ABSTRACTS

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INFLUENCE OF ASCORBIC ACID AND GLUTATHIONE ANTIOXIDANTS ON CANINE SEMEN CRYOPRESERVATION

Monteiro JC¹; Gonçalves JSA¹; Rodrigues JA¹; Lúcio CF¹; Silva LCG¹; Assumpção MEOA¹; Vannucchi CI¹

¹Department of Animal Reproduction, School of Veterinary Medicine and Animal Sciences, University of São Paulo, Brazil. Av Prof. Orlando Marques de Paiva, 87. 05508-270. São Paulo, Brazil.
E-mail: cacavann@usp.br

Introduction - Canine semen cryopreservation has been constantly researched in the past decade due to the expressive loss of viability and fertilizing capacity notified in post-thaw samples. One of the factors that affect semen quality is the excessive formation of reactive oxygen species by sperm oxidative stress (3). Sperm lipid peroxidation leads to acrosomal structural alterations, irreversible motility loss, metabolism change (1) and reduction of the fertilizing potential through inhibition of the oocyte-sperm binding capability (2, 4). The possible roles of antioxidant supplementation have been verified elsewhere for the boar (3) and bull (1) cryopreserved semen. Antioxidants such as glutathione and ascorbic acid are believed to exert a protective effect on spermatozoa by maintaining metabolic activity and sperm viability (1). The aims of this work were to evaluate and compare the effect of different concentrations of ascorbic acid (50μM and 250μM) and glutathione (1mM and 5mM) on post-thaw canine semen parameters.

Material and methods - The present study was performed in 2 steps with the use of 5 mature dogs of distinct breeds whose semen was collected twice in each step. After collection, semen was centrifuged, resuspended and the final volume divided into 3 aliquots in order to accomplish the following experimental groups: glutathione 1mM (Glu-1), 5mM (Glu-5) and control (C) in Step I and ascorbic acid 50μM (AA-50), 250μM (AA-250) and C in Step II. The extender Tris-fructose-citric acid was utilized with a final glycerol concentration of 5%. Thawing was performed at 37°C for 30 seconds. The fresh and frozen-thawed semen were appraised by sperm motility (%), forward progressive velocity (0-5) and morphology (%) with Eosin-Nigrosin and Spermac® stains. The sperm membrane status was also assessed by the carboxifluorescein diacetate/propidium iodide (CFDA-PI) fluorescent probes. Moreover, the thermal resistance test (TRT) was carried out in order to verify post-thaw sperm longevity for up to 120 minutes of incubation at 37°C. Data were analyzed using a repeated measure analysis of variance (ANOVA) and the post hoc Newman-Keuls comparison was conducted to establish overall differences among groups at p<0.05.

Results and Discussion - Although an expressive decrease in sperm viability was verified after cryopreservation, sperm motility of AA-50 group was significantly superior (72% ± 5.7) than control (62.3% ± 8.1), Glu-1 (61.0% ± 10.4) and Glu-5 (63.5% ± 12.4). There was a significant reduction of progressive forward velocity in all treatments, except for Glu-5 group (3.4 ± 0.5). Hence, 5mM glutathione supplementation maintained unaltered the post-thaw sperm velocity compared to fresh semen. Moreover, sperm velocity results were superior comparing to control (2.9 ± 0.1) and ascorbic acid groups (3.1 ± 0.1 for AA-50 and AA-250). In relation to sperm motility along the TRT, ascorbic acid supplementation showed to be beneficial for the first 30 minutes (51% ± 6.5). However, this positive effect did not last the role incubation period and the 5mM glutathione group presented significantly higher motility for up to 90 minutes after thawing (18% ± 14.4). For this reason, we assume that glutathione had a long last protective effect than ascorbic acid. Moreover, higher concentrations of glutathione (5mM) were capable of prolonging sperm viability for a longer period of time, whereas ascorbic acid showed a negative effect at 250μM concentration during incubation.
superior to 90 minutes. Sperm integrity evaluated by the CFDA-PI dyes showed extensive injuries in plasmatic and acrosomal membranes. When comparison was performed among groups, a beneficial influence on sperm integrity was verified for glutathione supplementation at 1mM (40.1% ± 6.2) and 5mM (42% ± 4.0). Sperm integrity index of Glu-1 and Glu-5 groups were significantly superior to control (26.2% ± 5.1) and ascorbic acid groups (30.2% ± 8.2 and 30.3% ± 7.3, respectively AA-50 and AA-250). Spermac® was considered a stain with better sensitivity to identify sperm pathology as it enables to detail acrosomal defects. No difference between post-thaw sperm defects of Glu-1 (22.2% ± 3.98) and fresh semen (14.4% ± 5.76) was notified. In addition, Glu-5 showed significantly lower sperm defect (22.5% ± 3.02) than the other groups. At the Eosin-Nigrosin stain, all groups showed significantly higher post-thaw sperm pathology compared to fresh semen. However, glutathione groups (16.1% ± 5.1 and 18.3% ± 5.4, respectively Glu-1 and Glu-5) presented a better protective antioxidant effect than other groups (25.2 ± 6.4; 24.5 ± 6.8 and 20.4 ± 4.3, respectively for AA-50, AA-250 and C).

**Conclusion** - Antioxidant supplementation to freezing extenders proved to maintain frozen-thawed semen parameters superior to control group, increasing spermatozoa longevity and viability. Moreover, glutathione demonstrated to act as a more efficient protector than ascorbic acid, mainly when it was employed at a 5mM concentration. On the other hand, the 250µM ascorbic acid concentration presented possible toxic effects when incubated at a prolonged period of time.

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**References**