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EFFECT OF THE BREED ON THE ACTIVITY OF THE ANTIOXIDANT ENZYMES – SOD AND CAT IN RAM SPERM, BEFORE AND AFTER CRYOPRESERVATION

Madlena Andreeva[1,2], Albena Alexandrova[3], Elina Tsvetanova[2], Nikola Metodiev[4], Rossen Stefanov[1]

[1]Institute of Biology and Immunology of Reproduction "Acad. K. Bratanov", BAS, Sofia, Bulgaria
[2]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]National Sports Academy „Vasil Levski", Sofia, Bulgaria
[4]Institute of animal science, Kostinbrod, Bulgaria
E-mail: madlena_andreeva_@abv.bg

ABSTRACT

The aim of this study was to evaluate the variations in the activities of antioxidant enzymes SOD and CAT in sperm from different breeds of rams and their changes after cryopreservation. For this purpose, the ejaculates of a total of 24 rams of Ile-de-France, Synthetic Population Bulgarian Milk (SPBM), Lacaune and Sofia (Elin-Pelin) during their breeding season. Enzyme activities were determined spectrophotometrically. Statistically significant differences in SOD activity were found between breeds before freezing (from $P \leq 0.001$ to $P \leq 0.05$), which persisted even after thawing ($P \leq 0.001$), whereas in CAT activity significant differences between breeds were detected only after cryopreservation (from $P \leq 0.001$ to $P \leq 0.01$). In conclusion, animals of the Ile de France breed had higher antioxidant enzyme protection of sperm, which probably makes it more resistant to oxidative stress and determines better reproductive abilities.

Key words: sperm, ram, SOD, CAT, cryopreservation.

Introduction

Reactive oxygen species (ROS) are normally generated in cells as by-products of many processes such as electron transport in mitochondria and microsomes, and activity of a number of enzymes (Halliwell and Gutteridge, 2007). In sperm, physiologically, ROS control maturation, hyperactivation, acrosome reaction and sperm fusion with the ovum. However, in pathological conditions, ROS are generated in excess inducing lipid peroxidation, protein oxidation and nucleic acid damage. Oxidative modification of molecules leads to damage to their structures and functions in which they are involved and can cause apoptosis of sperm (Kothari et al., 2010). Evolutionarily, in organisms a system that limits the overproduction of ROS has been developed. This antioxidant defense system includes enzymatic and nonenzymatic antioxidants that ensure ROS elimination and the repairation of damaged structures. As elsewhere in the organism the main enzymatic antioxidants in sperm include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Sikka, 2004).

Disturbance of the balance between the prooxidant processes and the antioxidant protection of the cell is referred to as oxidative stress (OS). Oxidative stress has been identified as a major etiological cause of male infertility (Agarwal et al., 2003). High levels of ROS can cause sperm membrane lipid peroxidation impeding motility and sperm-oocyte fusion and damage to the nuclear DNA leading to mutations and apoptosis (Shiva et al., 2011). In livestock breeding, the processing of semen for artificial insemination can also cause OS (Yoshida et al., 2003; Gallardo, 2007). The

processes of cooling and aerobic preservation create physical and chemical stress on the sperm membrane, which successively reduces the sperm viability and the ability to fertilize. Cold shock of sperm during the cooling process is associated with induction of oxidative stress (Thuwanuta et al, 2011). Excess generation of ROS and OS during the freezing process can lead to serious sperm damage (Anger et al., 2003). Therefore, the assessment of germ cell resistance to the effects of prooxidant factors and/or the strength of antioxidant protection is of great importance in assisted reproductive techniques.

Thus, the aim of the study was to determine the influence of the breed on the activity of the main antioxidant enzymes – SOD and CAT in ram sperm, before and after cryopreservation.

