

Effects of 17 α -ethinylestradiol in the immune system of the gilthead seabream (*Sparus aurata* L.) through mast cells

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17 α -ethinylestradiol (EE₂) is a synthetic estrogen used in most oral contraceptives pills and hormone replacement therapy that has been found widespread in the aquatic environment. Histamine is a well-known biomolecule released by mast cells and involved in the process of inflammation in vertebrates. The gilthead seabream, *Sparus aurata* L., is a marine hermaphrodite teleost of great commercial value which presents a high recruitment of mast cells located in the peritoneal exudate (PE). In previous studies, we demonstrated that the head-kidney (HK) immune cells of adult gilthead seabream express estrogens receptors (ERs) and the dietary intake of EE₂ alters the capacity HK immune cells to appropriately respond to infection. Besides, gilthead seabream was the first fish species shown to possess histamine-containing mast cells at mucosal tissues.

One the essential objectives of this thesis is the development of techniques that improve the study of functional mechanisms in gilthead seabream mast cells. Regarding this matter, we have developed a method to isolate mast cells and we have produced a monoclonal antibody specific to mast cells of gilthead seabream. Moreover, the histamine release by gilthead seabream peritoneal mast cells *in vivo* and *in vitro* assays is being analysed by ELISAs, capillary electrophoresis, HPLC-Tandem Mass Spectrometry, flow cytometry and microscopy after different treatments with histamine, compound 48/80 (C48/80, which promoted mast cell activation and histamine release) and different antigens and pathogens associated to molecular patterns. Also, the gilthead seabream histamine receptors (HRs) have been identified and we can confirm their presence in total leukocytes population of HK and PE. The presence and abundance of HRs and ERs are being characterized in isolated populations of the main leukocytes populations found in HK and PE.

The other main goal of this project is the analysis through functional studies of the effect of EE₂ in the immune system of gilthead seabream focusing on mast cells. On the one hand, the intraperitoneally injection of histamine and C48/80 immunized or not with *Vibrio anguillarum* showed that histamine alters the percentage of the HK and PE immune cell types. Moreover, both histamine and C48/80 increase the expression of the gene encoding *il1b* in response to *V. anguillarum* in both tissues. On the other hand, the dietary intake of EE₂ immunized or not with hemocyanin+ inject alum adjuvant let us to conclude that: i) EE₂ alters the vitellogenin expression levels; ii) EE₂ modifies the abundance of the leukocyte populations in HK and PE; iii) EE₂ changes the recruitment of leukocytes in PE; and iv) EE₂ increases the ROS peritoneal production.