ABSTRACTS

6th International Symposium on Canine and Feline Reproduction

&

6th Biennial EVSSAR Congress

European Veterinary Society for Small Animal Reproduction

"Reproductive biology and medicine of domestic and exotic carnivores"

University of Veterinary Sciences
9th – 11th July 2008
Vienna, Austria

Editors: G. England, P. Concannon, S. Schäfer-Somi

Reprinted in IVIS with the permission of the Symposium Organizers
CORRELATION OF CANINE SEMINAL PROTEIN PROFILE AND SEMEN FREEZABILITY OF DOGS

Martins MIM1*, Souza FF2,3, Chirinéa VH4, Tebet JM4, Lopes MD4
1Departamento de Clínicas Veterinárias, UEL, Cx. Postal 6001, Londrina, PR, 86051-900, Fone: +554333714559 Fax +554333714063, Brasil. 2FertCani, Botucatu, SP, Brasil. 3Universidade de Franca, UNIFRAN, Franca, SP, Brasil. 4Departamento de Reprodução Animal e Radiologia Veterinária, FMVZ, UNESP, Botucatu, SP, Brasil. *E-mail: imartins@uel.br

Introduction - The seminal plasma is a mean of transport and support of sperm consisting, in the dog, by testicular, prostate and epididymal secretions, and among the biochemical components the proteins are correlated to fertility. SOUZA (2003) found a total of 37 bands and identified a positive correlation between protein bands densitometry of the seminal plasma and in vitro fertility. In cattle and sheep the influence of the seminal plasma protein in the semen freezability has been studied.

Objectives - The objective of this study was to correlate the results of sperm freezability and protein profile of canine seminal plasma.

Materials and methods - Eight adult dogs of 3 breeds (2 Blood Hound, 2 Golden Retriever, 4 Springer Spaniel), aged between 1.2 and 6 years and weight between 12 and 35 kg, were used in the study. During 14 months, semen was collected every 15 days by the method of digital stimulation, using a female in "heat". Following the collection the ejaculates were evaluated for sperm motility and centrifuged. The supernatant was stored at -20º C and the pellet was diluted in one step with an extender described by PEÑA & LINDE-FORSBERG (2000) and modified by Martins (2005). Samples were packed in 0.5mL French straws with 40 x 10^6 sperm/straw, and cooled to 5ºC, during 60 minutes. After cooling the straws were placed 6 cm above liquid nitrogen for 20 minutes and then emerged into it. Thus, they were stored in criogenic container. Semen was thawed in water bath (70ºC/8sec) and evaluated for sperm motility and integrity of membrane by fluorescent probes (Cunha et al., 1996). The electrophoresis, SDS-PAGE was performed using two concentrations of polyacrylamide, 12% and 18% in the gel of separation. The gels were stained with coomassie brilliant blue R-250, the images were scanned and evaluated in an analyzer of images, and the molecular weight was estimated according to a standard applied in each gel. The integrated optical density (IOD) of each band for each sample was also estimated (Martins, 2005). The results were submitted to the Pearson correlation.

Results - The mean values of sperm motility, pre- and post-thawing, were 86.9% and 49.2%, respectively. Regarding the quality of sperm after cryopreservation, a great individual variation was observed. Golden Retrievers and Blood Hounds showed better results than Springer Spaniels. Assessment of the electrophoretic profile of the seminal plasma proteins showed a total of 31 bands with molecular weights between 139.63 and 2.71 kDa. These results differ from the study of SOUZA (2003) working with 20 animals, in which 37 bands with variations between 100.6 kDa and 3.6 kDa were identified. Difference was also found from the study by SOUZA & LOPES (2002) working with 5 animals, in which only 25 bands were identified with densitometry between 136 kDa and 3.5 kDa. Among the bands identified, the greater optical density was found in the band 19 (15.29 kDa) in only one animal of the breed Golden Retriever with a low densitometry, which coincidentally showed high scores in the semen post-thawing evaluation (76% motility and 61% of integrity of membrane). This protein was in low concentration in the seminal plasma, probably because of its linkage with the spermatic membrane, protecting it from the stress of cryopreservation. Another band of...
15.59 kDa with high optical density identified throughout the experimental period showed a positive correlation with sperm motility before and after thawing, and membrane integrity of post-thawing (r = 0.20, r = 0.26 and r = 0.29, respectively). A band of similar molecular weight (15.59 kDa) with high densitometry was identified by SOUZA (2003), who suggested that this band could represent one of the subunits of arginine esterase. These bands can be searched in subsequent studies as a possible marker of fertility and/or high freezability in dogs.

References

