Canine sperm quality in fast freezing protocol with melatonin

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INTRODUCTION

The oxidative stress is a recurring problem related to sperm freezing, due to many process involved in this biotechnology of reproduction. Therefore, new techniques are frequently evaluated to reduce pos-thawing cellular damage, such as addition of antioxidants to protocol of sperm cryopreservation. However, the aim of this study was evaluate the addition of melatonin, in different concentrations, in a protocol of canine sperm fast freezing, with the purpose of reducing oxidative stress and improve the pos-thawed sperm quality.

MATERIAL AND METHODS

Six males dogs, adults, with age of two to five years old were used in the study, with three sperm collection, every fifteen days. The extender used was composed by Tris-egg yolk and ethylene glycol 5%, and melatonin added in five different concentrations at 1 mM, 1.5 mM, 2 mM, 2.5 mM and 3 mM of melatonin. The semen evaluated for the kinetic parameters with computer analysis in software CASA-SCA, morphology, membrane integrity, and parameters of oxidative stress, such as catalase (CAT) by consumption of H_2O_2, superoxide dismutase (SOD) due to pyrogallol autoxidation, and malondialdehyde (MDA) by lipid peroxidation rates.

RESULTS AND DISCUSSION

Kinetic parameters (Table 1), morphology, membrane integrity and oxidative stress parameters (MDA, CAT and SOD) (Figure 1) in different concentrations with melatonin and control group were compared.

Table 1. Kinetic parameters of fresh and post-thawed canine sperm, with addition of different concentrations of melatonin

Treat-ment	T Mot (%)	P Mot (%)	VCL (µm/s)	VAP (µm/s)	VSL (µm/s)	BCF (Hz)
Fresh	85.5 ± 9.3*	41.3 ± 21.9*	123.6 ± 29.7*	69.9 ± 18.5*	40.6 ± 11.3*	13.4 ± 3.8*
0 mM	16.0 ± 9.9	1.4 ± 1.0	76.4 ± 8.6	37.1 ± 4.8	20.3 ± 5.1	8.7 ± 4.9
1 mM	14.9 ± 7.8	2.2 ± 1.3	76.1 ± 11.2	38.9 ± 6.6	23.3 ± 4.5	13.2 ± 3.1
1.5 mM	17.1 ± 10.3	2.3 ± 1.4	74.9 ± 12.5	41.2 ± 6.2	24.5 ± 3.0	12.9 ± 3.6
2 mM	22.6 ± 16.3	3.8 ± 3.1	85.5 ± 12.6	42.6 ± 5.1	23.6 ± 2.7	11.6 ± 6.2
2.5 mM	13.2 ± 8.9	1.95 ± 1.4	68.7 ± 9.9	37.1 ± 5.0	19.9 ± 4.4	9.7 ± 5.1
3 mM	22.1 ± 7.1	3.5 ± 1.8	83.6 ± 7.3	43.1 ± 3.8	25.2 ± 2.3	13.3 ± 1.0

T Mot: total motility; PMot: progressive motility; VCL: curvilinear velocity; VAP: average path velocity; VSL: straight-line velocity; BCF: beat-cross-frequency; treatment: melatonin in different concentrations (mM). *: different superscripts within the same column indicate statistical differences: P < 0.05 (ANOVA one way).

No significant correlation (p>0.05) among different treated groups and control group was observed. Proportion of normal cells was similar (p>0.05) in fresh and cryopreserved sperm in all the groups. Abnormal morphology was observed with mean of 11,25% of head, 9,32% of tail and 3,56% of midpiece, without difference (p>0.05) among these. Membrane integrity was significantly higher (p<0.05) in fresh sperm compared to cryopreserved sperm. Similar mean of membrane integrity was observed among control group and treated groups (p>0.05).

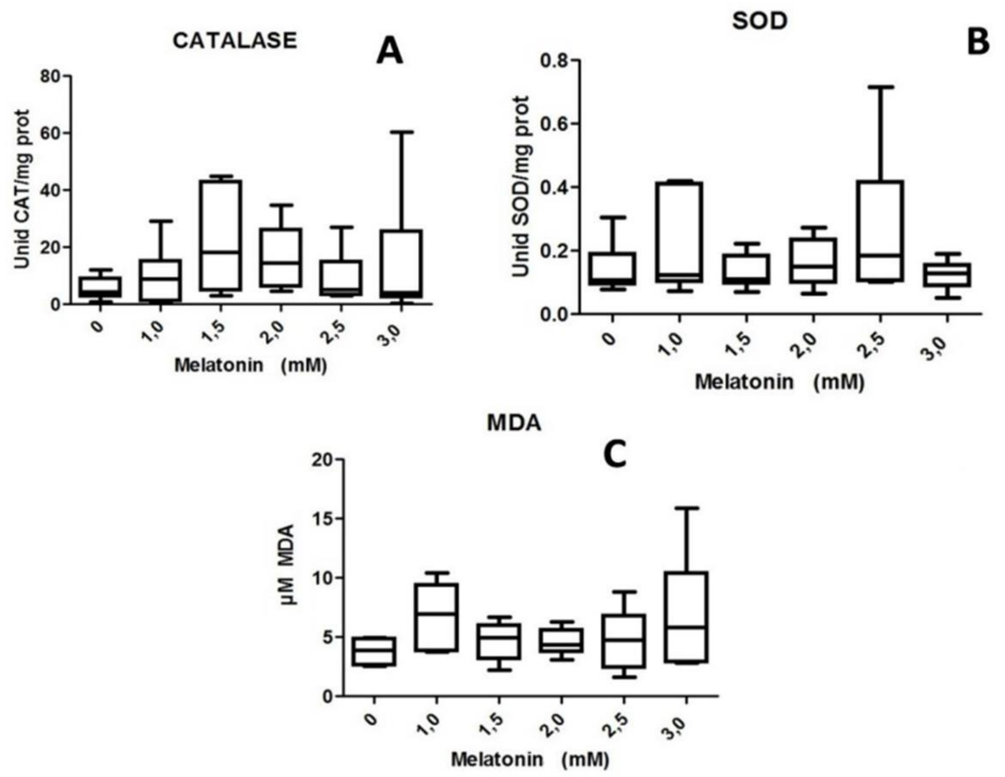

Figure 1. Levels of CAT (unid/mg protein) **(A)**, SOD (unid/mg protein) **(B)** and MDA (µM of MDA) **(C)** in dog sperm cryopreserved with melatonin (mM).

CONCLUSION

Melatonin addition in freezing canine semen didn't provide significant increases in pos-thawing quality semen and does not decrease oxidative stress. In this study it was possible to evaluate the efficacy of cryopreservation sperm protocol for Frenchie Bulldog.

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ACKNOWLEDGMENTS