

Wt1 and FoxL2 Genes Expression Pattern in Mesonephros-Gonad Complexes of Green Sea Turtle (*Chelonia mydas*) Embryos Incubated in Feminization and Masculinization Temperature

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Abstract : Green turtle (*Chelonia mydas*) is one of TSD (Temperature-dependent Sex Determination, TSD) animals which sex is determined by the egg's incubation temperature. GSD (Genotypic Sex Determination) homologous genes such as Wilms' Tumor (Wt1) and Forkhead Box L2 (FoxL2) play a role in TSD animal sex determination process. Wt1 plays a role in both male pathway, as a transcription factor for Sf1 gene and in female pathway, as a transcription factor for Dax1. FoxL2 plays a role specifically in female sex determination, and known as transcriptional factor for Aromatase gene. Until now, research on the pattern of Wt1 and FoxL2 genes expression in *C.mydas* has not been conducted yet. The aim of this research is to know the pattern of Wt1 and FoxL2 genes expression in Mesonephros-Gonad (MG) complexes of *Chelonia mydas* embryos incubated in masculinizing temperature (MT) and feminizing temperature (FT). Eggs of *C.mydas* incubated in 3 different stage of TSP (Thermosensitive Period) at masculinizing temperature ($26\pm 10^{\circ}\text{C}$, MT) and feminizing temperature ($31\pm 10^{\circ}\text{C}$ FT). Mesonephros-gonad complexes were isolated at Pre-TSP stage (FT at days 14th, MT at days 24th), TSP stage (FT at days 24th, MT at days 36th) and differentiated stage (FT at days 40th, MT at days 58th). RNA from mesonephros-gonad (MG) complexes were converted into cDNA by RT-PCR process, and the pattern of Wt1 and FoxL2 genes expression is analyzed by quantitative Real Time PCR (qPCR) method, β -actin gene is used as an internal control. The pattern of Wt1 gene expression in Pre-TSP stage was almost the same between MG complexes incubated at MT or FT, while TSP and differentiation stage, the pattern of Wt1 gene expression in MG complexes incubated at MT or FT was increased. Wt1 gene expression of MG complexes that incubated at FT was higher than at MT. There was a difference pattern between Wt1 gene expression in this research compared to the previous research in protein level. It could be assumed that the difference caused by post-transcriptional regulation mechanisms before mRNA of Wt1 gene translated into protein structure. The pattern of FoxL2 gene expression in Pre-TSP stage was almost the same between MG complexes that incubated at MT and FT, and increased in both TSP and differentiated stage. The FoxL2 gene expression in MG complexes that incubated in FT is higher than MT on TSP and differentiated stage. Based on the results of this research, it can be assumed that Wt1 and FoxL2 gene were expressed in MG complexes that incubated both at MT and FT since Pre-TSP stage. The pattern of Wt1 gene expression was increased in every stage of gonadal development, and so do the pattern of FoxL2 gene expression. Wt1 and FoxL2 gene expressions were higher in MG complexes incubated at FT than MT.

Keywords : *Chelonia mydas*, FoxL2, gene expression, TSD, Wt1

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