The role of the trophoblast in modulating pro-fibrotic processes in the mare's endometrium: a novel perspective on the underlying causes.

Endometrial fibrosis, defined as the pathological accumulation of excess extracellular matrix (ECM) components, primarily collagens, which results in impaired organ function, is an important cause of infertility in mares. While its clinical impact is well recognized, the molecular mechanisms driving fibrotic changes remain poorly understood. Recent findings highlight molecules such as TGF-β1, IL-33, and luteinizing hormone (LH) as contributors to gene expression differences between fibrotic and healthy endometrium. Additionally, M2a macrophages have been shown to modulate ECM composition, inflammation, and fibrosis-associated micro RNAs (miRNAs).

Strikingly, these same molecules—TGF-β1, IL-33, CD14, and CD68—are enriched at the feto-maternal interface during early pregnancy, alongside activation of the IL-4 signaling pathway, which is implicated in fibrotic processes. Given that mares are typically bred annually from ages 3 to 20, and pregnancy lasts ~11 months, their endometrium is exposed to trophoblast-derived signals for much of their reproductive lifespan. However, the potential link between trophoblast activity and fibrosis remains an unexplored question. Thus, this project will investigate, whether trophoblast signaling contributes to endometrial fibrosis. Specifically, it will:

1. Characterize the equine trophoblast and its secretome at single-cell resolution (addressing the current lack of data).
2. Identify and validate trophoblast–endometrium interactions, focusing on fibrosis-linked miRNAs and signaling pathways.
3. Compare endometrial profiles of multiparous and nulliparous mares to assess whether prior pregnancies predispose to fibrosis.
4. Evaluate how varying cell type compositions (e.g., fibroblasts, macrophages) influence in vitro endometrial responses to trophoblast signals.

This integrative study employs single-cell spatial transcriptomics, bulk RNA-seq, and LC-MS proteomics, bridging *in vivo* and *in vitro* work. It will be the first to explore how trophoblast signals reshape the endometrial miRNA landscape, providing novel insights into post-transcriptional regulation during early pregnancy.

Mares serve as a unique large animal model, as they spontaneously develop endometrial fibrosis and, like women, produce chorionic gonadotropin (CG), which is an advantage to rodent models. While fibrosis may be a natural consequence of repeated pregnancies, understanding its molecular basis is key to developing therapies that prevent pathological changes without disrupting essential reproductive processes.

By uncovering new trophoblast–endometrium interactions, this project will enhance our understanding of fertility loss in horses and inform broader research on uterine health across species. Additionally, combined with data from a previous project, the findings will contribute to building a cellular atlas of the equine feto-maternal interface, supporting future studies beyond fibrosis and advancing strategies to mitigate reproductive decline in mares.