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**Review of the Doctoral Dissertation**

**of Mr Mehmet Onur Aksoy, M.Sc.,**

**submitted in the form of a collection of scientific publications entitled:**

**„Transcriptional and epigenetic regulation of in vitro adipogenic differentiation of porcine Mesenchymal Stem Cells (MSCs)"**

The review was prepared at the request of the Scientific Council of the Discipline of Biological Sciences of the Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, pursuant to the resolution adopted on 7 July 2026.

Mr Mehmet Onur Aksoy, M.Sc., obtained his Master's degree in Biochemistry on 13 February 2020 from Anadolu University, Turkey. According to the submitted documentation, the doctoral candidate has not previously applied for the award of the doctoral degree, and therefore no previous doctoral proceedings have been conducted. His academic career has consisted of doctoral training at the Doctoral School of the Poznań University of Life Sciences. According to the submitted documentation, the doctoral candidate has not been employed at any other institution.

**General assessment of the dissertation**

The doctoral dissertation of Mr Mehmet Onur Aksoy, M.Sc., was prepared in the form of a coherent series of three thematically related scientific publications, with a total score of 380 points according to the Polish Ministry of Science and Higher Education evaluation system and a combined Impact Factor of 10.09. The doctoral candidate's contribution to the research presented in the dissertation should be considered substantial. He is the first author of all three publications included in the dissertation, demonstrating his leading role in the project, from the planning and execution of the experiments to the analysis and interpretation of the results. It should also be pinpointed that the research was carried out as part of the National Science Centre OPUS project (No. 2018/29/B/NZ2/00956), which further confirms the scientific value and relevance of the research topic.

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The dissertation follows the structure typically expected of a doctoral thesis. It includes an Introduction, Research Hypotheses and Objectives, Materials and Methods, Results, Discussion with a section on the limitations of the study and conclusions, and a final Summary. The dissertation has been prepared with great care in terms of both organization and presentation. One of its particular strengths is the graphical presentation of the research programme. The Author has prepared clear and informative diagrams illustrating both the experimental design and the relationships between the successive stages of the study and the results obtained. This considerably enhances the quality of the dissertation, as it makes it easier for the reader not only to follow the experimental workflow but also to understand the overall logic of the research project and to interpret the findings correctly.

Overall, the dissertation fulfils the requirements expected of a doctoral thesis submitted in the form of a series of scientific publications. It is conceptually coherent, well documented, and based on research findings published in peer-reviewed scientific journals.

**Scientific evaluation of the dissertation**

***Originality of the research topic and research objectives***

The dissertation addresses a highly relevant and timely research topic in the fields of molecular biology and cell biology. Although adipogenesis is one of the best-characterized models of cellular differentiation, many of the molecular mechanisms regulating this process remain to be fully understood. This is particularly true for the complex interactions between transcriptional regulators with well-established biological functions and the epigenetic mechanisms that control gene activity during adipocyte differentiation. Research conducted using the pig as an experimental model provides additional scientific value. For many years, the pig has been recognized as an important translational model for studies on metabolism, obesity, and human metabolic disorders, including diabetes and cardiovascular diseases. A better understanding of the molecular mechanisms controlling adipose tissue development may therefore contribute not only to developmental biology but also to animal genetics, the quality of animal-derived products, and biomedical research. In my opinion, the choice of the experimental model is fully justified, and the research topic addressed in the dissertation is both highly relevant and scientifically interesting.

The dissertation consists of three original scientific publications focused on the molecular mechanisms regulating the adipogenic differentiation of porcine mesenchymal stem cells. The first two publications investigate the functional roles of the *SREBF1* and *CEBPB* genes using CRISPR/Cas9 technology, whereas the third examines epigenetic changes occurring during adipogenesis by analysing selected histone modifications involved in the regulation of key adipogenic transcription factors.

The publications form a coherent and logically structured body of work. The Author systematically develops the research from the functional characterization of key regulators of adipogenesis, through the analysis of the effects of their inactivation, to the investigation of epigenetic mechanisms that may explain the observed changes in gene expression. This well-designed research strategy demonstrates careful planning and the ability to conduct a complex, multi-stage research project.

K. Rapacz-Kmita

The Introduction is well prepared and provides an appropriate overview of adipose tissue biology, mesenchymal stem cells, the transcriptional regulation of adipogenesis, and the fundamental principles of epigenetic regulation. The Author demonstrates a good knowledge of the relevant literature and clearly justifies the need for the research presented in the dissertation.

