Abstract

Endometritis, of both infectious and non-infectious origin, is one of the most common pathological conditions in the reproductive system of females, especially farm animals. It generates significant economic losses and leads to a reduction in reproductive indicators and significantly increases the cost of treatment. In case of women, it can lead to irreversible impairment of fertility. Additionally, the inflammation of the uterus is accompanied by mastitis combined with agalactia, which in the case of pigs translates into problems with feeding piglets.

The pathogenesis of this disease is complex and associated with an increase in the production of numerous pro-inflammatory substances by the inflamed uterus. These include the interleukins IL-1β, IL-6, IL-8, prostaglandins - PGF2α, PGE2, as well as leukotrienes - LTB4 and LTC4. The long-lasting inflammatory process leads to changes in the neural structures, both intrauterine as well as extrinsic ganglia innervating the uterus. One of the main sources of exogenous uterine innervation is the paracervical ganglion (PCG). The main characteristic of this ganglion is that it contains both sympathetic and parasympathetic neurons. In this study, the influence of uterine inflammation caused by intrauterine inoculation of a suspension of *Escherichia Coli* bacteria on the phenotype of paracervical ganglion neurons was investigated. The research was carried out in sexually mature sows. In order to accurately label the population of neurons present in the PCG innervating the uterus, the technique of retrograde neuronal tracing using the Fast Blue neuronal marker (FB) was used.

The tests were performed on 3 groups of animals. Control group (n = 4) and two experimental groups. The first (n = 4) of them received an intrauterine injection of a suspension of *E. Coli* bacteria, whereas the second (n = 3) was treated with the intrauterine injection of a physiological solution (0.9% NaCl). The FB neuronal tracer was administered to the animals of all groups into the right and left uterine horns. After a period of 28 days, the control animals underwent another laparotomy. At the same time, the animals from the *E. Coli* group received the aforementioned intrauterine inoculation of the bacterial suspension, while the animals from the second experimental group received an intrauterine injection of physiological fluid. Eight days after surgery (experiment day 36), all animals were euthanized. The cervix together with the broad ligament of the uterus were collected for further histopathological and immunofluorescence examinations.

In this study, the method of double immunofluorescence staining with the use of primary antibodies against selected biologically active substances and the histochemical Karnovsky-Roots method to demonstrate the activity of acetylcholinesterase were used. Additionally, a histopathological examination of the uterus was performed, which confirmed uterine inflammation. In the group of animals injected with *E. coli*, there was a reduction in the total amount of FB-positive neurons relative to the control group and the group with physiological fluid injection. In order to determine the phenotypic variability of sympathetic neurons, in this study, dopamine β-hydroxylase (DβH) was used as a marker, additionally determining the collocation of DβH-positive neurons with vasoactive intestinal peptide (VIP), NPY neuropeptide (NPY), somatostatin (SOM) and neuronal isoform of nitric oxide synthase (nNOS). Analysis of the sections under fluorescence microscope showed an increase in the population of neurons with the following phenotype: DβH+/VIP+, DβH+/NPY+, DβH+/ SOM+ and DβH+/nNOS+. In addition, a decrease in the number of VIP, SOM and nNOS-positive neurons was observed in the population of the non-adrenergic neurons, as well as a decrease in neurons expressing only DβH. Antibodies against the vesicular acetylcholine transporter (VAChT) were used to label the population of parasympathetic neurons. Somatostatin (SOM), vasoactive intestinal peptide (VIP), neuronal nitric oxide synthase isoform (nNOS) and galanin (GAL) were used as co-transmitters of parasympathetic neurons in this study. The experimental inflammation of the uterus resulted in an increase in the population of uterine paracervical ganglion neurons with the phenotype VAChT+/VIP+, VAChT+/VIP-, VAChT+/SOM+, VAChT+/SOM- VAChT+/GAL- and VAChT+/nNOS-. In addition, a *de novo* appearance of both cholinergic and non-cholinergic GAL synthesizing neuronal population, as well as an increase in the population of non-cholinergic VIP-, SOM- and nNOS-positive neurons was noted in the *E. coli* group. In contrast, the cholinergic neurons immunoreactive against nNOS decreased as a result of uterine inflammation. Additionally, as a result of inflammation of the uterus, a decrease in the number of neurons containing acetylcholinesterase was observed.

The obtained results provide, for the first time, accurate data on the variability of the phenotype of the sympathetic and parasympathetic neurons of the paracervical ganglion innervating the uterus of a sexually mature sow under the influence of intrauterine inoculation of a suspension of *E. coli* bacteria. These results confirm that the nervous system, as a result of inflammatory changes, undergoes functional adaptation to the unfavorable factors.