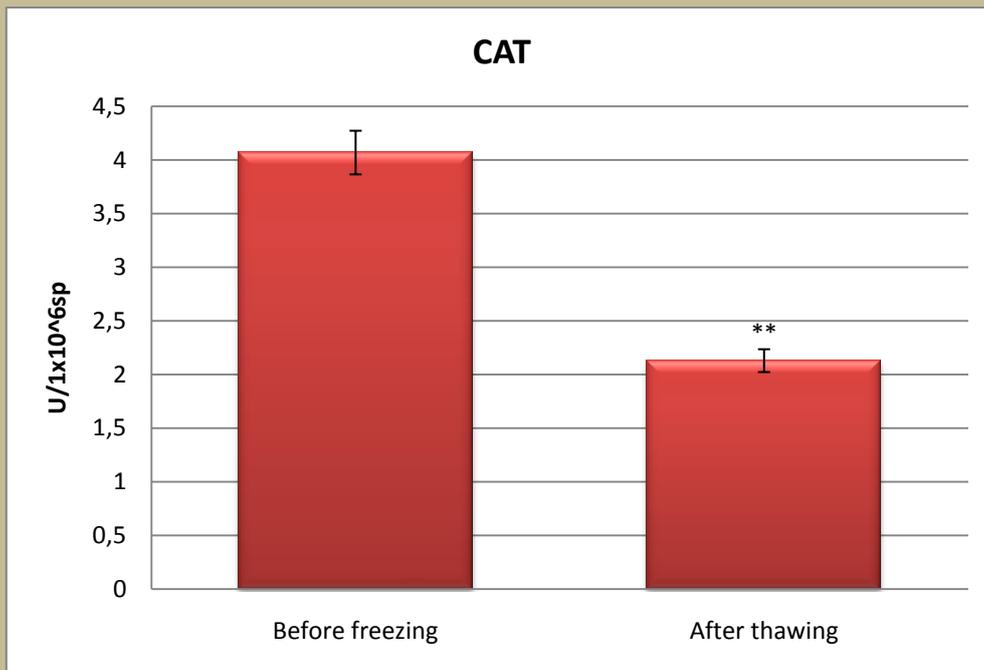


Fig.3 CAT enzyme activity

In conclusion, we can say that the ejaculates of rams breed Sofia (Elin-Pelin) have good cryotolerance, which is expressed in the ability to reduce the induction of oxidative stress in the ejaculate and preserve their fertility after the freezing-thawing process.

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