ABSTRACTS

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Comparative analysis of canine semen freezing methods and extenders

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OBJECTIVES AND METHODS: There are many different extender formulations and protocols for canine semen freezing but the scientific evidence of their efficacy is sparse. In addition, two different methods of storing cryopreserved semen specimens exist - in straws and in pellets. Therefore, the purpose of this study was to investigate the cryopreservation effect of four different semen extenders. We used one commercial canine extender designed for freezing spermatozoa in pellets (extender A), a second commercial canine extender for freezing spermatozoa in straws (extender B), commercial human extender for freezing human spermatozoa in straws (extender C) and Equex STAMP (extender D). Ten dogs were used, each of them giving at least four separate ejaculates with a resting period of a minimum of three days. Minimal required semen quality was 75% estimated progressive motility, 75% normal morphology and 250 x 10^6 total spermatozoa per collection. Every ejaculate was stored in straws and pellets with each one of the extenders tested. Semen quality parameters assessed before and after freezing were total subjective motility, progressive motility, concentration (spectrophotometer SpermaCue®), membrane integrity, the presence of the acrosomal reaction and eosin-nigrosin staining for viability and morphology. After thawing, semen quality of each collection was evaluated in triplicates under both freezing methods.

RESULTS: Forty separate ejaculates of appropriate quality were included in the study. Sperm quality was significantly decreased following freezing and thawing (p < 0.05) for all four extenders with extender D giving the best overall post-thaw parameters. While post-thaw progressive motility (PM) for extenders A (38.6 +/- 20.8 %; p < 0.05), B (49.0 +/- 13.1%; p > 0.05) and C (53.5 +/- 15.9%; p > 0.05) was better preserved in pellets, extender D performed better in straws (PM 60.0 +/- 17.3; p > 0.05) than in pellets (57.5 +/- 13.9%). In two dogs, PM after freezing semen in pellets was much better preserved using extender A (both dogs 70%) compared to extender B (20% and 35%), C (35% and 50%) and D (40% and 50%). In another dog, PM was better preserved in pellets with extender C (75%) compared to extender D (50%). Freezing–thawing process caused a significant decrease in plasma membrane integrity (PMI) in straws and pellets with all four extenders (A and B, p < 0.001; C and D, p < 0.05). For all extenders, PMI was better preserved when semen was frozen in straws (p < 0.05). Although the morphology of cryopreserved sperm did not differ significantly between four extenders, it seemed that extender D resulted in fewer spermatozoal anomalies (p > 0.05). The percentage of morphologically normal spermatozoa was not correlated with post-thaw motility or plasma membrane integrity.

CONCLUSION: None of the extenders used in our study performed equally well for all parameters in all dogs. Namely, even though it seemed that in the majority of dogs' semen quality post-thaw was better preserved with extender D, three dogs showed better results with extenders A or B. This suggests that differences exist in the ability of individual canine semen samples to withstand freezing when using the same extender. Surprisingly, a commercial human extender gave better after thawing results for canine semen compared to the commercial canine extenders in this study. Unexpected as well, canine and human extenders produced for freezing semen in straws gave better overall post-thaw results if used in pellets. Our results suggest that until the ideal semen freezing extender is found, alternative freezing protocols should be considered in dogs whose semen does not freeze well.