別紙４

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主　論　文　の　要　旨

論文題目 **The *cyp21a2* mutant medaka, a novel cortisol-deficiency disease model, reveals a new role of cortisol in reproduction**
（コルチゾル欠損病因モデルのメダカ *cyp21a2* 変異体を用いた生殖におけるコルチゾルの役割の解析）

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論　文　内　容　の　要　旨

The *cytochrome P-450, 21-hydroxylase (cyp21a2)*, is a key enzyme in the cortisol biosynthetic pathway. In humans, mutations in the *cyp21a2* locus has been considered to be the major cause of the congenital adrenal hyperplasia condition also known as CAH. This condition is often accompanied with a compromised fecundity and accumulation of many follicles in the ovary known as polycystic ovary syndrome (PCOS).

Our understanding about the mechanisms leading to this condition and, especially those that directly impact the reproductive aspects are limited mostly because of the severity and the life-threating nature of this condition. In this study, an already Lab established *cyp21a2* mutant medaka (*Oryzias latipes*) was used to conduct a comprehensive analysis of the phenotypes and compare those to the *cyp21a2*-deficiency condition in humans. A synteny analysis revealed that medaka has a single active copy of the *cyp21a2* gene on chromosome 16, regardless the whole genome duplication that teleost underwent during evolution. This encodes a protein that shares about 38% of homology with the human CYP21A2, the reason why a mutant allele was generated with the CRISPR/Cas9 system.

Homozygous mutant medaka did not show embryonic lethality, but low survivability with about 10% reaching adulthood. The deficiency of cortisol was confirmed by mass-spectrometry, which also revealed high levels of 17α-hydroxyprogesterone and 21-deoxycortisol (cortisol metabolic precursors) in mutant medakas. Upregulation of ACTH (*pomca*) and interrenal hyperplasia were also observed in the homozygous mutant. These phenotypes were successfully rescued by cortisol treatment of medaka mutants during embryogenesis.

Interestingly, the analysis of the upregulation of *pomca* in the pituitary by confocal microscopy in this study, allowed to clearly recognized two *pomca*-expressing cell populations in the pituitary of medaka (RPD and PI). Subsequent analysis, revealed that these two populations have a different response mechanism to the levels of cortisol, and that the upregulation of *pomca* observed in mutants, mainly relies on changes observed in those from the RPD.

Unlike in humans, mutant medaka did not show high tendency in sex reversal. In fact, only 2 out of more than 100 homozygous mutant females underwent female-to-male sex reversal. On the other hand, males were fertile and not defects in either tissue and cellular level were observed in the testis. Females, however, were infertile and never spawned eggs. Mutant females were found to develop an ovarian enlargement with accumulation of many mature follicles. Simultaneous development of multiple follicles was obvious in mutant females, which resembled the PCOS-like phenotype in humans.

Likewise, mutant follicles showed defects in ovulation and, intriguingly, mutant oocytes underwent spontaneous parthenogenetic activation. The activation happens without fertilization, and eventually stop before zygotic genome activation. A disruption in gonadotropins fluctuation particularly in LH, high levels of androgens and maturating inducing hormone, are presented in this study as possible causes that may explain the defects in reproduction as a consequence of a systemic cortisol deficiency in vertebrates.

Collectively, the *cyp21a2* mutant medaka is proposed as a new disease model for the study of the mechanism leading to steroid hormones imbalances, interrenal hyperplasia and interestingly, the different response mechanisms employed by the two *pomca*-expressing cell populations in the pituitary of medaka to the cortisol levels, identified and described in the present thesis. Besides, this work sheds light on the possible role of cortisol in regulating the final steps of oogenesis. Particularly, oocyte maturation, ovulation, and meiotic arrest.