Early pregnancy in all placental mammals (including pigs), is a critical period characterized by a high mortality of embryos. Up to 35% of pregnancies do not develop beyond the embryo implantation period. One of the reasons of early pregnancy loss is impaired function of endometrium. Hypoxia, a condition characterized by reduced oxygen levels, is a critical regulator of endometrial physiology, mediating tissue breakdown and repair, angiogenesis, embryo implantation and development, as well as immune modulation. Our preliminary results indicate that in response to pregnancy and to main embryonic signal (estradiol) differentially expressed genes in whole porcine endometrium are significantly linked to hypoxia response. *In vitro* endometrium models are essential tools for studying its complex biology and pathologies. Traditional two-dimensional (2D) cell cultures have been widely used due to their simplicity and cost-effectiveness. However, they fail to replicate the structural and functional complexity of the *in vivo* endometrium. Recently, 3D *in vitro* cell cultures started to fill in the gap between 2D cell cultures and whole-animal systems. In our preliminary study, we have developed *in vitro* model using scaffolds allowing long-term culture and mimicking architecture of endometrial tissue consisting of epithelial and stromal cells. We plan to extend this study and perform improvements and validation of the model.

In the presented project, we plan to determine if porcine trophoblasts and their signal (estradiol), as well as hypoxia conditions, may induce changes in transcriptome in various cells of endometrium and composition of proteins (secretome) secreted by endometrial and trophoblast cells. In addition, we will develop and validate the 3D scaffold based model of the porcine endometrium. Thanks to the use of scaffolds, we will be able to recreate the complex architecture of endometrial tissue consisting of epithelial, stromal and immune cells. This complex approach to unlock secrets of embryo implantation process and discover this blueprint for early beginning of life by 3D *in vitro* models.

Thus, the aim of current project is to characterize and validate the 3D *in vitro* model of pig endometrium using advanced imaging techniques such as confocal microscopy, live-cell imaging and histological analysis to visualize the 3D culture and assess cellular organization. During early pregnancy, the mother's immune response to the developing embryo is also very important. The co-culture of novel 3D endometrial model with immune cells will better mimic the *in vivo* environment and allow for the study of immune-endometrial interactions. Studying the attachment/implantation process *in vivo* is complicated, so creating a 3D implantation model will allow for a better understanding of this process. For this purpose, our 3D endometrium model will be combined with a 3D embryo model (spheroids made from trophoblast cells). The proper development of early pregnancy is controlled by many factors. One important factor involved in this process is hypoxia. To elucidate the role of hypoxia in the mechanisms involved in embryo-endometrial interactions in the novel 3D embryo implantation model we will use single-cell RNA sequencing. This modern technique will allow us to determine changes in the transcriptome (gene expression) of different types of endometrial and trophoblast cells. Moreover, we will also determine the effects of hypoxia on the secretome of trophoblast and endometrial cells in 3D *in vitro* co-culture model using a mass spectrometry. Importantly, the results obtained during the implementation of this project will be compared to the results obtained from whole-animal systems (*in vivo* models), to determine its relevance for studying specific aspects of endometrial biology.

Our research will be performed in the well-equipped and modern laboratories (laboratory of molecular biology and laboratory of cell and tissue analysis and imaging) of the Institute of Animal Reproduction and Food Research of PAS in Olsztyn and in the top international research units collaborating with our team. The planned research aligns with the latest trends in reducing the use of animals in studies, in accordance with current guidelines known as the **3R principle (Replacement, Reduction, Refinement).** By addressing the current limitations of 2D and other 3D *in vitro* endometrial models, this project will provide a more physiologically relevant platform for studies of key processes in early pregnancy. Thanks to this it will be possible to reveal fundamental processes that could significantly improve fertility treatments and reduce pregnancy losses in domestic animals.