

# LIPID COMPOSITION OF EPIDIDYMAL SPERMATOZOA FROM DOMESTIC CATS

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## Background

Sperm lipids determine important physico-chemical properties of the sperm membranes and may serve as energy substrates for the cell. In a recent comparative study on the lipid composition of ruminantia and feloideae spermatozoa we have shown by matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) that feloideae sperm lipids mainly contain diacyl phospholipids but only marginal amounts of plasmalogens which are characteristic components of spermatozoa from ruminantia and other mammalian species (Fuchs et al., 2009).

Here we collected epididymal semen samples from 71 castrated domestic cats to analyse the individual variability of their lipids by MALDI-TOF MS. The total number of available sperm cells per animal varied from 7 to 278 x 10<sup>6</sup> spermatozoa. The high sensitivity of MALDI-TOF MS allows the analysis of lipid extracts from small semen samples.

After lipid extraction from washed spermatozoa according to Bligh and Dyer (1957), the chloroform phase was split into aliquots (in dependence of the cell number) and dried under nitrogen. In case of sufficient cell numbers, we additionally determined the cholesterol and phospholipid content of the spermatozoa.



Healthy domestic cat.



Cat epididymis prepared before cutting and slicing of the cauda.

## Quantitative analysis of cholesterol and phospholipid

Cholesterol was solubilized by use of methyl- $\beta$ -cyclodextrin and measured by a fluorescence-based enzyme assay (Amplex Red Cholesterol Assay Kit A12216, Invitrogen GmbH, Karlsruhe, Germany).

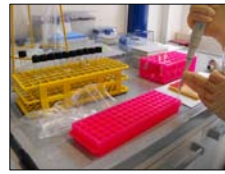
A minimum of about 10 x 10<sup>6</sup> cat spermatozoa cells gave reliable results and a mean **cholesterol** content of **0.30 ± 0.21 nmol per 10<sup>6</sup> sperm cells** was determined in 62 samples of domestic cats.

The **phosphate** content of cat spermatozoa could be determined according to Böttcher et al. (1961) in an extract corresponding to a minimum of 30 x 10<sup>6</sup> cells. Samples of 24 animals were measured and revealed a mean of **1.03 ± 0.64 nmol per 10<sup>6</sup> sperm cells**.

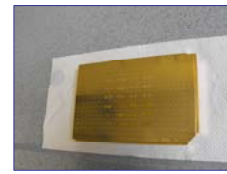
*The wide variation in the amounts of cholesterol and phospholipids among the animals is possibly caused by the inaccuracy of cell counting and/or by the diet of the animals.*

From the data of 24 animals, a mean molar **cholesterol/phospholipid** ratio of **0.30 ± 0.06** could be calculated. This ratio is comparatively low, but in the range of 0.26 to 0.45, given by Martinez and Morros (1996) and Jones (1998) for different species.

## Qualitative analysis of lipid extracts by MALDI-TOF mass-spectrometry



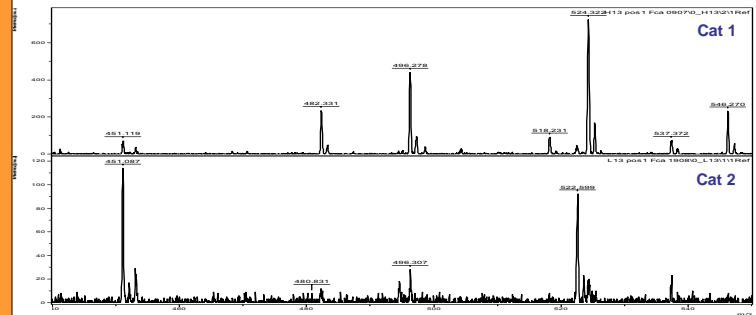
The samples were diluted with DHB matrix and 9-AA matrix.



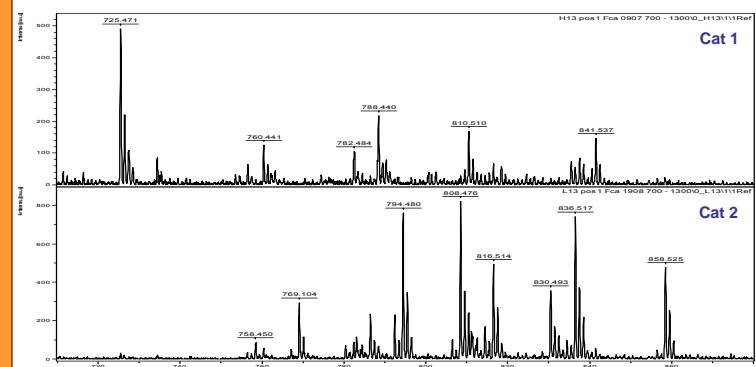
The samples were applied onto a gold target and dried.



Positive and negative ion MALDI-TOF MS were acquired on an Autoflex workstation (Bruker, Germany).



Positive MALDI-TOF mass-spectra (9-aminoacridine matrix) in the organic lipid extracts of domestic cats spermatozoa. m/z 482.3/504.3: H<sup>+</sup>- and Na<sup>+</sup>-adducts of lyso-phosphatidylcholine (LPC, 16:0 alkyl), 496.3/518.3: LPC (16:0), 522.3/544.3: LPC (18:1), 524.3/546.3: LPC (18:0).



Positive MALDI-TOF mass-spectra (9-aminoacridine matrix) in the organic lipid extracts of domestic cats spermatozoa. m/z 725.6: Na<sup>+</sup>-adduct of sphingomyelin (16:0), 758.6/780.6: H<sup>+</sup>- and Na<sup>+</sup>-adducts of phosphatidylcholine (PC, 16:0, 18:2), 760.6/782.6: PC (16:0, 18:1), 786.6/808.6: PC (18:0, 18:2), 788.6/810.6: PC (18:0, 18:1), 794.6/816.6: PC (16:0 alkyl, 22:5), 808.6/830.6: PC (16:0, 22:5), 810.6/832.6: PC (18:0, 20:4), 836.6/858.6: PC (18:0, 22:5), 841.6: seminolipid.

Spectra of 71 samples from domestic cats could be detected and MALDI-TOF MS revealed a high variability of lipid species from the individual animals. This especially concerns (i) the occurrence of lysophospholipids and (ii) the predominant fatty acyl residues in phosphatidylcholine.

## Outlook

Future analyses will evaluate the data in the context of parameters for spermatogenesis, sperm quality and animal condition.