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REVIEWER OPINION

Gödöllő, January 10, 2026

Reviewer: Elen Gócza

Academic Title: PhD, DSc, Corresponding Member of the Hungarian Academy of Sciences

Institution: Hungarian University of Agriculture and Life Sciences (MATE), Hungary

Candidate: Mariam Ibrahim

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

Field of Study: Dissertation for the degree of Doctor of Medical Sciences and Health Sciences in the discipline of health sciences

Supervisor: Dr hab. Katarzyna Stadnicka

INTRODUCTION

This doctoral thesis examines the intergenerational and transgenerational effects of early developmental exposure to epigenetic modulators, with particular emphasis on nutriepigenetic factors in an *in ovo* chicken model. The topic is highly relevant and original, situated at the intersection of developmental biology, epigenetics, transcriptomics, and germline research.

ASSESSMENT OF DISSERTATION STRUCTURE AND COMPLIANCE WITH TITLE

The dissertation is prepared as a monothematic cycle of five publications, supplemented by a comprehensive integrative text. The structure is clear, logical, and consistent with the title and research aims. The bibliography is extensive and up-to-date, predominantly consisting of international peer-reviewed journals in epigenetics, developmental biology, nutrigenomics, and animal biotechnology.

COHERENCE

The individual publications are logically connected and address a common overarching research question concerning the heritability of nutriepigenetic effects. The integrative text provides clear objectives, a consistent description, and a detailed discussion of methodology and results across studies.

ASSESSMENT OF METHODOLOGY

The applied experimental and bioinformatic methods, including RNA sequencing, RRBS, and PGC characterisation, are appropriate, modern, and scientifically exact. The statistical analyses are correctly selected and adequately interpreted.

ASSESSMENT OF RESEARCH RESULTS

The most important findings demonstrate tissue-specific inter- and transgenerational transcriptomic and epigenetic changes induced by synbiotic and choline treatment. The stability of primordial germ cells after cryopreservation further supports their suitability as a model for epigenetic inheritance studies.

ASSESSMENT OF DISCUSSION

The discussion is comprehensive, well-referenced, and critically evaluates the results in the context of current international literature. Study limitations and future perspectives are clearly addressed.

REFERRED ARTICLES

1. **Primordial Germ Cells as a Potential Model for Understanding (Nutri)Epigenetic–Metabolic Interactions: A Mini Review**

Mariam Ibrahim, Ewa Grochowska, Katarzyna Stadnicka

Frontiers in Cell and Developmental Biology, 2025, 13, 1576768

https://doi.org/10.3389/fcell.2025.1576768

Impact Factor: 4.6; Ministerial Points: 100

This paper introduces primordial germ cells (PGCs) as a valuable model for studying how early-life dietary influences epigenetic reprogramming and metabolic outcomes. PGCs arise early in embryonic development and are the precursors of gametes (sperm and oocytes). As the only cells that transmit genetic and epigenetic information to the next generation, they represent a critical window through which environmental factors, such as diet, can shape both immediate and inherited traits. Accordingly, this paper establishes the theoretical and mechanistic basis for using PGCs to investigate intergenerational and transgenerational inheritance of behaviours influenced by nutritional and other bioactive epigenetic factors.

2. The Effect of Short- and Long-Term Cryopreservation on Chicken Primordial Germ Cells

Mariam Ibrahim, Ewa Grochowska, Bence Lázár, Eszter Várkonyi, Marek Bednarczyk, Katarzyna Stadnicka

Genes, 2024, 15(5), 624

https://doi.org/10.3390/genes15050624

Impact Factor: 2.8; Ministerial Points: 100

The second study evaluates the technical possibility of maintaining primordial germ cells (PGCs) for long-term experimental use. It investigates the impact of short- and long-term cryopreservation—an interference that may influence epigenetic regulation—on PGC pluripotency and germ cell–specific marker expression. The results show that PGCs retain their characteristic identity after both short- and long-term freezing, with upregulation of several marker genes following long-term storage. Altogether, these data confirm that PGCs constitute a stable and reliable model for studies of germline biology and transgenerational epigenetic inheritance.

3. Inter- and Transgenerational Effects of In Ovo Stimulation with Bioactive Compounds on Cecal Tonsils and Cecal Mucosa Transcriptomes in a Chicken Model

Mariam Ibrahim, Marek Bednarczyk, Katarzyna Stadnicka, Ewa Grochowska

International Journal of Molecular Sciences, 2025, 26(3), 1174

https://doi.org/10.3390/ijms26031174

Impact Factor: 4.9; Ministerial Points: 140

This study presents experimental evidence for both intergenerational and transgenerational effects of *in ovo* exposure to bioactive compounds, applied either as synbiotics alone or in combination with choline, on immune-related tissues. The paper demonstrates that prenatal stimulation induces persistent alterations in gene expression profiles of the cecal tonsils and cecal mucosa across successive generations, identifying the gut–immune axis as a significant target of early nutritional programming and revealing distinct transcriptomic signatures associated with transgenerational inheritance.

