SUMMARY

THE EFFECT OF VISFATIN ON THE FUNCTION OF PORCINE PITUITARY GLAND (*SUS SCROFA DOMESTICA* L.)

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Doctoral thesis was carried out in the Department of Animal Anatomy and Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn under the guidance of prof. dr hab. Tadeusz Kaminski and dr Marta Kiezun.

The doctoral thesis comprises two papers published in 2023 (1 – Scientific Reports, DOI: 10.1038/s41598-023-45255-4; 2 – Cells, DOI: 10.3390/cells12242835), focused on the expression of visfatin in the pituitary gland of domestic pigs (*Sus scrofa domestica* L.) during the oestrous cycle and early pregnancy, the influence of selected hormones on the concentration and secretion of visfatin. Moreover, the effect of visfatin on gonadotropin secretion, apoptosis, proliferation and phosphorylation of INSR, AKT/PI3K, MAPK/ERK1/2 and AMPK in this gland is discussed.

The pituitary gland plays an important role in controlling basic biological processes. The activity of this gland is regulated by numerous hormones and other biologically active factors, including adipokines. This broad group of compounds also includes visfatin, which exists in two forms: extracellular and intracellular. The visfatin receptor has not been identified, however, it has been suggested that visfatin may bind and activate the insulin receptor (INSR), chemokine receptor CCR5 and toll-like receptor 4. Visfatin is expressed in all structures of the hypothalamus-pituitary-gonadal axis (HPG): in the pig and mouse hypothalamus, in the mouse pituitary and the follicular cells of women and pig ovaries. Visfatin is involved in the regulation of inflammation, cell differentiation and control of energy homeostasis as well as appears to be involved in the regulation of female fertility. The effect of visfatin on the secretory functions of the pituitary gland has not yet been investigated.

The aim of this study was to verify the research hypothesis assuming that the gene expression and concentration of visfatin protein in pig pituitary depend on the hormonal status of the animals related to the phase of the estrous cycle and the period of early pregnancy. Moreover, a hypothesis was put forward that visfatin participates in the control of proliferation and apoptosis of pituitary cells, and by activating/inhibiting the INSR, AKT/PI3K, MAPK/ERK1/2 and AMPK signaling pathways, it influences the regulation of the secretory functions of pig pituitary cells. The hypotheses were verified by:

1) assessment of the level of gene expression and concentration of visfatin protein in the anterior (AP) and posterior (NP) pituitary of domestic pigs in four phases of the oestrous cycle (days 2-3, 10-12, 14-16, 17-19 of the cycle) and four periods of early pregnancy (days 10-11, 12-13, 15-16, 27-28 of pregnancy) using real-time PCR and Western Blot methods;
2) determination of visfatin colocalization with LH, FSH, ACTH, TSH, PRL and GH on days 10-12 of the oestrous cycle by fluorescence immunohistochemistry;
3) assessment of the influence of GnRH, LH, FSH and INS on the concentration of visfatin protein by Western Blot and on the secretion of the adipokine by ELISA;
4) assessment of the visfatin effect in various concentrations and/or the visfatin blocker FK866 on basal and GnRH and/or INS-induced LH and FSH secretion by AP cells during the oestrous cycle using ELISA method;
5) determination of the effect of visfatin and its blocker FK866 on the proliferation and apoptosis of AP cells during the oestrous cycle;
6) assessment of the visfatin effect on the activation/inhibition of INSR, AKT/PI3K, MAPK/ERK1/2 and AMPK signaling pathways in AP cells on days 10-12 of the oestrous cycle by Western Blot;
7) determination of the visfatin effect, an INSR antagonist and inhibitors of the INSR, AKT/PI3K, MAPK/ERK1/2 and AMPK signaling pathways on the secretion of LH and FSH by AP cells by ELISA.

As a result of the research conducted:

- it was shown that the expression of the visfatin gene and the concentration of visfatin protein in AP and NP depend on the phase of the oestrous cycle and the period of early pregnancy, which suggests a relationship with the hormonal status of female pigs;
- visfatin was found to colocalize with LH, FSH, ACTH, TSH, PRL and GH in AP;
- the influence of GnRH, FSH, LH and INS on the concentration and secretion of visfatin protein was confirmed;
- the effect of visfatin on the basal and GnRH and/or INS-induced secretion of gonadotrophins by AP cells was found;
- visfatin has been shown to have a stimulating effect on the proliferation of AP cells;
- the effect of visfatin on the activity of INSR, AKT/PI3K, MAPK/ERK1/2 and AMPK signaling pathways in the porcine pituitary was found.

The obtained research results indicate that visfatin, by influencing the secretion of gonadotrophins, is involved in the regulation of reproductive processes in gilts.

keywords: visfatin, oestrous cycle, proliferation, apoptosis, pig