Materials and methods

Animals: Sperm samples were taken from 24 rams, breeds: Ile de France, Synthetic Population Bulgarian Milk (SPBM), Lacaune and Sofia (Elin-Pelin). The rams were with normal reproductive organs, in age of 2–4 years placed under the same regime of nutrition, care and sexual use, in accordance with the normative requirements. The ejaculates were collected from the rams by a trained technician using an artificial vagina. Two ejaculates were obtained from each ram – a total of 48 ejaculates were examined. In the experiments were included ejaculates from clinically healthy animals during their breeding season. After collection, the samples were transferred to the laboratory where were stored in a thermostatic water bath at 37°C until examination.

Cryoconservation of ejaculates: The obtained samples were diluted 1:12 with colloidal extender 6 AG (sodium citrate, lactose, sucrose, egg yolk and glycerol) – prepared at the Institute of Biology and Immunology of Reproduction "Acad. Kiril Bratonav" – BAS and frozen by the straw technology of Cassou (1964). The evaluation of the cryopreserved semen was performed by thawing the straws in a water bath at 37°C for 30 s.

Enzyme activities

Superoxide dismutase (SOD) activity was determined by the method of Peskin and Winterbourn (2017) – The superoxide radicals, generated in xanthine-xanthine oxidase system, reduced the water-soluble tetrazolium (WST-1) to insoluble blue colored formazan. One unit of SOD activity is defined as the amount of enzyme needed to inhibit 50% of the WST-1 reductio.

Catalase (CAT) activity was assayed according to Aebi (1970) – The enzyme catalyzes the decomposition reaction of hydrogen peroxide to water and oxygen. The decrease in extinction at 240 nm corresponds to the degradation of H_2O_2 and serves as a measure of catalase activity.

Statistical analysis

Data were analyzed with SPSS 23 to compare sperm characteristics using statistical tests of ANOVA and Paired T-test. The significance of the differences between groups was evaluated by t-criterion of Student. Findings were considered statistically significant if $P< 0.05$.

Results and Discussion

Our results showed that before freezing there were significant differences in SOD activity between the breeds (Fig. 1), but not in the activity of CAT (Fig. 3). The enzymes SOD and CAT are main antioxidant enzymes acting within cells. They form the body's first line of defense against ROS (Ighodaro and Akinloye, 2018) as SOD dismantles superoxide radicals (O_2-) to hydrogen peroxide (H_2O_2), and catalase breaks down the latter to harmless molecules (H_2O and O_2). Although O_2- are

relatively less reactive radicals and it has been shown that they are not able to directly initiate lipid-, DNA-, and protein oxidation, they are generated in large amount in cells. About 1% to 3% of the O_2 used in mitochondria may form O_2^- (Halliwell and Gutteridge, 2007). At least 9 key points have been identified in the mitochondria, of possible O_2 generation (Andreyev et al., 2005). Another major source of O_2- in spermatozoa is the activity of membrane-bound NADPH oxidase (Aitken and Vernet, 1998). The generated O_2^- dismutate rapidly to H_2O_2. There are many studies suggesting that H_2O_2 is the most toxic oxidizing species for spermatozoa (De Lamirande and Gagnon, 1992; Aitken et al., 1993; Griveau and Le Lannou, 1997). However, it is more likely this toxicity to be due to the hydroxyl radicals' generation from H_2O_2 in the presence of metal ions. Hydroxyl radicals are thought to be the most damaging agents within cells (Halliwell and Gutteridge, 2007). Thus, the high activity of CAT is crucial for the protection of biological molecules from oxidative damage. The results obtained in this study show that the sperm of the animals of the breed Ile de France had statistically significantly higher SOD activity, followed by this of Sofia (Elin-Pelin) breed. The same pattern was preserved with regard to CAT activity, although there were no significant differences between the different breeds. Our results for the activity of this enzyme before freezing (Fig. 3) are close to the results obtained by Asadpour (2012) for inbred rams.

