Proceedings of the 8th International Symposium on Canine and Feline Reproduction
ISCFR

June 22-25, 2016
Paris, France

In a joint meeting with the XIX EVSSAR Congress

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Effect of cholesterol loaded cyclodextrins on post-thaw semen quality in dogs
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Cryopreservation provokes cholesterol depletion in the plasma membrane of sperms leading to decreased fertility of frozen-thawed semen. Cholesterol-loaded cyclodextrins (CLC) have been shown to improve post-thaw semen parameters in stallion, bull, buck, ram and rabbits. However, no such study is available on cryoprotectant potential and optimal inclusion level of CLC in canine semen. The aim of this study was to investigate the effectiveness of CLC on the post thaw semen quality in dogs. In addition, different concentrations were compared to establish the optimum inclusion level in the extended semen. Semen collection, through digital manipulation, was conducted once a week in 4 adult German shepherd dogs (n=20 ejaculates; 5 ejaculates/dog). Only samples with mass motility>3 (0: without movement; 5: fast progressive movement), motility>70% and concentration >200 × 10^6/ml were pooled and further processed. Semen was extended according to each treatment group (Control vs 1, 2 and 3 mg of CLC/ml of extender) to obtain the final sperm concentration of 120 × 10^6/ml. The extended semen was cooled, equilibrated and frozen according to the standard procedure. All semen straws were assessed for post-thaw parameters including percentage motility (phase-contrast microscopy), normal/abnormal and live/dead ratio (eosin-nigrosin staining) plasma membrane integrity (hypo-osmotic swelling test), acrosome integrity (normal apical ridge test) and DNA integrity (acridine orange assay). The effects of different inclusion levels of CLC on various post-thaw quality parameters were compared using ANOVA and further analyzed by the Tukey’s range test, if applicable. The addition of CLC showed an overall improvement in post thaw semen quality as compared to the control group. Among various treatment groups, and when compared to the control group, the percentages of motile (55.5%), viable (65%), plasma membrane intact (56.7%), acrosomal intact (49.2%) and DNA intact (98%) spermatozoa were significantly higher in 2 mg/ml CLC group (P<0.05). The percentages of morphological abnormalities, though lowest (5.9%) in 2mg/ml CLC group, were not significantly different between treated and control groups. It is concluded that the cholesterol incorporation in semen extenders prior to freezing results in a beneficial increase in the cryosurvival of canine spermatozoa. Further studies are needed to explore the mechanism of interactions between cholesterol and sperm membrane which result in improved semen quality parameters.