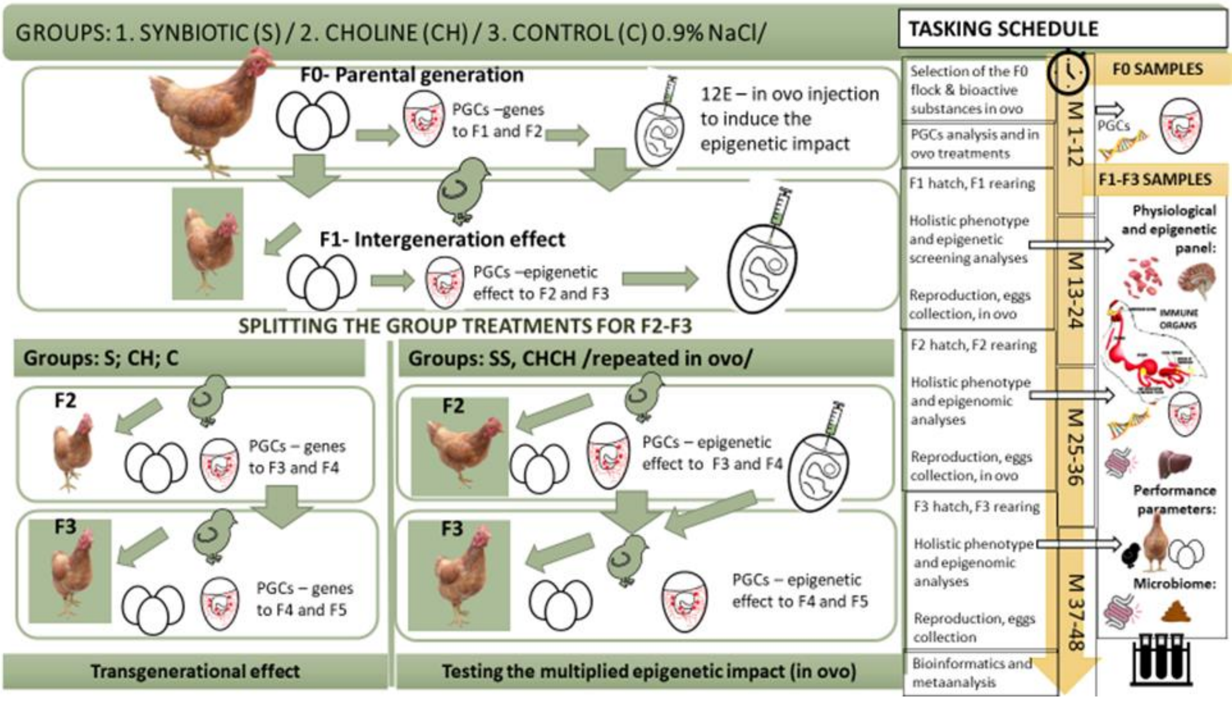
The term "epigenetic modifications" refers to phenotypic modifications in an individual caused by mechanisms that are not related to changes in the DNA sequence. In vertebrates, crucial events of epigenetic reprogramming occur during the stages of early embryogenesis and germ cell development.

We hypothesized that when administered *in ovo* by a single injection, synbiotic and choline, both of which are bioactive substances, can stimulate embryonic development and produce a life-long impact on the phenotype of chicken (such as gut health, immune system, performance, mortality, and physiological traits).
We hypothesized also that epigenetic changes that were acquired in one generation, as a result of the *in ovo* impact on embryonic and long-term postembryonic development, can be permanently inherited and propagated in future generations.

The first aim of this project is to analyze whether epigenetic changes that were acquired in one generation, as a result of the prenatal *in ovo* impact on embryonic and long-term postembryonic development, can be stably inherited and propagated in future generations. **The second aim is** to investigate the physiological relevance of intergenerational and transgenerational inheritance. **The final aim is** to identify the molecular mechanisms behind this type of information transfer by using a high-informative multi-omics approach.
The proposed project will be realized on the chicken model (Gallus domesticus), Green-legged Partridgelike chicken, a dual-purpose Polish breed. Compared to mammals, birds have several advantages, especially for studying transgenerational epigenetic inheritance. The major advantage is that a bird's embryo develops outside of the mother, and the maternal influence is reduced only to the egg composition. Moreover, chicken embryo can be easily manipulated both *in vitro* and *in ovo*.

The general project schedule



In our project, we propose a multi-omics approach based on modern Next-Generation Sequencing assays, including: analysing the genome-wide methylation profiles; total RNA sequencing; and analysing protein interactions with DNA. To better understand the effects of bioactives (symbiotic, choline) administered *in ovo* at the early stages of embryonic development in poultry, it is necessary to analyse the direct effects (phenotypic traits such as performance, physiological functions, immune status, and microbiome), as well as the molecular background, and their intergenerational and/or transgenerational inheritance.
The knowledge of host–microbiome interaction will allow a better comprehension of the epigenetic modifications and will aid in the studies aimed at defining the epigenetic roles in improving animal production and disease resistance. Most importantly, we believe that adopting improved epigenetic strategies for prevention against disease will subsequently pave the way for a more focused and efficient application of marker-assisted selection or genomic selection in animal breeding programs in the near future.
To the best of our knowledge, the transgenerational effects of an in ovo-administered synbiotic on the changes in methylome, transcriptome, and chromatin landscape of progenies have never been investigated for any tissue type in chicken, and at a genome-wide scale in particular.