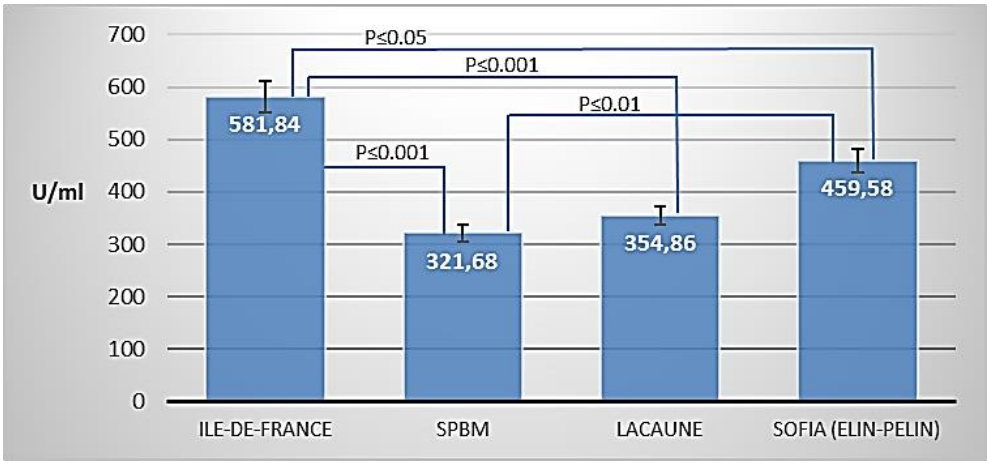

Figure 1: Significant differences between breeds on the enzyme activity of the enzyme SOD before freezing.

When measuring the activity of the studied enzymes after freezing and subsequent thawing of the semen, the obtained values (Fig. 2) were about 40 – 50% lower than those before freezing (Fig. 1). These results confirm the data on lower activity of these enzymes after cryopreservation of sperm in animals: ram (Marti et al., 2013), boars (Bilodeau et al., 2000), goats (Ismail et al., 2020), and humans (Lasso et al., 1994). For the studied SOD and CAT we found interbreed variabilities in their activity after thawing of the ejaculates (Fig. 2 and Fig. 3). To the best of our knowledge there are no research evaluating the rams' interbreed variabilities in the activity of antioxidant enzymes after cryopreservation. Our results established the highest SOD and CAT activities preserved after freezing-thawing for the sperm of the breeds Ile-de- France and Sofia (Elin-Pelin). It can be assumed that the semen with higher activity of antioxidant enzymes will be more resistant to oxidative stress and will have better reproductive capabilities, given the numerous data on the presence of an inverse relationship between the degree of oxidative stress and sperm quality (Bansal and Bilaspuri, 2011; Aitken et al., 2016; Lucio et al., 2016).

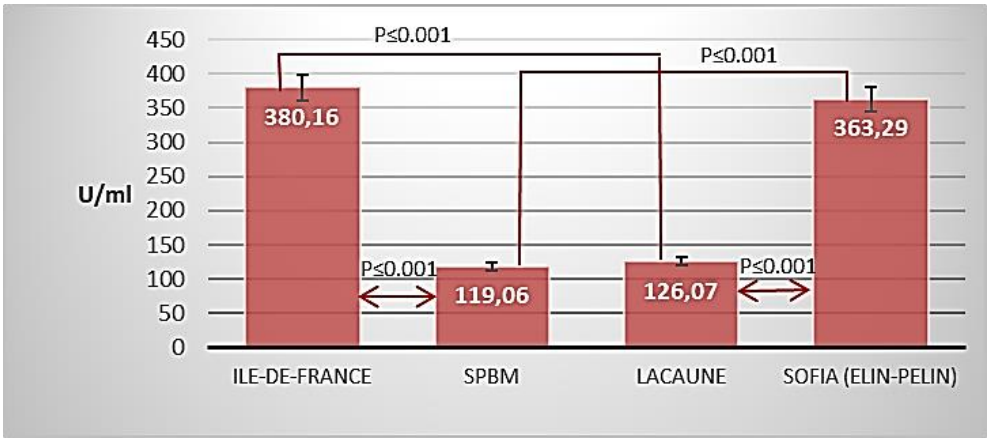

Figure 2: Significant differences between breeds on the enzyme activity of the enzyme SOD after thawing.

