GHRELIN AND KISSPEPTIN: TWO NOVEL ENDOCRINE MODULATORS OF OOCYTE MATURATION IN FISH

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Introduction:
Ghrelin is an orexigenic hormone that has been proposed to link the hypothalamo-pituitary-gonadal axis, thus integrating energy balance and reproduction in fish. Ghrelin stimulates luteinizing hormone secretion in fish. However, the role of ghrelin on gonadal physiology remains poorly understood. Similarly, kisspeptin is another recently discovered reproductive hormone and appears to have many important regulatory roles in mammals and non-mammalian vertebrates. Kisspeptin and its receptor have been found to play a role in regulating reproduction by acting locally in the gonads of mammals. Kisspeptin has been identified in several fishes and it was found to regulate reproduction by influencing the hypothalamic and pituitary derived reproductive hormones. However, whether kisspeptin has a direct role in regulating gonadal functions is yet to be determined. We hypothesized that both ghrelin and kisspeptin acts directly on the ovary of fish to regulate oocyte maturation. Our specific objectives were to characterize the presence of ghrelin, ghrelin receptor, kisspeptin and kisspeptin receptor in the ovary of goldfish and zebrafish, and to study the direct effects of ghrelin and kisspeptin on oocyte maturation in zebrafish.

Methods:
We used RT-PCR and fluorescence immunohistochemistry to detect the expression of mRNAs or proteins in the ovary, respectively. Quantitative PCR was used for assessing mRNA abundance in tissues. In vitro oocyte maturation assays on freshly collected oocytes from zebrafish were used to test the effects of ghrelin and kisspeptin on oocyte maturation. All primers for PCRs were designed based on native mRNA sequences. Mammalian antibodies were used for immunolocalization of ghrelin, kisspeptin and its receptors.

Results and Discussion:
We found ghrelin and ghrelin receptor mRNA expression in the ovaries of goldfish and zebrafish. Ghrelin receptor mRNA expression in the ovary was relatively lower during the sexually mature stages of goldfish. Ghrelin-like and ghrelin-receptor like immunoreactivity was found in the follicle cells in paraffin embedded cross sections of goldfish ovary. These results suggest that the ghrelin system is present in fish ovary and this peptide could contribute to the regulation of ovarian physiology. In agreement with this, we found that the incubation with native synthetic ghrelin at 50ng/mL and 100ng/mL concentrations significantly inhibited zebrafish basal oocyte maturation in vitro at 24 hours post-incubation. Ghrelin also inhibited maturation inducing hormone (MIH) stimulated oocyte maturation. This result indicates that ghrelin has an inhibitory role in zebrafish oocyte maturation. We also found kisspeptin and its receptor expressed in goldfish and zebrafish ovaries, suggesting its role in oocyte maturation. As expected, zebrafish synthetic kisspeptin-10 (zfKP-10) at 10 ng/mL and 100 ng/mL acted directly on oocytes to induce its maturation in vitro. In addition, zfKP-10 significantly increased luteinizing hormone receptor, follicle stimulating hormone receptor and 20beta-hydroxysteroid dehydrogenase mRNA expression in the oocytes. These results clearly indicate a direct role for kisspeptin in inducing oocyte maturation in zebrafish. It appears that kisspeptin affects local regulators of gonadal physiology to elicit its functions.

Conclusions:
Overall, our results provide several lines of supportive data for the presence of ghrelin and kisspeptin systems in the ovaries of both zebrafish and goldfish. The presence of ghrelin and kisspeptin receptors in oocytes clearly suggest direct actions of both peptides on the gonadal tissue. While ghrelin has an inhibitory role on zebrafish oocyte maturation in vitro, kisspeptin has a prominent stimulatory role. It was also found that kisspeptin affects oocyte maturation by influencing the local effectors of ovarian functions. Collectively, our results for the first time indicate a direct role for ghrelin and kisspeptin in the ovarian physiology of fish.