

## **Abstract**

To our knowledge, peptides such as the leptin or ghrelin associated with the regulation of food intake and synthesized in peripheral tissues, , are also involved in the modulation of growth and reproduction processes. Obestatin, the another peripheral peptide exhibiting activity at the peripheral and central nervous system (CNS), together with ghrelin, leptin and neuropeptide Y (NPY), could create a neuroendocrine network modulating the growth and reproductive hormones activity. So far, the mechanism of obestatin activity in the hypothalamo-pituitary axis has been studied mainly in rodents, and the results are ambiguous. This issue, particularly in farm animals, needs further intensive research.

The aim of this study was to investigate the mechanism of obestatin activity in the regulation of somatotrophic and gonadotrophic hormones secretory activity in peripubertal sheeps. The research hypothesis assumes that obestatin can modulate gene expression, storage and release of somatotrophic and gonadotrophic hormones at the hypothalamus and pituitary levels.

In order to verify the assumptions, the experiment on 32-week old Polish Merino sheeps (n=28) before obtaining sexual maturity (September-October) was conducted. The animals were randomly divided into two experimental groups: control group (n=14) receiving infusion of Ringer-Locke solution, and obestatin group (n=14) receiving infusion of obestatin diluted in Ringer-Locke solution. For three consecutive days, the Ringer-Locke solution or obestatin infusions were made to the III ventricle of sheep brain. On the day before the infusion (day 0) and on the third day of infusion (day 3) the blood samples were collected to determine the concentration of growth hormone (GH), luteinizing hormone (LH) and follicle stimulating hormone (FSH). Immediately after the last infusion, the sheeps were euthanized in order to obtain the selected hypothalamic structures and anterior part of pituitary gland. Hypothalamus and pituitary were collected and secured in accordance with Real Time RT qPCR protocol and immunohistochemical assays.

The obtained results showed that obestatin increases the NPY gene expression in the medio-basal hypothalamus (MBH) but at the same time decreases the neurosecretory activity of NPY neurons in periventricular nucleus and ARC. Moreover, it was found that

exogenous obestatin can change somatotrophic axis activity at all organization levels. Obestatin changed the activity of growth hormone releasing hormone (GHRH) and somatostatin (SOM) neurons. These changes led to increase in the GH gene expression and in the total amount of immunoreactive GH material stored in the somatotrophic pituitary cells, and in consequence to increase of its concentration in the peripheral blood, due to an increase the amount of GH pulses. It was also shown that exogenous obestatin changes the selected gene expression of gonadoliberine (GnRH) pulse generator, decreases the secretory activity of GnRH neurons, resulting from the inhibition of GnRH release from median eminence terminal nerves, and also decreases the GnRH receptor gene expression in pituitary. Obestatin inhibits the LH $\beta$  gene expression and affects the accumulation of LH in gonadotrophic pituitary cells. In consequence, obestatin reduces the LH concentration in the peripheral blood and affects its releasing profile. Moreover, performed studies have shown that obestatin stimulates both FSH $\beta$  gene expression and FSH protein levels in gonadotrophic pituitary cells, but has no effect on its release into the peripheral blood.

On the basis of the obtained results it can be concluded that obestatin may be involved in the modulation of growth and reproduction processes in animals at the level of the central nervous system. However, the mechanism of its action requires further research, especially identifying the obestatin receptor itself.