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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Comparative membrane analysis of cat and boar sperm
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Sperm membranes are largely accountable for cell injury during chilling and freezing. Therefore, sperm membranes are the main target for the protective effects of extender lipids (e.g. in egg yolk), whereupon the details of lipid impact are still not understood. Whereas neither epididymal nor electro-ejaculated cat sperm are susceptible to cold shock if cooled at moderate cooling rates[1], ejaculated boar sperm are generally preserved at 16°C because they are extremely sensitive to cooling to 4°C. This different behaviour might be caused by the different lipid composition between these species. Whereas boar sperm membranes comprise a significant amount of ether lipids including plasmalogenes[2], cat sperm membranes mainly consist of “common” ester lipids with only a minor population of plasmalogenes[3]. To evaluate how the different lipid composition influences the physico-chemical membrane properties, we incorporated spin-labeled (SL) lipid analogues into the cell membrane of washed epididymal cat (n=9 individuals for SL-SM, n=7 for SL-PC and SL-PS) or ejaculated boar sperm (n=9 individuals for SL-SM, n=6 for SL-PC, n=7 for SL-PS) and recorded the ESR spectra of labeled unprotected sperm (i.e. medium free of lipids and protein) at 38, 22, 16, 0°C as well as after rewarming to 38°C. Those analogues reflect the behavior of endogenous lipids. SL-sphingomyelin (SL-SM) has been shown to accumulate solely in the outer sperm membrane leaflet, whereas SL-phosphatidylserine (SL-PS) is actively transported to the cytoplasmic leaflet. SL-phosphatidylcholine (SL-PC) is found in both leaflets. From the ESR spectra, the rotational correlation time (τc) of the spin-label was determined, which is inversely correlated to the micro-fluidity in the near analogue vicinity. Non-parametric statistical analyses were performed by Kruskal-Wallis test for unrelated samples followed (in case of data at different temperatures) by respective post-hoc tests. At 38°C, the mobility of SL-SM and SL-PS was higher in cat than boar sperm. The mobility of SL-PC was similar between both species. Upon cooling to 0°C, the fluidity decreased but this decrease was less pronounced in in cat sperm. In boar sperm, τc increased for SL-SM to significantly higher values at 22, 16, and 0°C compared to cat. This was also the case for SL-PC at 22 and 16°C. However, when sperm labeled with SL-SM were further cooled to -30°C, τc was significantly higher in cat sperm indicating a far more pronounced loss of fluidity upon freezing below 0°C in these cells. After rewarming sperm to 38°C, mobility of all labels was restored to the values before cooling, suggesting that no transversal relocation of the analogues had occurred during the experiment. In conclusion, membrane fluidity reported by SL-lipid analogues revealed a different response to cooling in cat and boar sperm. It remains to be analysed whether the less pronounced loss of fluidity upon cooling in cat sperm is related to their better chilling tolerance. According to this rationale, results suggest that the freezing process is the more critical part for cryopreservation of cat sperm. SL-lipid analogues will be applied in future studies to analyse the effects of protective extender components on changes in membrane fluidity upon cooling.