The research objectives are clearly defined, logically derived from the current state of knowledge, and consistent with the scope of the experimental work. The Author correctly identifies the key questions requiring further investigation, particularly the functional roles of *SREBF1* and *CEBPB* during the adipogenic differentiation of porcine mesenchymal stem cells, as well as the contribution of epigenetic mechanisms to the regulation of adipogenesis. Based on these assumptions, one main hypothesis and four specific hypotheses were formulated, each supported by a corresponding research objective and an appropriate experimental approach. The logical consistency between the hypotheses, objectives, and methodology is one of the strengths of the dissertation. The planned experiments, including the functional analysis of *SREBF1* and *CEBPB*, the assessment of selected histone modifications, and the investigation of the relationship between epigenetic changes and transcriptional activity during adipogenesis, are well aligned with the stated research objectives.

The first two hypotheses, concerning the functional roles of *SREBF1c* and *CEBPB*, are clearly formulated and can be directly tested using the CRISPR/Cas9 approach. In contrast, the third and particularly the fourth hypothesis are more general in nature. In my opinion, the fourth hypothesis largely extends the main hypothesis rather than representing an independent research hypothesis. It proposes that chromatin remodelling, together with the activity of transcription factors, regulates the sequential activation of adipogenic genes. This is a broad biological concept, whereas the experimental methods applied in the dissertation allow it to be addressed only partially.

The ChIP-qPCR analyses and gene expression profiling demonstrate an association between changes in chromatin structure and transcriptional activity. However, they do not provide sufficient evidence to establish a causal relationship between these processes. The results indicate that chromatin modifications are linked to changes in gene expression, but they do not clarify whether these modifications drive transcriptional activation or occur as its consequence. The Author also acknowledges that this remains an unresolved question in adipogenesis research. Therefore, I believe that the extent to which this hypothesis can be considered experimentally verified should be interpreted with appropriate caution.

***Evaluation of the experimental model and research methodology***

One of the strongest aspects of the dissertation is its well-designed experimental strategy. The Author employed a complementary set of molecular biology techniques that enabled the investigation of both the functional roles of selected genes and the chromatin changes associated with adipogenic differentiation. A particular strength of the study is its methodological consistency. All three publications are based on the same biological model, (porcine mesenchymal stem cells), use a comparable adipogenic differentiation protocol, and apply a similar set of analytical methods. As a result, the findings form a coherent body of evidence and can be interpreted collectively.

K. Rejko-Holik

The experimental work was carried out using a range of modern molecular biology techniques, including CRISPR/Cas9 genome editing, porcine mesenchymal stem cell culture, induction of adipogenic differentiation, RT-qPCR analysis of gene expression, immunofluorescence, lipid accumulation assays, sequencing of genome editing products, and chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) to analyse histone modifications. The introduced genomic modifications were appropriately validated by PCR and Sanger sequencing. In my opinion, the selection of experimental methods is highly appropriate, reflects current standards in functional genomics research, and provides a reliable basis for assessing the biological roles of the investigated genes.

***Evaluation of the research findings, their interpretation, and the achievement of the research objectives***

The results presented in the dissertation form a coherent and logically organized body of experimental evidence that is fully consistent with the overall research concept and effectively addresses the stated objectives. The findings are presented in a clear and well-structured manner. The figures are carefully prepared, and the numerous diagrams and illustrations facilitate both the interpretation of the results and the understanding of the relationships between the successive stages of the study.

The first publication (Aksoy et al., 2024; International Journal of Molecular Sciences) focuses on the functional analysis of the *SREBF1* gene using CRISPR/Cas9-mediated genome editing. The experimental strategy enabled the selective deletion of a 567-bp fragment within the 5′UTR regulatory region specific to the *SREBF1c* isoform, while preserving the integrity of the *SREBF1a* isoform. This represents a well-designed experimental model for investigating the distinct biological roles of the two transcript variants. The successful validation of the deletion by Sanger sequencing and its precise localization within the first intron of *SREBF1a* confirm both the careful experimental design and the high technical quality of the study.