4. Transgenerational Effects of In Ovo Stimulation with Synbiotic and Choline on Gonadal Tissue Across Three Generations

Mariam Ibrahim, Ewa Grochowska, Marek Bednarczyk, Katarzyna Stadnicka

Scientific Reports, 2025, 15, 30940

https://doi.org/10.1038/s41598-025-16387-6

Impact Factor: 3.9; Ministerial Points: 140

The fourth study establishes a direct connection between the PGC-based theoretical framework and functional analyses of the germline. Examination of gonadal tissues demonstrates that *in ovo* administration of a synbiotic and choline leads to stable, multigenerational alterations in both transcriptomic and DNA methylation profiles, thereby providing exciting evidence for germline-mediated epigenetic inheritance across three successive generations.

5. Multigenerational Transcriptomic Changes in Embryonic Blood Following In Ovo Stimulation with Nutriepigenetic Factors (*in preparation*)

Mariam Ibrahim, Katarzyna Stadnicka, Marek Bednarczyk, Ewa Grochowska

This study provides a systemic perspective by investigating gene expression profiles in embryonic blood, a tissue that contains circulating primordial germ cells during early development. Analysis of this compartment enables the assessment of both somatic and germline-associated transgenerational responses in F3 and F4 embryos following nutritional stimulation applied in the F1 generation.

Together, these studies represent a coherent, integrated body of work that significantly advances our understanding of how embryonic nutritional interference can induce inter- and transgenerational molecular adaptations. Primordial germ cells serve as a central model for revealing the essential epigenetic mechanisms.

CONCLUSIONS

Based on the results of this doctoral research, I can accept the following conclusions:

1. In ovo exposure to synbiotics, alone or combined with choline, induces both intergenerational (F2) and true transgenerational (F3–F4) effects in somatic (cecal tonsils, cecal mucosa, embryonic blood) and germline (gonadal) tissues.

2. Cecal tonsils show intense and persistent transgenerational responses, whereas cecal mucosa exhibits mainly transient intergenerational changes.

3. Embryonic blood displays moderate transgenerational transcriptomic alterations in F3, which decrease in F4.

4. Gonads are particularly sensitive to combined synbiotic–choline treatment, showing more pronounced and persistent transcriptomic and epigenetic changes than synbiotic alone.

5. Transcriptomic patterns indicate generational skipping, suggesting that epigenetic effects may be latent, re-emerge, or attenuate across generations.

6. RRBS analysis of gonads supports RNA-seq data, indicating that the observed transcriptional changes are at least partly epigenetically regulated.

7. Repeated *in ovo* stimulation does not lead to a consistent cumulative amplification of transcriptomic effects.

8. The results highlight the key role of nutritional and microbial factors in influencing long-term metabolic and immune functions via inter- and transgenerational mechanisms.

9. Chicken primordial germ cells remain stable after short- and long-term cryopreservation, preserving germline identity, pluripotency, and viability, and thus represent a robust model for studies of epigenetic inheritance.

SUMMARY AND RECOMMENDATION

Early-life nutritional and microbial interferences can induce long-lasting molecular and physiological effects across generations. Translation of these findings to human health suggests that prenatal and early postnatal modulation of the microbiome and epigenetic landscape may offer novel strategies to influence metabolic, immune, and developmental programming.

The doctoral dissertation represents an original, high-quality, and methodologically rigorous scientific work that fully meets the formal requirements. I therefore recommend that Ms Mariam Ibrahim be admitted to the subsequent stages of the doctoral proceedings and, upon successful public defence, be awarded the PhD degree. Given the high quality of the thesis, I recommend summa cum laude.

QUESTIONS TO THE CANDIDATE

1. Which of your results provides the most considerable evidence for true transgenerational inheritance, and how can direct exposure effects be excluded based on your experimental design?

2. Your data reveal distinct tissue-specific differences in transcriptomic responses among cecal tonsils, cecal mucosa, gonads, and embryonic blood. What biological mechanisms could underlie this tissue specificity?

3. You propose primordial germ cells as a key model for investigating epigenetic transmission. Based on your cryopreservation experiments and transcriptomic analyses, what evidence supports the stability of PGC epigenetic identity, and how does this help the interpretation of germline-mediated inheritance?

4. One of your initial hypotheses was that repeated in ovo exposure across generations would result in an amplification of transcriptomic effects compared with a single ancestral exposure. As your results do not consistently confirm this, how do you interpret this observation, and what does it suggest about the regulation and determination of epigenetic inheritance mechanisms?

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Elen Gócza, head of department

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