

Endometriosis is a mysterious disease, characterized by the presence and growth of tissue similar to the lining of the uterus outside its usual location. It most commonly occurs in the peritoneal cavity but can also be found in distant parts of the body, such as the brain, lungs, pleura, or even the nose. It affects about 10% of women of reproductive age, which refers over 190 million worldwide. The disease is associated with chronic inflammation, severe lower abdominal pain, and reduced fertility. Its symptoms impact not only women's overall health and well-being but also place a significant financial burden on healthcare systems, comparable to other chronic diseases. Despite its prevalence, the underlying mechanisms of endometriosis are still not well understood, mainly due to the lack of suitable models for research. Modern three-dimensional (3D) *in vitro* disease models are promising tools, as they better reflect tissue architecture and cell responses to treatment than traditional cell cultures.

Researchers increasingly emphasize that one of the main challenges in treating endometriosis is progesterone resistance, which limits the effectiveness of standard hormonal therapies. A new class of drugs is under investigation. This includes selective progesterone receptor modulators (SPRMs). However, these too are not always effective. As a result, natural plant-derived compounds are gaining attention. Phytoprogestins, non-steroidal, plant-based analogs of SPRMs are a promising, yet still understudied, alternative in the treatment of hormone-related conditions such as endometriosis.

This project aims to investigate whether the flavonoids kaempferol, apigenin and naringenin, which belong to the group of phytoprogestins, can support the prevention and treatment of endometriosis using a functional, well-characterized 3D *in vitro* disease model. The research consists of three main stages:

- 1) **Developing a modern 3D *in vitro* model of endometriosis (spheroids)**, which closely mimics the cellular and molecular changes observed in the disease. The model will be analyzed in terms of morphology, metabolism, and gene expression, including key endometriosis-related markers.
- 2) **Evaluating the effect of plant-derived phytoprogestins on progesterone metabolism and hormonal pathways (steroidogenesis)** within the 3D developed model.
- 3) **Investigating how phytoprogestins influence the key cellular processes** involved in disease development, such as excessive cell proliferation, programmed cell death (apoptosis), inflammation and metastasis.

The project will begin with the establishment of a 3D *in vitro* endometriosis model using a microwell-based system that facilitates the formation of spheroids and natural cell-to-cell interactions. This model will then undergo analysis with microscope to assess the morphology, its morphology, metabolism, and gene expression profile characteristic of the disease. The next phase will examine the impact of phytoprogestins on progesterone biosynthesis by analyzing gene and protein expression (qRT-PCR, Western blotting and ELISA), as well as investigating gene expression changes after compound exposure using RNA-seq. In the final stage, the impact of these compounds on critical processes such as proliferation, apoptosis, inflammation and metastasis will be assessed using advanced molecular tools and flow cytometry with imaging.

The implementation of the project will allow to show the discoveries related to the uncharacterized mechanisms of action of phytoprogestins on endometriosis, with the identification of specific genes and proteins and specific cellular, molecular and hormonal events modulated by analyzed compounds. The results may provide new fundamental knowledge for the development of natural, long-term dietary therapeutic strategies in endometriosis. In addition, the developed 3D *in vitro* model can become an effective, modern and ethical research tool to evaluate the actual therapeutic effects of bioactive compounds without the need for animal testing.