



THE ANTIOXIDATIVE ACTION OF VITAMIN C AND ROSMARINIC ACID ON DNA INTEGRITY FROM BOAR SEMEN

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Abstract: Chemical structure make boar semen sensitive to cryoconservation and storing at room temperature. The reactive oxygen species (ROS) in high concentrations are leading to apoptosis and fertility decrease. Adding antioxidants such as Vitamin C and rosmarinic acid may reduce the deleterious effects displayed during assisted reproductive techniques. In this paper were assayed the antioxidative effects of Vitamin C and rosmarinic acid on DNA integrity from boar semen. The assessment was performed on semen samples from Large White and Pietrain boars, kept at room temperature ranging between 20-22°C. In control group (CO) no antioxidant were added mean while in Vitamin C group (C) 0.5 mM/L of C Viatmin was included and the same was performed with 105 µM/L rosmarinic acid (RA). In sample 1 the decrease of normal sperm DNA after 48 hours is less intense in C and RA groups compared to CO. In the samples 2 and 3 obtained from Pietrain boars the best results were noticed subsequent vitamin C use versus sample 1 where rosmarinic acid generated better results. The DNA integrity is crucial for fertilization and the antioxidative substances may contribute to its normal status.

• Introduction

Boar semen is more sensitive to cryopreservation and storing at room temperature than other species, due to the high content of unsaturated phospholipids and the low level of cholesterol in the plasma membrane. Thus, during cooling-thawing and assisted reproductive techniques (ART), changes in the sperm membrane lead to a destabilization, affecting calcium homeostasis, acrosome integrity, but also a disorganization of the lipid membrane [1]. Sources of ROS during ART procedures could be either endogenously (immature spermatozoa, leukocytes, oocyte, cumulus mass cells, follicular fluid, embryos) or exogenous environmental factors (visible light, culture media, pH, temperature, oxygen concentration, centrifugation, cryopreservation)[2].