The results demonstrate that *SREBF1c* expression increases only during the later stages of adipogenesis, whereas its expression was completely abolished in cells carrying the regulatory region deletion. In contrast, *SREBF1a* expression was maintained, and its expression profile suggests a partial compensatory response that was nevertheless insufficient to support normal adipocyte differentiation. These findings provide convincing evidence that *SREBF1c,* rather than *SREBF1a*, acts as the major regulatory isoform controlling adipogenesis in pigs.

I particularly appreciate the results concerning the expression of genes belonging to the core adipogenic regulatory network. In cells lacking *SREBF1c* expression, the Author observed a marked reduction in the expression of *PPARG* and *FABP4*, accompanied by decreased transcript levels of *CEBPA* and *CEBPD*, while *CEBPB* expression was increased. In contrast, *GATA2* expression remained unchanged. This transcriptional profile is consistent with the current understanding of adipogenic regulation and indicates that *SREBF1c* is primarily involved in activating the later stages of adipocyte differentiation. The increased expression of *CEBPB* may reflect compensatory mechanisms triggered in response to impaired differentiation. The Author correctly interprets this finding as evidence of disrupted sequential activation of adipogenic transcription factors rather than the isolated activation of a single gene.

The molecular findings are further supported by the phenotypic observations. BODIPY staining showed that control cells accumulated lipid droplets from day 4 of differentiation,

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whereas cells carrying the *SREBF1c* regulatory region deletion exhibited almost no lipid accumulation and failed to differentiate into mature adipocytes. These results provide strong functional confirmation that the observed changes in gene expression translate into a profound impairment of adipogenesis, thereby strengthening the validity of the conclusions drawn

The second publication (Aksoy et al., 2026; Journal of Applied Genetics) investigates the functional role of the *CEBPB* gene. The Author generated a 575-bp deletion within the promoter region of *CEBPB*, establishing both homozygous and heterozygous cell lines. A particularly important finding was the approximately 11-fold reduction in *CEBPB* expression in heterozygous cells compared with wild-type cells, while expression was almost completely abolished in the homozygous lines. Notably, the homozygous cells exhibited such severe proliferative defects that they could not be used for further adipogenic differentiation experiments, providing strong evidence for the essential role of *CEBPB* in maintaining mesenchymal stem cell function. The expression analysis of key adipogenic regulators clearly demonstrated that reduced *CEBPB* expression led to a marked decrease in *CEBPA, PPARG,* and the mature adipocyte marker *FABP4,* accompanied by a complete absence of lipid droplet accumulation. A particularly valuable aspect of this study is the extension of the analysis to genes involved in cell proliferation, including *CCND1, MCM2,* and *PCNA.* The Author demonstrated a significant reduction in the expression of all three markers in both heterozygous and homozygous cells, indicating that *CEBPB* regulates not only adipogenic differentiation but also the proliferation of mesenchymal stem cells. These findings represent an important extension of the current knowledge regarding the biological functions of this transcription factor.

The third publication (Aksoy et al., 2026; Genes) completes the research cycle by investigating chromatin changes occurring during adipocyte differentiation. The study demonstrates that adipogenesis in porcine mesenchymal stem cells is accompanied by dynamic, stage-dependent, and gene-specific changes in histone modifications (H3K9ac, H4K8ac, and H4K20me3) within the promoter and exon regions of the *PPARG, GATA2, CEBPA*, and *CEBPB* genes. The activating histone acetylation marks either preceded or accompanied changes in gene expression, whereas H4K20me3 showed no clear association with transcriptional activity, highlighting the complex and gene-specific nature of epigenetic regulation during adipogenesis.

One of the major strengths of this study is the attempt to interpret changes in gene expression together with alterations in chromatin modifications. In epigenetic studies, these two aspects are often analysed separately. The Author rightly assumes that integrating both levels of regulation provides a more comprehensive understanding of adipogenesis. At the same time, this part of the dissertation presents the greatest interpretative challenges.

It should be emphasized that the ChIP-qPCR technique allows the assessment of histone modification enrichment only within predefined genomic regions. It does not provide information on the global organization of chromatin or on epigenetic changes occurring outside the analysed loci. Consequently, the presented results document the presence of specific epigenetic marks but do not allow a comprehensive characterization of chromatin remodelling during adipogenesis. Although the Author acknowledges this limitation, some statements in the Discussion may suggest that the observed mechanisms have broader implications than the data support. In my opinion, it should be stated more explicitly that these findings apply only to the

selected regions of the four analysed genes and should not be generalized to the entire epigenetic programme regulating adipogenesis.

