Abstract for General Public

Semen cryopreservation is one of the most important assisted reproductive techniques (ART) used in animals, both productive, companion and wild. Preservation of cells in very low temperatures (usually in liquid nitrogen, -196ºC) stops all the biological processes and keeps cells in the state of so called anabiosis (no-life). Thanks for that, cells can be safely stored for prolonged time without changing their properties. This enables creation of gene banks and facilitates shipment of biological material worldwide – it is much easier to transport a container with frozen semen that a living animal.

However, cryopreservation is a very harmful process. During freezing cells must face abrupt changes in their environment, formation of intracellular ice crystals, which mechanically damage their structures, disorganization of plasma membrane and attacks of reactive oxygen species. As a result, on average only half of sperm cells survive cryopreservation.

In domestic animals protocols for semen cryopreservation has been optimized for years, to ensure the best post-thawed semen quality. However, even with the superior procedure and good fresh semen quality, there are differences in sperm freezability between individuals – spermatozoa from some males can easily survive freezing and thawing and from the others will mostly die. Basing on the post-thaw semen quality sires are typically divided into good and bad ‘freezers’. Driven by scientific curiosity and economic reasons (loses generated by ‘bad freezers’), researchers have tried to explore the secrets of cryo-resistance for years. Although the studies has been performed in many different species, the exact causes and underlying mechanisms are still an enigma. There are several hypotheses explaining different cryoresistence of spermatozoa from different individuals, including genetically determined variations in the cell shape, cytoplasmic membrane properties, expression of proteins etc. A lot of effort is put into finding markers in fresh semen, which will allow to predict cryopreservation outcome.

In cats this area has been definitely understudied, and no such markers exist. As there is a growing demand from the cat breeders to cryopreserve semen from their tomcats, markers of freezability would be a valuable tool. Also, domestic cat is a model animal for its wild relatives. As most of species of Felidae family are endangered, semen cryopreservation sometimes is the only solution to preserve genetic material from rare and precious individuals and therefor wild cats could benefit from the development of knowledge in this field.

The aim of this study is to identify factors responsible for different cryosurvival of domestic cat spermatozoa and to search for the potential markers for freezability prediction.

To achieve this goal, a wide range of tools and assessments will be applied for semen evaluation – from computer assisted sperm morphology analysis, through osmotic challenge tests, measurement of expression of different proteins (aquaporins, proAKAP4) to thorough examination of sperm membranes structure and evaluation of its properties by fluorescence spectrophotometery and flow cytometry. The results of these analyses will be compared between cats classified as good or bad ‘freezers’. This comprehensive approach will increase the chance for finding markers for sperm cryoresistence, which may be further applied in clinical practice. Also, the project will deepen our understanding of feline sperm physiology and cryobiology, which can serve as a theoretical basis for further studies aiming the improvement of semen cryopreservation, both in the domestic cat and endangered wild feline species.