Intensification of industrial progress and aggressive human expansion into the natural environment resulted in significant reduction of ecological niches of wild animals and the progressive extinction of individual species. This problem also applies to wild felids. Significant environmental pollution, inbreeding and infectious diseases reduce reproductive capability within wild cats populations, leading to a considerable decline in biodiversity. The low population of Eurasian Lynx in Poland indicates an urgent need for wildlife preservation. Similarly, all species of wild cats are threatened with extinction in some areas of their natural occurrence. In such critical situation, we should realize that the genetic material of each individual is worth its weight in gold. Acquiring material from shot, dead or euthanized animals allows to posthumously preserving of their reproductive and genetic potential. For wild felids preservation, assisted reproduction technology (ART) is applied. One of ART approach is cryopreservation of gonadal tissue and gametes. Procedures for freezing male gametes in cats are well-developed, in contrast to the outcome of cryopreservation of female gametes. The low efficiency of mechanical recovery of isolated oocytes (up to several dozen oocytes from one individual) and poor cryopreservation procedures lead to significant loss of valuable material. A promising solution may be the freezing of native ovarian tissue together with the entire pool of oocytes. Promising results of human ovarian tissue freezing (up to now 130 births) provides the perspective for the development of ovarian tissue preservation and the protection of genetic material in wild felids.

The aim of the project is to reduce the cryo-induced stress of cells undergoing *in vitro* procedures for the preservation of genetic material from rare and endangered species of the Felidae family. The project will examine the impact of the composition of the transport medium, physicochemical parameters of the freezing protocol and the implementation of post-thaw cells restoration step to the biological parameters of ovarian follicles.

Ovaries of domestic cats will be obtained by routine sterilization, properly prepared and submitted to *in vitro* procedures. Initially, the effect of preservation solutions, size of ovarian tissue fragments and supplementation of antioxidants and cell death inhibitors (superoxide dismutase, N-acetyl-L-cysteine, glutathione, calpain protease inhibitor, caspase inhibitor) on survival, structure and apoptosis of ovarian follicles will be evaluated. The developed method of transporting ovarian tissue will be assessed in relation to the effectiveness of freezing. Another task is multifactorial optimization of the parameters of the quick-freezing procedure - vitrification, i.e.: incubation time, cryoprotectants and size of ovarian tissue fragments. Detailed evaluation of oxidative stress and ovarian follicle apoptosis will be performed after the use of *in vitro* tissue culture. Subsequently, we will develop a medium for post-thaw cell restoration for vitrified ovarian tissue. The effect of media supplementation will be assessed as using the methodology described above (histology, immunohistochemistry and florescence microscopy).

Proposed experimental studies of media supplementation for transport and post-thaw cell restoration will reveal the possibility of reducing cellular stress induced by storage of ovarian tissue at low temperatures. Hence, research contributes to the development of cryobiology and biology of reproductive cells in felids. Optimization of the feline ovary tissue vitrification protocol, due to the low requirements and simply procedures, will enable it to be carried out in wildlife centres conditions. It needs to be mentioned, that the equipment facilities and laboratory capabilities of wildlife centres, or even zoological gardens is not as advanced as in scientific ART centres. By developing an easy and fast method of freezing, we will gain a valuable tool to preserve the genetic material of wild felids for many years. Therefore, in addition to the most important aspect of the project - media supplementation for cryopreservation of wild felids ovarian tissue, we aim to shed light on the mechanisms of cell defence against extreme sub-zero temperatures. Moreover, obtained results also will contribute to the creation of a cryo-bank of genetic and reproductive resorces of endangered felid species.