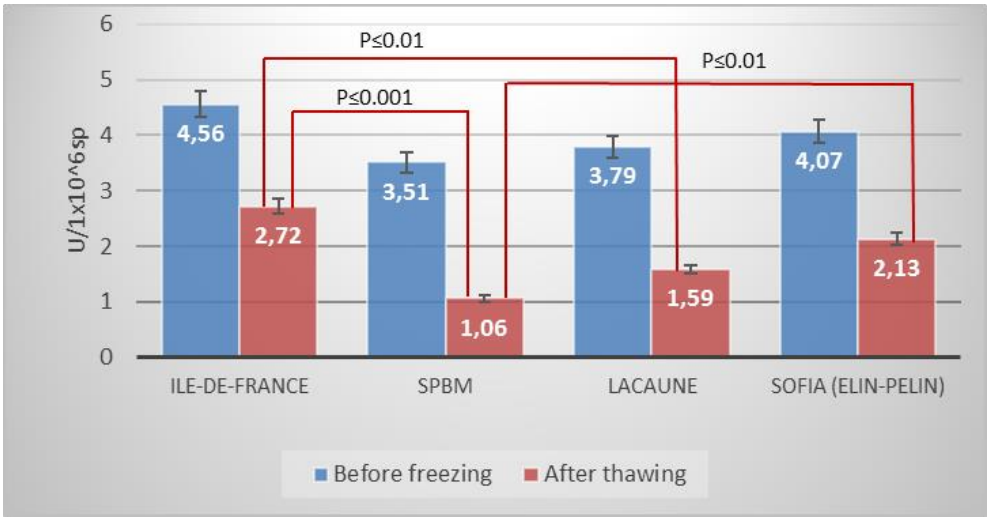

Figure 3: Significant differences in catalase activity (CAT) in sperm from the studied breeds before freezing and after thawing.

Conclusion

1. Cryopreservation caused a decrease in the activity of enzymes from the antioxidant defense system of sperm – SOD and CAT.
2. Significant interbreed differences were found before freezing and after thawing in the sperm SOD activity of the studied breeds.
3. Significant interbreed differences were found in seminal CAT activities only after cryopreservation.
4. The indigenous Bulgarian breed (Sofia (Elin-Pelin)) and the breed for meat Ile de France displayed higher antioxidant enzymatic protection of sperm than the studied breeds for milk – SPBM and Lacaune. It can be assumed that semen with better antioxidant protection remains more stable during processing and has better fertility.

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References

1. Aebi H. (1984). *Catalase in vitro*. Methods Enzymol. 105:121–126.
2. Agarwal A., Saleh R., Bedaiwy M. (2003). *Role of reactive oxygen species in the pathophysiology of human reproduction*. Fertil Steril., 79: 829–843.
3. Aitken R.J., Buckingham D., Harkiss D. (1993). *Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa*. J. Reprod. Fertil. 97:441–450.
4. Aitken R.J., Vernet P. (1998). *Maturation of redox regulatory mechanisms in the epididymis*. J. Reprod. Fertil. Suppl., 53:109–118.
5. Aitken R.J., Gibb Z., Baker M.A., Drevet J., Gharagozloo P. (2016). *Causes and consequences of oxidative stress in spermatozoa*. Reproduction, Fertility and Development, 28, 1–10 http://dx.doi.org/10.1071/RD1532.
6. Andreyev A., Kushnareva Y., Starkov A. (2005). *Mitochondrial metabolism of reactive oxygen species*. Biochemistry, 70(2):200–214.
7. Anger J.T., Gilbert B.R., Goldstein M. (2003). *Cryopreservation of sperm: Indications, methods and results*. The Journal of Urology, 170: 1079–1084.
8. Asadpour R., Pourseif M.M., Moghaddam G.H., Tayefi H. (2012). *Activity of antioxidant enzymes and malondialdehyde in seminal plasma and theirrelationship with semen characteristics in cross-bred rams*. Indian Journal of Animal Sciences, 82(7):710–712.
9. Bansal A.K., Bilaspuri G.S. (2011). *Impacts of Oxidative Stress and Antioxidants on Semen Functions*. Veterinary Medicine International, Article ID 686137, https://doi.org/10.4061/2011/686137.
10. Bilodeau J.F., Chatterjee S., Sirard M.A., Gagnon C. (2000). *Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing*. Mol. Reprod. Dev., 55:282–288.
11. Cassou R. (1964). *La method des paillettes en plastique et son adaption la generalion la generalization de la congelation*. C.R. Acad/ Agric., N 50, p. 881–887.
12. De Lamirande E., Gagnon C. (1992). *Reactive oxygen species and human spermatozoa. II. Depletion of adenosine triphosphate plays an important role in the inhibition of sperm motility*. J. Androl., 13:379–386.
13. Gallardo M. (2007*). Evaluation of antioxidant system in normal semen*. Rev. Invest. Clin., 59 (1): 42–47.
14. Griveau J.F., Le Lannou D. (1997). *Reactive oxygen species and human spermatozoa: physiology and pathology*. Int. J. Androl., 20(2):61–69.
15. Halliwell B., Gutteridge J.M.C. (2007). *Cellular responses to oxidative stress: adaptation damage repair senescence and death*. In Free Radicals in Biology and Medicine 4th edn. pp. 187–268. Oxford: Oxford University Press.
16. Ighodaro O.M., Akinloye O.A. (2018). *First line defence antioxidants–superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid*. Alexandria Journal of Medicine, 54 (4): 287–293.

