ABSTRACTS

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Evaluation of sperm DNA peroxidation in fertile and subfertile dogs

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OBJECTIVES AND METHODS: Spermatozoa are sensitive to oxidative stress because they possess limited endogenous antioxidant protection while presenting abundant substrates for free radical attack in the form of unsaturated fatty acids and DNA (1). The expanding research interest in the last two decades on reactive oxygen species (ROS), oxidative stress, and male infertility has led to the development of various techniques for evaluating oxidative DNA damage in spermatozoa. Measurement of 8-hydroxydeoxyguanosine (8-OHdG) offers a specific and quantitative biomarker on the extent of oxidative DNA damage caused by ROS in sperm (2). Among various oxidative DNA adducts, 8-OHdG has been selected as a representative of oxidative DNA damage due to its high specificity, potent mutagenicity, and relative abundance in DNA (3). Embryonic development and pregnancy rate can be influenced by the rate of peroxidation found in DNA (4). As oxidative stress occurs when there is an imbalance between the concentrations of ROS and antioxidants, it is believed that a dietary supplementation with antioxidants may reduce the sperm DNA damage. The aim of this work was to compare peroxidation damage of DNA from sperm cells of fertile and subfertile dogs after oral supplementation with vitamin C and E. Ten healthy and sexually mature dogs were used in this study. They were from different breeds and age ranging between 2-8 years. The dogs were fed with a commercial dog food (Maxi adulto; Royal Canin) and water ad libitum. Based on the results of five previous semen analyses, dogs were divided in two groups: fertile dogs (G1): five dogs (n=5) that had a normal spermiogram; subfertile dogs (G2) six dogs (n=6) that had low sperm count <2x10^6 sperm/mL and/or more than 30% of total sperm pathology. After the first semen collection (M1), all dogs received an oral supplementation of 500mg/day of vitamin C and 500mg/day of vitamin E for 60 days. After 60 (M2) days since the beginning of oral supplementation, semen was collected. The degree of DNA peroxidation was quantified using a commercial kit (DNA Damage Elisa Kit – Assay designs). This kit is a fast and sensitive competitive immunoassay for the detection and quantitation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Statistical analysis was performed using SAS system. In case of difference between groups, it was used the Student-Newman-Keuls test.

RESULTS: Before oral supplementation with vitamin C and E (M1): fertile dogs (G1): 63.66 ng/mL of 8-OHdG and subfertile dogs (G2): 64.88 ng/mL of 8-OHdG. After 60 days of oral supplementation: G1: 59.69 ng/mL of 8-OHdG and G2: 67.44 ng/mL of 8-OHdG. No significant difference was observed between groups and moments. Those results are in disagreement with others previous reported, where the levels of 8-OHdG in sperm DNA from infertile men were significantly higher than in the control, and a combination of antioxidant supplements given to those infertile men resulted in a significant reduction of 8-OHdG level in sperm DNA (5). Using only vitamin C for 28 days Fraga et al. (6) also observed a decrease on sperm DNA damage. Dogs, unlike humans can synthesize vitamin C, this could be the reason why the oral supplementation with this vitamin did not have the same as effect as it could be seen in men.

CONCLUSION: the oral supplementation with 500mg of vitamin C and E do not decrease DNA peroxidation in fertile and subfertile dogs.

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