In light of the results presented, it can be concluded that the objectives of the dissertation have been fully achieved and that the research hypotheses have been successfully verified through the experimental work. The conclusions are logically formulated, well supported by the findings, and provide a valuable contribution to our understanding of the molecular mechanisms regulating adipogenesis in pigs.

In the Discussion, the Author demonstrates a good knowledge of the relevant literature. The cited references are current, well selected, and include both the classical studies on the roles of C/EBP transcription factors and SREBF1, as well as recent publications addressing the epigenetic regulation of adipocyte differentiation. I also appreciate that the Author does not discuss the individual publications separately but instead integrates their findings into a common model of adipogenic regulation by combining data on gene expression, phenotypic changes, and selected histone modifications. This demonstrates the Author's ability to take a comprehensive view of the research problem and to integrate findings obtained at different levels of biological organization.

At the same time, I believe that the interpretation of the results reveals some limitations of the dissertation. The Author demonstrates a good ability to relate the findings to the existing literature, but less attention is given to considering alternative explanations for the observed results. In many parts of the Discussion, the interpretation primarily supports the proposed research hypotheses, whereas I would have expected a more explicit discussion of the limitations of the experimental model and the interpretative constraints associated with the applied methodology. This is particularly relevant to the epigenetic analyses.

The Author demonstrates a clear association between changes in selected histone modifications and the transcriptional activity of the analysed genes. However, the presented results primarily document the coexistence of these phenomena rather than providing sufficient evidence to establish a causal relationship. In several parts of the dissertation, the observed chromatin changes are discussed as if they directly regulate gene activity, whereas the methodology used does not allow this conclusion to be drawn unequivocally. In my opinion, the Author should distinguish more clearly between conclusions directly supported by the experimental data and the proposed biological model, which, although well justified, remains a hypothesis requiring further experimental validation.

A similar comment applies to the interpretation of the results obtained following the disruption of *SREBF1* and *CEBPB* function. The Author convincingly demonstrates the importance of these genes in adipogenesis but gives relatively little consideration to the possibility of compensatory mechanisms involving other transcriptional regulators. Based on our current understanding of transcriptional regulatory networks, it is likely that at least part of the observed phenotype reflects not only the direct consequences of disrupting the target gene but also secondary changes in the activity of other regulatory pathways. A broader discussion of this possibility would further strengthen the interpretation of the findings.

K. Rejduk-Pol

**Final Conclusions**

The doctoral dissertation of Mr Mehmet Onur Aksoy constitutes an original solution to a scientific problem concerning the molecular mechanisms regulating adipogenesis in pigs. The doctoral candidate has demonstrated the ability to design and conduct experimental research, to apply modern molecular biology methods, and to critically interpret the results obtained. The findings presented in the dissertation extend the current state of knowledge regarding the functional roles of transcriptional regulators and epigenetic mechanisms involved in adipocyte differentiation. The comments raised in this review relate primarily to the scope of data interpretation and the extent to which some of the conclusions are generalised, without calling into question the reliability of the results obtained or their scientific value.

I conclude that the doctoral dissertation of Mr Mehmet Onur Aksoy, entitled "Transcriptional and Epigenetic Regulation of Adipogenic Differentiation In Vitro of Porcine Mesenchymal Stem Cells (MSCs)", fulfils the requirements for doctoral dissertations stipulated in Article 187 of the Act of 20 July 2018 – Law on Higher Education and Science (Journal of Laws of 2020, item 85, as amended). Accordingly, I recommend that Mr Mehmet Onur Aksoy be admitted to the subsequent stages of the proceedings for the award of the doctoral degree in the discipline of Biological Sciences.

At the same time, I recommend to the Scientific Council for the Discipline of Biological Sciences of the Faculty of Veterinary Medicine and Animal Science at Poznań University of Life Sciences that the dissertation be awarded with distinction. This recommendation is justified by the high scientific value and originality of the results, which significantly advance our understanding of the molecular mechanisms regulating adipogenesis in pigs. The doctoral candidate has demonstrated the ability to independently design and conduct experimental research using modern methods, as well as to critically analyse and interpret the results in the context of current knowledge. The high scientific standard, clear presentation, and research maturity demonstrated throughout the dissertation fully justify its distinction.

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