Effect of selected dopamine antagonists on reproduction and immunity of crucian carp females (*Carassius carassius* L.)

Increasing the supply of aquaculture products is largely determined by the development of controlled fish reproduction techniques. Controlled reproduction is usually done with the use of pharmacological preparations, which enable synchronization of spawning, improve the quality of gametes and minimize fish dysfunction resulting from stress. In practice, various preparations, including gonadoliberin (GnRH), are used to induce gamete maturation, ovulation and spermiation. However, in many species of fish, especially cyprinids (Cyprinidae), administration of exogenous gonadoliberin is followed by increased dopamine secretion. Dopamine reduces the release of GnRH and gonadotropins (GtH). This unfavorable effect can be counteracted by administering dopamine receptor blockers. Such properties are demonstrated by dopamine receptor antagonists (DA). This explains the increased interest in DA preparations in terms of the possibility of using them, alone or with GnRH, to induce reproduction processes in fish. However, it should be emphasized that research in this area is scarce and the results are incomplete and inconclusive. The need to deeper the knowledge about DA action on fish, and the potential prospects for improving the welfare of spawners (DA's exhibit calming properties), and the increase of the efficiency of the production of stocking material became the inspiration to undertake the research. The research was conducted on a crucian carp (*Carassius carassius* L.), so far, the only species in which ovulation was recorded and high-quality gametes were obtained after the use of metoclopramide.

The aim of this study was to investigate and compare the effects of chosen commercial preparations containing both GnRH analogues and dopamine receptor antagonists (Ovopel: mGnRHa + metoclopramide and Ovaprim: sGnRH + domperidone), and DA's acting alone (Metoclopramide and Domperidone) on the hypothalamic-pituitary-gonads axis (HPG). Additionally, the research covered evaluation of oocyte maturation stages; cortisol level in blood plasma; selected biochemical and hematological indicators and the oxygen burst level and phagocytic activity of leukocytes as markers of stress and immunity level in female's crucian carp.

Mature females of crucian carp (n=60) were acquired in the pre-spawning season, from the Fish Breading Center "Żurawia" (Biała Rawska, Poland). The HPG axis was studied by determining the level of *cgnrh-II* gene expression in the brain and *lhβ* and *fshβ* genes expression in the pituitary gland, and levels of hormones in blood plasma: LH, FSH, 17β-estradiol (E) and

17α20β-dihydroxyprogesterone (17α20β-DPH). The impact of the tested preparations on the oocyte maturation was assessed under a microscope, using the *in vivo* method to determine the degree of maturity of female cyprinids.

In vivo and *in vitro* methods were used to evaluate the effects of Ovopel, Metoclopramide, Ovaprim and Domperidone on the stress level and immunity of crucian carp females. *In vivo* experiment included determination of cortisol level in the blood plasma and the values of blood biochemical parameters: glucose (GLU), total protein (TP), albumin (ALB), globulin (GLOB), bilirubin (BIL), creatinine (CREA), ammonia (NH_3). Selected enzymes and ions and basic hematological indices were also determined. Leukogram, phagocytic activity and the parameters of an oxygen burst were examined in an *in vitro* experiment.

Results of this study showed that the tested pharmacological preparations: Ovopel, Ovaprim, Metoclopramide and Domperidon, had a limited impact on the functioning of the HPG axis. Their impact on the gene expression: gonadoliberin (*cgnrh-II* gene) and gonadotropin (*lhβ* and *fshβ* genes), and FSH and LH plasma concentrations was not found ($p > 0,05$). There were significant differences between the Ovopel (OM group) and Ovaprim (OD group) groups in the gene expression level of the *cgnrh-II* and *fshβ*, and between the OM group and the group of females administered Domperidone (group D) for the *fshβ* gene expression. The applied preparations changed the level of estradiol and 17α20β-dihydroxyprogesterone in blood plasma. Compared to the control group (group K), significantly higher concentrations of E were found in the OM group, while an increase in 17α20β-DHP was noted in the OD group. The results of oocyte maturation analysis were similar to the level of hormones (E and 17α20β-DHP). In relation to the control group, a significantly higher degree of oocyte maturity was observed after injection with preparations containing GnRHa and DA.

The concentration of cortisol in blood plasma was elevated in all groups. There was no difference between the type of pharmacological preparation used and the concentration of cortisol in blood plasma. Few biochemical parameters showed changes after administration of tested preparations. Differences were found in the concentration of ammonia (group K *vs* groups M and D), creatinine (group K *vs* groups OM), potassium ions (group K *vs* groups OD and D) and the ratio of Na^+/K^+ (group K *vs* group D). From the analyzed hematological indicators, only Metoclopramide caused changes in blood counts, namely an increase in RBC values. The effect of the tested preparations on the level of oxygen burst and phagocytic activity was much clearly seen. The oxygen burst grew most rapidly in the group exposed to Metoclopramide, reaching the highest value among all tested variants ($p \leq 0.05$). Moreover,

Metoclopramide caused a significant increase in the phagocytic activity of leukocytes (IF), relative proportion of phagocytic cells (PhC) and the mean cidal activity (MCA). Contrary, the maximum value of oxygen burst (PIK) as well as IF and PhC values were significantly lower compared to the control group following Ovopel exposure. Exposure to Ovaprim or Domperidone resulted in increases in IF and MCA levels.

Concluding, dopamine receptor antagonists administered alone to female crucian carp did not stimulate the HPG axis and did not affect oocyte maturation as effectively as Ovopel or Ovaprim. The cortisol level analyses as well as hematological and biochemical indices showed that the tested preparations had no effect on the stress level in female crucian carp. In contrast, the results of *in vitro* studies clearly shows that Metoclopramide itself stimulates the immune system of female crucian carp, while Ovopel has a suppressive effect.

Key words: dopamine receptor antagonist, oocyte maturation, crucian carp, immunity, hypothalamic-pituitary-gonads axis, stress