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The use of reduced glutathione (GSH) as antioxidant for cryopreserved sperm in dogs

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Semen cryopreservation is known to induce the overproduction of reactive oxygen species (ROS) by spermatozoa. Despite the physiological effect of ROS in sperm (i.e. capacitation process), excessive amounts may be potentially deleterious. Thus, the addition of antioxidant agents in the cryopreservation extender may reduce the deleterious effects of oxidative stress improving post-thaw sperm quality. The addition of reduced glutathione (GSH) in the extender for semen cryopreservation in dogs had satisfactory results in the concentration of 5mM. However, systematic studies of the direct effect of GSH in sperm are scarce. Therefore, the aim of this study was to evaluate the effect of supplementation with different concentrations of GSH (0; 5; 7.5; 10mM) in the extender for cryopreservation in dogs with evaluations performed after both glycerolization (chilled) and thawing (thawed). For this purpose, the study was conducted using 8 dogs of several breeds, body weights (10-30 kg) and ages (2 to 7 years). Two semen collections were performed in a weekly interval, totaling 16 semen samples. Semen of each individual was divided to contemplate the GSH concentrations (0; 5; 7.5; 10mM). The cryopreserved protocol was based on one step technique, using the egg yolk based extender containing 5% of glycerol with GSH. Samples of both chilled and thawed semen were evaluated by automatic sperm motility (CASA) and flow cytometry analysis of mitochondrial potential (JC1 dye) and membrane/acrosome integrity (FITC-PI dyes). Furthermore, seminal plasma was evaluated for lipid peroxidation (TBARS concentration). Statistical analysis was performed using repeated measures procedure (PROC MIXED; SAS). Significance was considered when p<0.05. No effects of treatments were observed on TBARS analysis and CASA parameters after glycerolization. On the other hand, flow cytometry analysis revealed lower percentages of sperm with damaged plasma membrane and acrosome in control group (72.98±3.40%) and samples treated with 7.5mM (65.43±3.62%) and 10mM (73.13±1.98%) when compared to those treated with 5mM (61.63±3.61%). Also, higher percentage of cells with low mitochondrial potential was observed in 0mM group (23.2±3.69%), when compared to 5mM (14.33±2.25%) and 7.5mM (13.89±1.82%), indicating a protective effect of GSH for both membranes and mitochondria of chilled samples. For the thawed samples, CASA analysis showed higher ALH in control group (6.3±0.36µm) when compared to 7.5mM (4.78±0.65µm). Similarly, groups treated with 0mM (28.96±0.69Hz), 5mM (29.7±1.4Hz) and 10mM (29.4±0.3Hz) had higher BCF when compared to those treated with 7.5mM (22.04±2.9Hz). Furthermore, the flow cytometry analysis of thawed samples showed higher percentages of sperm with intact plasmatic membrane and damaged acrosome in 0mM group (1.62±03%) when compared to 5mM (0.8±0.1%). These latter results of motility (higher BCF and ALH) and plasma and acrosome membrane status (higher percentage of cells with intact membrane and damaged acrosome) in the control group may have occurred to an early capacitation of thawed samples, which could be avoided by the antioxidant treatment. Also, corroborating with this assertion, the percentage of sperm showing both acrosome and membrane damaged were superior in the group treated with 0mM (51.02±5.6%) when compared to that treated with 10mM (33.2±3%). In conclusion, these results show a protective antioxidant effect of GSH in both chilled and thawed spermatozoa. However more studies are necessary, searching the ideal concentration of GSH in each condition.