The effect of macrophages on the miRNA expression profile in equine endometrial fibroblasts

Endometrosis (endometrial fibrosis) is a chronic endometrial degeneration of equine *endometrium*, which is responsible for infertility in mares. The pathogenesis of endometrosis is still not clear. Principally fibrosis is characterized by excessive extracellular matrix (ECM) component deposition and fibroblast as well as myofibroblast activation. Inflammation is associated with the development of fibrosis in a paracrine way by the secretion of profibrotic cytokines and other factors from injured tissues and inflammatory cells.

Macrophages (Mϕ) are the main immune cells that phagocyte pathogens. Macrophage polarization refers to the process by which Mϕs produce distinct functional phenotypes as a result to specific microenvironmental stimuli and signals. Macrophages are not homogenous, and they are generally categorized into two broad but distinct subsets as either classically activated (Mϕ1) or alternatively activated (Mϕ2). Macrophages Mϕs represent a continuum of highly plastic effector cells, resembling a spectrum of diverse phenotype states. Much research suggests that Mϕ1 and Mϕ2a play a role in fibrosis development, and our data suggest that Mϕs participate in processes associated with the development of endometrosis in mares.

MicroRNAs (miRNAs) are small non-coding RNA (~ 22 nt) that can regulate gene expression at the translational level. miRNAs, by regulating the expression of target genes, regulate physiological but also pathological processes like fibrosis. Recently our group has shown that endometrial miRNA expression profile differs between stages of endometrosis. Those results suggest that miRNA participates in equine endometrosis development. The expression of miRNAs is highly dynamic and responsive to cytokine signals. Research results also show that there is a dual cross-talk between miRNAs and inflammatory mediators secreted by immune cells. However, there is no study about the effect of mediators secreted by Mϕs on miRNA profile in equine endometrial fibroblasts. **This project investigates** the effect of paracrine factors of MΦ1 and MΦ2a on the whole miRNA profile in equine endometrial fibroblasts.

We hypothesize that (1) paracrine factors secreted by activated Mϕ1 and Mϕ2a induce changes in the miRNA profile in equine endometrial fibroblasts (2) and their effect differs between Mϕ1 and Mϕ2a via secreted mediators. This project aims to investigate the effect of different types of Mϕs on miRNA profile in equine endometrial fibroblast in processes associated with the development of endometrosis. In the proposed project, the research material will be equine endometrial fibroblast with mild fibrosis and monocytes isolated from peripheral blood for generating Mϕ conditioned medium**. In our first aim,** we will determine the effect of mediators secreted by Mϕ1 and Mϕ2a on miRNA expression profile in endometrial fibroblasts by using RNA-seq followed by qPCR and miRNA-FISH. In addition, we will perform *in silico* analysis of potential molecular processes in which genes regulated by miRNAs are involved in processes related to the development of equine endometrosis. **In the second aim,** we will determine the paracrine factors of Mϕ1 and Mϕ2a responsible for changes in miRNA expression in equine endometrial fibroblasts. For this purpose, we will choose the most significantly higher levels of cytokines produced by Mϕ1 and Mϕ2a and then we will neutralize selected cytokines. Then the expression of selected miRNA in endometrial fibroblasts will be done using qPCR and miRNA-FISH.

As part of the project, we will expand the basic knowledge on the impact of MΦ1 and MΦ2a via acting miRNA on the gene expression in processes related to the development of fibrosis. This may become a tool for other researchers to study this process in fibrosis in *endometrium* and other organs and other species, which may lead to a better understanding of fibrosis development. Additionally, respecting that planned analyses will provide a wide range of molecular metadata, they may settle a direction for our future studies.