C1. DESCRIPTION FOR GENERAL PUBLIC

Endocrinal disorders, including **equine metabolic syndrome (EMS)**, are a serious problem in veterinary medicine and horse breeding. As it is an international disorder, it also affects population of horses in Poland. More importantly, it was observed that EMS also affects sport horses fed with diet rich in carbohydrates. In consequence, these horses also develop laminitis and **pancreas disorders** resulting in **type II diabetes** and **insulin resistance**.

Moreover, it was discovered that EMS horses accumulate excessive amounts of stress factors at systemic and local level (adipose tissue) which impairs immune responses. This is not without a consequence for **stem cell** population residing in the adipose tissue (Adipose-Derived Mesenchymal Stromal Stem Cells - ASC). In those unfavorable conditions ASC lose their most important features, which include multipotency, immunomodulating activity and pro-regenerative character. As a result, ASC isolated from EMS horses are characterized by **decreased proliferation potential, mitochondrial dysfunction, excessive accumulation of reactive oxygen species (ROS) with simultaneously decreased anti-oxidative capacity and increased DNA methylation status.** All that contribute to their decreased regenerative potential as increased accumulation of oxidative stress negatively affects mitochondrial functions and accelerates the process of cell aging while deteriorating cell functions.

The aim of the project is to deliver a detailed analysis of phenotype and molecular characteristics of ASC_{EMS}. **Bisulfite sequencing** will enable investigation of their methylation status and will be helpful in determining how EMS affects ASC population and their regenerative potential. Certain enzymes, called methyltransferases (DNMT), are responsible for DNA methylation, mainly by nitrogen bases modification, especially at cytosine. One of the DNMT inhibitors is **5-azacitidine** which contributes to DNA demethylation indirectly and directly. Studies conducted by other teams and our own indicate that 5-azacitidine not only **enhance ASC proliferation** and pluripotency-related gene expression, but also **increases the effectiveness of differentiation** into chondrogenic, osteogenic and neural lineages. Additionally, increased activity of superoxide dismutase (SOD) and **decreased ROS** and nitric oxide (NO) amounts were observed. Moreover, cells supplemented with 5-azacitidine were characterized by increased secretion of membrane-derived microvesicles (MVs). Bearing in mind the utility of ASC_{EMS} in cellular therapy, an attempt to reverse the aged phenotype of ASC_{EMS} seems to be reasonable.

Resveratrol, which is an organic polyphenol, belongs to the most powerful antioxidants. It was shown that resveratrol supplementation results in gene expression pattern similar to the one observed on low calorie diet. It reduces signs of cellular senescence including apoptosis and inflammation and lowers blood cholesterol level. It was also demonstrated that resveratrol has beneficial effects on mitochondrial metabolism. It increases mitochondrial biogenesis and SOD expression by the activation of transcription factor SIRT1. More interestingly, the newest research indicates that resveratrol plays a role in increasing insulin sensitivity. Taking the above into account, we plan to investigate the influence of resveratrol and 5-azacitidine on ASC_{EMS}. We assume that, thanks to anti-oxidative and demethylative activities, these substances will abolish cellular senescence and reverse epigenetic alterations in *in vitro* culture of ASC. The supplementation of ASC with resveratrol and 5-azacitidine may be a crucial step in **cellular pharmacology** in the future. We also assume that addition of these agents to ASC culture will inhibit apoptosis, improve mitochondrial metabolism and, in consequence, increase secretory activity of ASC cells. It is expected that combination of anti-oxidative and demethylating agents in proper concentrations will decrease the amount of accumulated in ASC_{EMS} stress factors e.g. ROS, NO and simultaneously increase the expression of anti-oxidative enzymes like SOD, catalase and thioredoxin reductase**. Improved anti-oxidative capacity** of ASC_{EMS} may lead to enhanced viability, proliferation, multipotency and secretion of MVs. Synthesized and secreted by ASC MVs play an important immunomodulating role and may reduce inflammation in the course of EMS, possibly through activation of regulatory T cells (T_{REG}), which may be crucial in the future for EMS treatment with autologous ASC or MVs in equine veterinary medicine. Additionally, we want to investigate the mechanism of internalization and/or influence of MVs on impaired pancreatic β cells isolated from EMS horses. Our research hypothesis assumes that MVs, enriched in anti-inflammatory cytokines and growth factors, will be **cytoprotective** towards **β cells** and will improve their function by ameliorating mitochondrial function.

Pharmacological treatment of impaired stem cells isolated from EMS horses, by their pre-incubation with anti-oxidative and demethylative agents may become the first step to improve their therapeutic value and to discover an **effective treatment strategy of metabolic syndrome in horses**.