Primordial germ cells - a new approach for epigenetic research in chicken

Epigenetics is the study of changes in gene function that are mitotically and/or meiotically heritable and that do not entail a change in DNA sequence. In vertebrates crucial epigenetic reprogramming events occur during germ cell development and early embryogenesis. The precursors of the gametes are primordial germ cells (PGCs) as the only ones capable to transmit information in genetic material from generation to generation. In proposed project chicken embryo will be used, as a source of PGCs, as it is readily available, possible to culture in the laboratory conditions, and above all allows the isolation of cells at early stages of development. An important argument in favor of using this model is that some PGCs can maintain potential to create germline, even after a long-term *in vitro* cultivation. Some exogenous PGCs, when introduced into the bloodstream of recipient embryo are sometimes capable to differentiate into germ cells in *in situ* conditions, which makes PGCs a new, good model for the study of developmental biology. Mechanisms of these processes, and above all factors that make that only some of PGCs possess the aforementioned properties are not known.

The scientific aim of this project is to understand the molecular changes in PGCs isolated from chicken donor, cultured *in vitro* and injected into recipient embryos and subjected to interaction between donor/recipient PGCs in the environment of recipients. Another aim is to determine whether those changes are epigenetic character or not, and how it affects the properties of germline chimeras' offspring. Four experiments were planned in detail, which aims are to compare the expression of selected genes of donors PGCs isolated at different developmental stages of embryos from both sexes; to evaluate of the effect the length of *in vitro* culture (short or long-term) and the culture conditions (different culture media) and to determine the influence of recipient embryo environment /hetero- and homogeneous cell transfer/ on expression of specific genes in donors' PGCs. Following genes responsible for: oxidation stress (*GPX1*, *GPX4*, *GPX7*); apoptosis (*CASP2*, *CASP3*; *CASP8*; *CASP9*; *XIAP*); related with pluripotency (*cPOUV*, *Nanog*, *Sox2*); related with germline (*Dazl*, *CDH*, *CVH*); and related with migration (*CXCR4*) will be subjected to analysis. Finally, the impact of the environment of recipient embryo (different sex, allogeneic transfer) on the expression of transcriptome using high-through analysis of RNA (whole-genome RNA analysis) will be analyzed. Migration of donor PGCs through the recipient embryo hypothetically could affect the pattern of DNA methylation of chimeras offspring. In order to verify this hypothesis, we selected the panel of ten genes for epigenetic analysis and methylation patterns (method MSRE-qPCR; LightCycler 480 II) between the initial PGCs from donors and PGCs from progeny of germline chimeras. Research carried out on early migrating PGCs will provide an understanding of the epigenetic reprogramming, pluripotency and transgenerational epigenetic inheritance.

From a pragmatic point of view, results of this project could form the basis of a novel strategy to maintain endangered species in birds, by using the available domesticated birds and germline chimeras, producing fecund offspring. In addition, an improved epigenetic strategies will enable effective disease prevention and pave the way for more focused and efficient application of marker-assisted selection (MAS) or genomic selection in animal breeding program in the future.