Artificial reproductive assisted technologies (ART) are potentially precious tools in cattle breeding. Pregnancy development depends on the developmental competence of the embryo, and obtaining sufficient number of embryos qualified for embryo transfer depends on the developmental competence of the oocyte, defined as the ability to undergo nuclear and cytoplasmic maturation. In proposed project, next-generation genome sequencing (NGS) based multi-omics studies will be carried out to understand and explore the molecular processes and mechanisms of oocyte maturation and early embryonic developmental stages of bovine fetus in Polish HF cattle.

The research hypothesis assumes that:

- there is a close and contrast relation between the developmental competence and gene expression changes in oocyte maturation and early embryonic stages of fetus which can be detected by multi-Omics NGS technologies and bioinformatics methods in Polish HF cattle;
- using sc*RNA-seq* and *methyl-seq* analysis of oocytes and embryos will be helpful in identification of potential biomarkers and candidate genes for bovine reproduction and fertility trait in Polish HF cattle.
- we expect that using advanced NGS tools of sc*RNA-seq* and *methyl-seq* will identify the novel gene expression variation of potential candidate genes, gene networks, co-expression gene networks and metabolic pathways, novel biomarkers for bovine reproduction and fertility trait.

This study will answer the following key questions:

- What is the physiological state of oocytes and early embryonic developmental stages of bovine fetus in context to single cell RNA sequencing (sc*RNA-seq*) and epigenome (*methyl-seq*) profiling in Polish-HF cattle?
- How does physiological state of oocytes and embryos sc*RNA-seq* and *methyl-seq* profiling in Polish-HF cattle will have the impact and effect on bovine fertility and other reproductive traits?
- Does oocytes/embryos transcriptome and epigenome physiological state in Polish-HF cattle significantly affect the prenatal gene expression of crucial candidate genes and proteins which could be used as candidate genes and biomarkers for bovine fertility and reproductive traits?

In the planed studies, the scientific objectives with particular attention to the innovative nature of the research project will be done by achieving three research tasks in the form of work-packages [WP1-WP3].

- In WP1, collection of oocytes and embryos at early developmental stages in Polish-HF cattle will be done to execute the experimental design. Two pilot experiments will be conducted in WP1. In experiment 1, In vitro morphological examinations and assessments of oocytes and embryos will be executed. In experiment 2, In vivo morphological examinations and assessments of embryos will be conducted.
- In WP2, laboratory research tasks on single cell RNA sequencing (sc*RNA-seq*)) and epigenome sequencing (*methyl-seq*) of oocytes and embryos at early developmental stages in Polish-HF cattle will be conducted. After the NGS sequencing, we will do the validation of single cell m*RNA-seq* and mi*RNA-seq* experiments by RT-PCR.
- In WP3, three bioinformatics pipelines based on the m*RNA-seq*, mi*RNA-seq* and *methyl-seq* data will be developed. Our bioinformatics analysis will be based on the (i) embryos comparison between Exp-1 vs Exp-2 in three pipelines (sc-m*RNA-seq* sc-mi*RNA-seq*, and *methyl-seq* data); and (ii) within Exp-1, comparison of oocytes and embryos of in three pipelines *RNA-seq* data. In WP3, we will utilize the following wide range of advanced bioinformatics tools: viz., SAMtools mPileUp package for call SNPs and indels, three Bioconductor R packages: EdgeR, DEseq and DESeq2 to identify differentially expressed genes (DEGs), an R package for weighted correlation network analysis (WGCNA) for co-expression of hub genes, GO and KEGG for gene ontology enrichment and pathway analysis, R package ChAMP integrative pipeline analysis, including QC, desntifyPlot, dendrogram etc. ChAMP.norm to standardize the *methyl-seq* data, ChAMP.DMP and Champ.DMR to filter single CpG site and cluster of CpG sites, Champ.GSEA for function enrichment and pathway analysis, visualization of data by CIRCOS software, Unique DNA methylation patterns and CpG sites correlation will be analyzed using R package “coMET“.

Expected investigated results will allow us: i) to characterize the biological and molecular mechanisms oocytes maturation and early embryonic development in Polish-HF; ii) identification of the candidate genes/biomarkers for bovine fertility and reproduction trait; iii) results will fill the knowledge gap regarding integration of transcriptome and epigenome profiling of oocytes maturation and early embryonic development.