Successful pregnancy establishment requires reciprocal interactions between conceptuses and the endometrium. These interactions drive the unique gene expression pattern in the endometrium that define optimal conditions for conceptus development and implantation. It is still not clear however, how these genes are precisely regulated and coordinated to manifest the global reaction of the endometrium. The dynamic of histone acetylation/deacetylation is fundamental for the precise timing and level of gene transcription. However, the enzymes modulating histone acetylation code in endometrium and their effect on conceptus development and implantation are poorly understood in pigs.

Sirtuins, a NAD+-dependent class III histone deacetylases which target both histone and non-histone proteins, have been found in porcine endometrium during early pregnancy; but still relatively little is known about their role in reproduction. Our initial data revealed the dynamic regulation of endometrial sirtuin expression by conceptus secretions, and relatively high levels of these enzymes in conceptus trophoblasts of the pig during implantation and early placentation. Therefore, we hypothesize that sirtuins improve uterine-conceptus interaction via preparation of endometrium for implantation and by protecting conceptuses against oxidative stress in the uterus.

The main goal of the project is to determine the role of sirtuins in the uterine endometrium and conceptus trophoblast of the pig around the time of implantation. To achieve this, we plan to:

1) identify genes which expression is modulated by sirtuins, as a consequence of deacetylation of histone and/or non-histone proteins in porcine endometrium at the time of implantation;
2) examine the role of conceptus secretions on transcription and enzymatic activity of sirtuins;
3) determine the role of sirtuins in endometrial luminal epithelial (LE) cell functions;
4) examine the potential protective role of sirtuins against oxidative stress in trophoblast (TR) cells.

Task 1. Endometrial samples from the peri-implantation period will be used to determine expression and activity of sirtuins (qPCR, Western blot, fluorometric analyses). Moreover, endometrial explants will be incubated with sirtuin activators and subjected to RNA-Seq and ChIP-seq analyses.
Task 2. Endometrial explants and LE cells will be cultured with conceptus products (E2, IFNγ, IL1β, PGE2) to determine expression and deacetylase activity of sirtuins (qPCR, Western blot, fluorometric analyses).
Task 3. LE cells will be pre-treated with control medium or inhibitors or cells will be subjected to siRNA transfection, followed by incubation with activators of respective sirtuin. Cells will be used for: qPCR of genes selected based on integrating data from ChIP-seq and RNA-Seq (from Task 1), proliferation and cell viability/apoptosis assays, ELISA of Caspase-3, and Western blot of BAX, BID.
Moreover, LE cells will be transfected with siRNA or DNA plasmids targeting individual sirtuins, then TR cells will be plated onto a confluent layer of these cells to perform adhesion assay.
Task 4. TR cells will be pretreated with control medium or inhibitors or cells will be subjected to siRNA transfection, followed by treatment with H_20_2 to induce oxidative stress. Then, cells will be exposed to sirtuin activators, and used for: qPCR of genes related to mitochondria function, cellular ROS, cell viability/apoptosis, proliferation, adhesion and cell cycle assays, and quantitation of DNA content by flow cytometry. Incubation media will be used to examine concentrations of proinflammatory factors with EIA/ELISA.

Realization of this project will provide new information about factors, genes, epigenetic modifications, and processes involved in the peri-implantation events in pigs. In particular, understanding the molecular and epigenetic changes induced by sirtuins in endometrium, and their role in trophoblast cell functions will enrich the basic knowledge about regulatory processes and signaling sequence events required for successful implantation in this species. Reduced fertility is a serious problem in animal reproduction, as it significantly decreases the profitability in animal production. In pigs, a high embryonic mortality occurs before day 30 of pregnancy that may result from inappropriate interactions between conceptuses and the maternal endometrium. Therefore, results of the project may be helpful for future attempts to improve conceptus implantation.