Intergenerational and transgenerational effects of epigenetic factors applied in early developmental stages – insights from in ovo model

Abstract

Epigenetic modifications, shaped by environmental inputs, regulate gene expression and contribute to phenotypic and clinical variability. Some of these changes are transgenerationally transmitted, though their persistence may differ across tissues. This thesis aimed to investigate how prenatal exposure to potential epigenetic modulators can influence the transcriptome of somatic and germline tissues across successive generations, and to evaluate the stability of primordial germ cells (PGCs) as an experimental model for studying epigenetic transmission by comparing the potential epigenetic effects of different conditions (freezing–thawing and in vitro cultivation) on the expression of germ cell–specific markers. Using an in ovo chicken model, we applied a synbiotic (PoultryStar®) alone or with choline to F1 embryos on day 12 of incubation and tracked effects in subsequent generations (F2–F4 generations). Treatment groups included control, synbiotic alone, or synbiotic plus choline, with lineages receiving either a single F1 exposure (to assess inter- and transgenerational effects) or repeated exposures in every generation (to test cumulative effects of repetitive injections). Cecal tonsils, cecal mucosa, and gonads were sampled from adult males (21 weeks old), while embryonic blood was collected at Hamburger–Hamilton (HH) stages 14–16. RNA sequencing was performed on all tissues, and RRBS was applied to gonads. In ovo exposure induced both intergenerational (F2) and transgenerational (F3 and F4) transcriptomic changes that were tissue-specific. Cecal tonsils exhibited robust and persistent transgenerational responses in F3, cecal mucosa showed transient intergenerational effects in F2, and embryonic blood displayed moderate effects in F3 that declined in F4. Gonads were particularly sensitive to synbiotic plus choline, demonstrating pronounced transcriptomic and epigenetic alterations in F2 and F3 generations. In general, enriched pathways included metabolism, immune signaling, proteostasis, stress responses, cytoskeletal dynamics, and cell growth and development. These findings highlight dynamic transmission patterns, indicating that epigenetic effects are non-linear. Notably, repeated

exposures did not consistently amplify effects across generations. In parallel, we investigated the stability of chicken PGCs under short- and long-term cryopreservation. Cryopreserved PGCs maintained viability, germline competence, and transcriptomic stability, confirming their utility for biobanking and as a model for studying epigenetic transmission. Overall, this thesis demonstrates that prenatal stimulation with bioactive compounds (synbiotic and choline) can program gene expression across generations, while cryopreserved PGCs provide a robust platform for germline preservation and functional studies. Together, these findings highlight the influence of microbial and nutritional factors on long-term metabolic and immune outcomes and reinforce the central role of germline biology in both experimental and applied contexts of transgenerational epigenetic regulation.