ABSTRACTS

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Effect of different concentrations of reduced glutathione on frozen-thawed canine semen

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OBJECTIVES AND METHODS: Sperm are particularly susceptible to oxidative stress as their plasma membrane contains large amounts of polyunsaturated fatty acids and their cytoplasm has a low concentration of protective enzymes. The process of sperm cryopreservation induces the formation of free radicals, thus decreasing sperm performance. Therefore, the supplementation of the extender for cryopreservation with antioxidants may improve post-thaw sperm quality. The aim of this study was to compare the effects of different concentrations of reduced glutathione (GSH) on frozen-thawed canine semen. For that purpose, eleven semen samples obtained from six sexually mature dogs of distinct breeds, aged from 2 to 7 years, were each equally divided into 3 groups: GSH10 (10 mM glutathione), GSH20 (20 mM glutathione) and Control (C - without glutathione). A two-step cryopreservation protocol with Tris-fructose-citric acid-egg yolk extender with 5 % glycerol was employed. Glutathione supplementation was performed according to the experimental group. Prior to freezing, semen was analyzed pursuing adequate seminal parameters. Post-thawed semen was evaluated for: sperm motility (%), forward progressive velocity (arbitrary scale from 0 to 5), specific patterns of sperm motility (computerized motility analysis-CASA), oxidative stress through thiobarbituric acid reactive substances assay (TBARS), mitochondrial activity through the oxidation of 3,3'-diaminobenzidine (DAB, %), flow cytometry analysis using mitochondrial membrane potential - JC1 dye (%), plasma and acrosomal membrane integrity - FITC/PI dyes (%) and DNA fragmentation - SCSA (Sperm Chromatin Structure Assay, %). Data were analyzed by ANOVA and LSD at p ≤ 0.05. This study was approved by the Bioethics Committee of the Faculty of Veterinary Medicine - USP.

RESULTS: There was statistical decrease in post-thaw sperm motility (C: 45% ± 5.8; GSH10: 29% ± 5.7; GSH20: 11% ± 2.6) as glutathione concentration increased. Only forward progressive velocity of GSH20 (1.5 ± 0.2) was statistically inferior to control (2.3 ± 0.3). CASA detected a statistical decrease in post-thaw total (MOT) and progressive (PROGR) motility in GSH20 (11% ± 2.2 and 6% ± 1.3 respectively) and increased static sperms (72% ± 7.5) compared to control (MOT: 28% ± 3.0, PROGR: 16% ± 2.5 and static: 53% ± 5.2). DAB assessment showed a higher percentage of sperm with low mitochondrial activity in GSH20, whereas no change in cytometric analysis for JC1 was verified. GSH20 group presented a higher percentage of DNA fragmentation compared with C group (3.8% ± 2.6 and 3.2% ± 1.67, respectively), indicating a deleterious effect of excessive concentration of GSH. No difference among groups for oxidative stress was verified. Regarding sperm membrane integrity, GSH groups showed a lower percentage of acrosomal damage - FITC/PI (C: 25.0% ± 5.5, GSH10: 13.6% ± 2.3 and GSH20: 13.1% ± 1.5). In a previous experiment, Monteiro et al. (1) reported a protective effect of glutathione (5mM) on semen cryopreservation. In this experiment, 10mM GSH showed no consistent improvement on post-thaw sperm quality and 20mM GSH led to deleterious effects on mitochondrial activity. Nevertheless, the acrosomal membrane protective effect of treated groups was also observed previously (2, 3).

CONCLUSION: GSH supplementation of semen extenders promoted protective effect on acrosomal membrane. However, excessive concentration of GSH (20 mM) may lead to sperm damage and deleterious effect on sperm mopho-functional parameters. Hence, it is of utmost importance to test different concentrations of this antioxidant in order to achieve a beneficial effect, without the pro-oxidative effect.

(2) Stradaioli G, Noro T, Sylla L, Monaci M. Decrease in glutathione (GSH) content in bovine sperm after cryopreservation: Comparison between two extenders. Theriogenology2007; 67; 1249–1255.

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