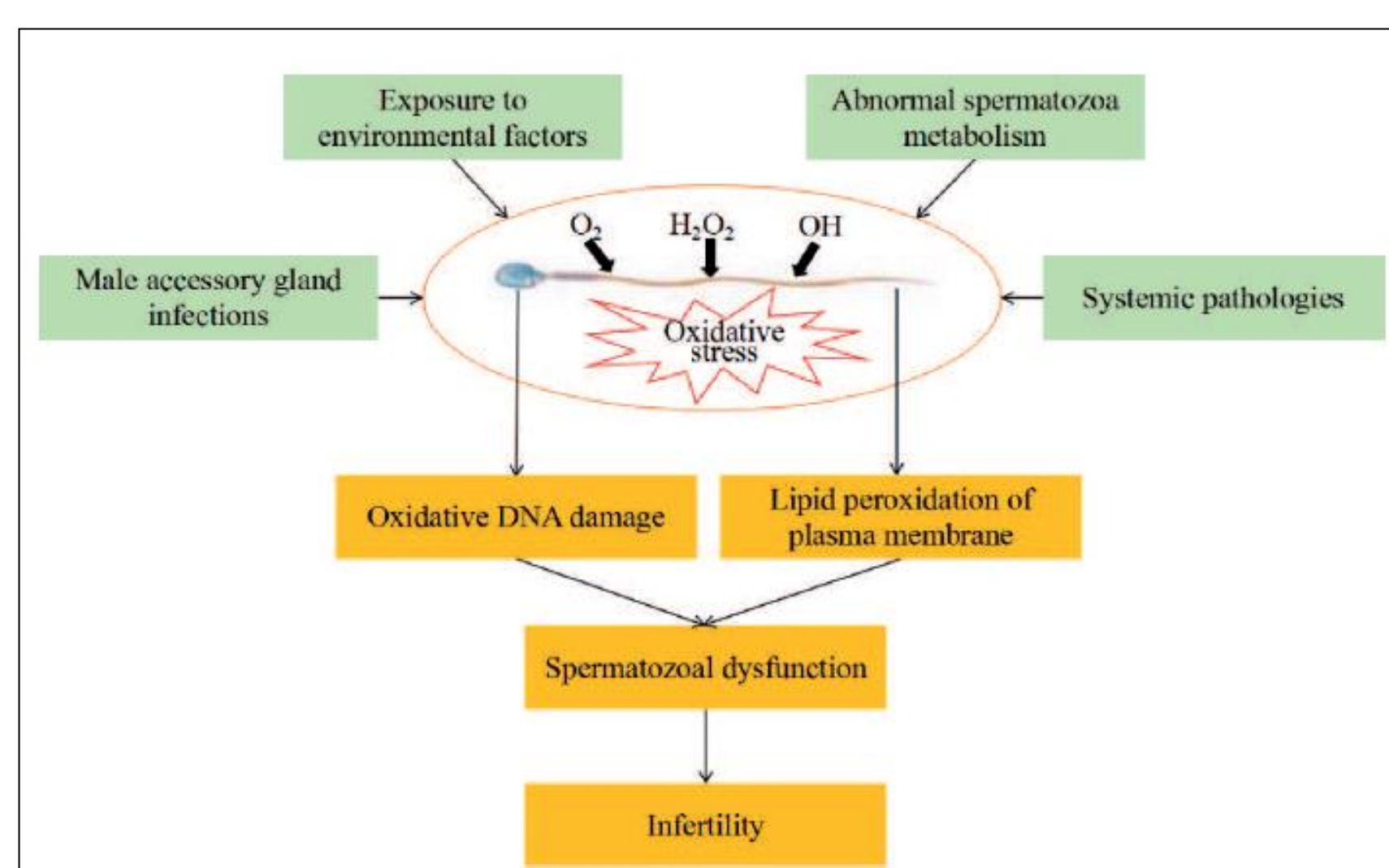


Figure.1. The main factors that contribute to the appearance of male infertility by inducing oxidative stress [3]

Antioxidants are the main defense factors against oxidative stress induced by free radicals. The supplementation of diluents used in cryopreservation with antioxidants ensures a protective effect and minimizes the effects of ROS in all species.

The aim of the paper is to evaluate the antioxidant effects of vitamin C and rosemary acid on the integrity of sperm DNA in diluted boar semen, after 48 hours, the samples being kept at room temperature 20-22°C.

• Material and method

Boar semen samples from the Great White (P1) and Pietrain (P2 and P3) breeds diluted with Androstar Plus, kept at a temperature of 20-22°C for 48 hours were used. Evaluation of sperm DNA integrity was performed using the Halomax kit. This assessment is based on the differential response of sperm chromatin with or without fragmented DNA to a protein depletion treatment. In the absence of massive DNA breakage, the removal of nuclear proteins produces intensely stained nucleoids with very small haloes of DNA loops emerging from a central and compact core.

Bibliography

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• Results and discussions

The results obtained after evaluating the integrity of sperm DNA in boar semen samples without antioxidants (control group at T = 0) and then at 48 h with and without antioxidants are presented in the table 1 and figure 2.

Table 1. Percentage of boar sperm with intact DNA "+" or affected DNA "-" before and 48 hours after use of antioxidants

ID probes	Group CO at T0		Group CO at 48h		Group C at 48h		Group RA at 48h	
	DNA "+"	DNA "-"	DNA "+"	DNA "-"	DNA "+"	DNA "-"	DNA "+"	DNA "-"
P1	93.91	6.09	85.82	14.18	89.87	10.13	92.42	7.58
P2	96.75	3.25	95.59	4.41	95.83	4.17	89.25	10.75
P3	97.20	2.80	96.64	3.36	97.18	2.82	92.12	7.88

