ABSTRACT

Influence of visfatin on the transcriptomic and proteomic profile of the luteal cells in domestic pig (*Sus scrofa domestica* L.) during the implantation period.

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The work was conducted under the supervision of prof. dr hab. Nina Magdalena Smolinska at the Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn.

The doctoral dissertation consists of two papers published in 2024:

1) *Visfatin Affects the Transcriptome of Porcine Luteal Cells during Early Pregnancy*. International Journal of Molecular Science, 25(4): 2339, doi.org/10.3390/ijms25042339 **IF[1]=5.6; IF[2]=6.2; MEiN[3]=140**

1) *Visfatin impact on the proteome of porcine luteal cells during implantation*. Scientific Reports, 14, 14625, doi.org/10.1038/s41598-024-65577-1

   IF[1]=4.6; IF[2]=4.9; MEiN[3]=140

in which transcriptomic and proteomic changes occurring in the luteal cells of domestic pigs under the influence of visfatin during the implantation period have been described.

Besides storing energy reserves, adipose tissue is also an important endocrine organ secreting over 600 biologically active substances called adipokines. Some are involved in appetite control, maintaining energy homeostasis, and regulating reproductive functions in humans and animals. Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT), was first described in 2005. So far, a specific receptor for this hormone has not been identified. However, it is suggested that visfatin may interact with target tissues by activating the insulin receptor (INSR). In addition to adipose tissue, visfatin expression has been demonstrated in various mammalian hypothalamic-pituitary-gonadal axis (HPG) structures, such as the mouse hypothalamus, pituitary glands of both male and female mice, and selected ovarian structures in humans, cows, and mice. This may suggest that visfatin belongs to the

group of adipokines involved in regulating the function of the female reproductive system. Furthermore, it has been noted that the concentration of this adipokine in human adipose tissue depends on hormonal status and changes during pregnancy. This hormone appears to exhibit pleiotropic properties. Like other adipokines, visfatin may act as an indicator of metabolic balance, thus playing an important role in regulating fertility in females. It is suggested that this hormone may play a significant role in the process of implantation and placental formation. Previous studies on the effect of visfatin on ovarian function have been limited to granulosa cells of ovarian follicles. It has been shown that visfatin is involved in regulating the steroidogenesis process in granulosa cells, and this effect is associated with its influence on the expression of steroidogenic enzymes genes and proteins. Similar studies have not yet been conducted in other steroidogenically active types of ovarian cells, such as luteal cells.

The lack of data regarding the role of visfatin in regulating ovarian function during the oestrous cycle and early pregnancy has inspired research on the influence of this hormone on the global gene and protein expression in the luteal cells of pigs.

This study aimed to verify the hypothesis that visfatin, a pleiotropic hormone, participates in the control of ovarian function by regulating gene and protein expression in the luteal cells of domestic pigs during the implantation period.

To verify these assumptions, the following research tasks were planned:

1. determining the effect of visfatin on the transcriptome and spliceosome of luteal cells, including the analysis of genes with altered expression profiles (DEGs), long non-coding RNAs (lncRNAs), and alternative splicing events (ASEs) using Next Generation Sequencing (NGS, RNA-Seq) method;
2. determining the effect of visfatin on the proteomic profile of luteal cells using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS).

In our studies, we have shown that:

1. Under the influence of visfatin, the expression profile of 170 genes changed. These genes were assigned to 45 functional annotations. Additionally, 40 lncRNAs with altered expression under the influence of this adipokine were demonstrated. We also

identified 168 transcripts exhibiting alternative splicing variants under the influence of visfatin.

2. Under the influence of visfatin, 511 proteins showed a diverse expression profile. These proteins were associated with 162 functional annotations.

We have demonstrated that visfatin plays an important role in regulating the function of luteal cells by regulating the expression of genes involved in steroidogenesis, proliferation, angiogenesis, inflammatory processes, cycle regulation, cell migration, and adhesion. Analysis of the proteomic profile of luteal cells exposed to visfatin showed that by regulating the expression of specific proteins, this hormone may be involved in steroidogenesis, lipogenesis, cholesterogenesis, and regulation of the insulin pathway. Additionally, visfatin regulated the expression of proteins associated with proliferation, apoptosis, angiogenesis, cell differentiation and tissue remodelling.

The obtained results contributed to deepening the knowledge about the influence of visfatin on the global gene and protein expression in the luteal cells of pigs. Based on the obtained results, we have shown that visfatin may be an important factor regulating reproductive processes and the proper functioning of the ovaries during the implantation period, and thus the course of pregnancy.

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[1]IF – Impact Factor of the journal in the year of publication according to Web of Science

[2]IF – five-year Impact Factor of journals from the year of publication of the work according to Web of Science

[3]Number of points for publication according to the Announcement of the Minister of Education and Science on the list of scientific journals and reviewed materials from international conferences of January 5, 2024