17. Ismail A.A., Abdel–Khalek E., Khalil W.A., Yousif A.I., Saadeldin I.M., Alshammari G.M., El–Harairy M.A.(2020). *Effects of mint, thyme, and curcumin extract nanoformulations on the sperm quality, apoptosis, chromatin decondensation, enzyme activity, and oxidative status of cryo-preserved goat semen*. Cryobiology, 97:144–152.
18. Kothari S., Thompson A., Agarwal A., Plessis S. (2010). *Free radicals: their beneficial and detrimental effects on sperm function*. Indian J.Exp.Biol., 48(5):425–435.
19. Lasso J., Noiles E., Alvarez J., Storey B. (1994). *Mechanism of superoxide dismutase loss from human sperm cells during cryopreservation*. J. Androl., 15:255–265.
20. Lucio C.F., Regazzi F.M., Silva L.C.G., Angrimani D.S.R., Nichi M., Vannucchi C.I. (2016). *Oxidative stress at different stages of two–step semen cryopreservation procedures in dogs*. Theriogenology, 85 (9) 1568–1575.
21. Marti E., Marti J.I., Muiño–Blanco T., Cebrián–Pérez J.A. (2013). *Effect of the cryopreservation process on the activity and immunolocalization of antioxidant enzymes in ram spermatozoa*. J. Androl., 29 (4):459–467.
22. Peskin A.V., Winterbourn C.C. (2017). *Assay of superoxide dismutase activity in a plate assay using WST–1*. Free Radic., Biol. Med., 103:188–191.
23. Shiva M., Gautam K., Verma Y., Shivgotra V., Doshi H., Kumar S. (2011). Association between sperm quality, oxidative stress, and seminal antioxidant activity. Clin. Biochem. 44(4):319–324.
24. Sikka S.C. (2004). *Role of oxidative stress and antioxidants inandrology and assisted reproductive technology*. Journal of Andrology, 25:5–18.
25. Thuwanuta P., Chatdarong K., Bergqvist A.S., Söderquist L., Thiangtum K., Tongthainan D., Axnér E. (2011). *The effects of antioxidants on semen traits and in vitro fertil–izing ability of sperm from flat–headed cat (Prionailurus planiceps)*. Theriogenology, 76:115–125.
26. Yoshida K., Yamasaki T., Yoshiike M., Takano S., Sato I., Iwamoto T. (2003). *Quantification of seminal plasma motility inhibitor/semenogelin in human seminal plasma*. Journal of Andrology, 24:878–884.