DNA "+" integrity of DNA is not affected, DNA "-" integrity of DNA is affected

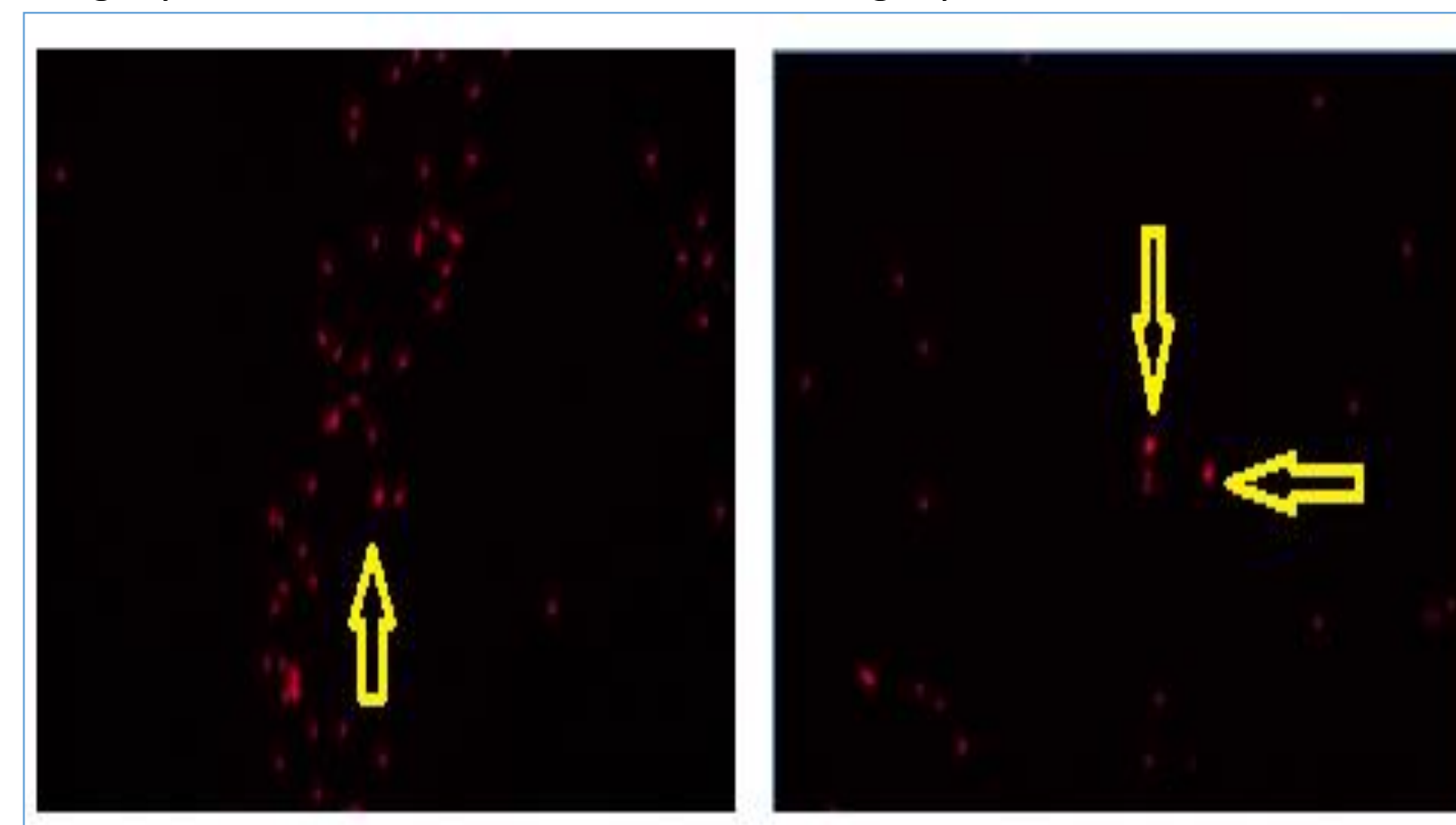


Figure 2. Boar spermatozoa stained with Fluorescein (arrow indicates spermatozoa with DNA integrity affected)(20X)

In sample 1 the decrease of normal sperm DNA after 48 hours is less intense in C and RA groups compared to CO. In the samples 2 and 3 obtained from Pietrain boars the best results were noticed subsequent vitamin C use versus sample 1 where rosmarinic acid generated better results.

• Conclusions

The antioxidative substances, vitamin C and rosmarinic acid may contribute to boar semen DNA integrity normal status.