Summary

Matrix metalloproteinases (MMPs) are a large groupof zinc- and calcium-depended proteolytic enzymes involved in degradation of extracellular matrix components as well as in activation and releasing of signalling proteins. Under physiological conditions, the activity of MMPs is mostly controlled by specific tissue inhibitors of MMPs (TIMPs). A growing body of evidence indicates the roles of MMPs in remodelling and functioning of mammalian ovary; however, the function and regulation of MMPs in the chicken ovary is largely unknown. The present studies were attempted to answer the question whether selected elements of the MMP system are involved in the regression of the avian ovary during a pause in reproductive activity and whether estrogen and gonadotropins regulate the expression and/or activity of MMPs. In order to answer these questions the following items were determined: (1) the expression, localization and activity of selected MMPs (MMP-2, MMP-9, MMP-10, MMP-13) and their tissue inhibitors (TIMP-2, TIMP-3) in hen ovarian follicles (*Gallus gallus domesticus*) during a pause in egg laying induced by feed deprivation, 2) the participation of estrogen in the regulation of expression and activity of chosen MMPs in chicken ovarian tissues, 3) the involvement of gonadotropins in regulation of expression and activity of selected members of MMP system (MMP-2, MMP-7, MMP-9, MMP-10, MMP-13) in the avian ovary.

The mRNA expression of selected MMPs and TIMPs as well as MMP-2 and MMP-9 proteins and activities were found in all ovarian tissues, i.e. in the white (WF), yellowish (YF) and small yellow (SYF) follicles, as well as in the granulosa and theca layers of the largest yellow preovulatory follicles (F3-F1) in hens of control groups, subjected to pause in laying, treated with tamoxifen (TMX; estrogen receptor blocker) or treated with equine chorionic gonadotropin (eCG).In general, in feed-deprived hens, atresia of preovulatory follicles was accompanied by a marked decrease in mRNA expression of the examined MMPs and TIMP-3 as well as in protein abundances and activities of MMP-2 and MMP-9 compared to healthy follicles. Simultaneously, in these follicles an increase in TIMP-2 transcript levels was observed. There was also a decrease in MMP-2 and MMP-9 protein abundances in the WF, and an increase in total activity of MMP-2 and MMP-9 in the YF. Specific immunoreactivity for MMP-2 and MMP-9 was demonstrated in the wall of yellow preovulatory healthy and atretic follicles with distinct cell- and tissue-specific localization patterns.TMX treatment caused: (1) an in crease in mRNA expression of MMP-2 and MMP-10 in the theca layer of F1 follicle, MMP-9, MMP-10 and TIMP-3 in the YF, MMP-13 in F3-F2follicles and the theca

layer of F1 follicle and TIMP-3 in the F3follicle; (2) a decrease in MMP-9 andMMP-10 transcript abundances in most tissues and TIMP-2 in the theca and granulosa layers of F3 follicle and granulosa layer of F2-F1 follicles; (3) a decrease in the relative abundance of MMP-9 protein in most examined ovarian tissues; (4) a decrease in the total MMP-2 activity in the WF and SYF and MMP-9 in the theca layer of F3-F1 follicles; (5) an increase in total MMP-2 activity in the theca layer of F3 and granulosa layer of F2 follicle and MMP-9 in the granulosa layer of F3 follicle. In addition, after eCG injections mRNA and/or protein expression depended on the stage of follicle development, tissue and type of MMPs. eCG treatment caused an increase in mRNA expression of MMP-2 in the YF, MMP-7 in the SYF and theca layer of F1 follicle, MMP-10 in the WF and YF,MMP-13 in the YF and granulosa layer of F2-F1 follicles, TIMP-2 in the granulosa layer of F1 follicle and TIMP-3 in most of the examined tissues. At the same time, after eCG injections, a decrease in the expression of MMP-2 in the theca layer of F3-F1 and granulosa layer of F3 and F1 follicles, MMP-9 in most of the ovarian tissues, MMP-10 in the theca layer of F3 and F2 follicles and TIMP-2 inthe WF, SYF and theca layer of F3 follicle were observed. eCG increased abundances of MMP-2 protein in the granulosa layer of F1 follicle and MMP-9 in the theca layer of F2 follicle, and decreased MMP-9 protein expression in the YF. Total MMP-2 activity measured after eCG injections was increased in the granulosa layer of F1 follicle and decreased in the YF, theca layer of F2 and F1 and granulosa layer of F2 follicle. In the case of MMP-9, lower activity in the YF and theca layer of F1 follicle was noted.

The results demonstrating expression and activity of selected components of the MMP system in all ovarian tissues indicate that the avian ovary is a site of synthesis and action of selected MMPs and TIMPs. Observed decrease in expression and activity of chosen MMPs in ovarian compartments during its regression, suggest that these enzymes may not be involved in the regulation of advanced stage of atresia of the largest yellow preovulatory follicles in the chicken ovary. Tissue- and stage-dependent changes in the mRNA expression of MMP-2, MMP-9, MMP-10, MMP-13, TIMP-2 and TIMP-3 as well as in the activity of MMP-2 and MMP-9 after injections of an estrogen receptor blocker indicate that estrogen is involved in the regulation of transcription, translation and/or activity of these components of the MMP system.Alterations in the expression of selected elements of the MMP system as well as in the activity of gelatinases (MMP-2 and MMP-9) in hen ovarian follicles after eCG injections suggest that gonadotropins, mainly FSH, regulate the expression and/or activity of these enzymes.