# Modeling the Possible Connection Between the Variation of Homozygous Birc6 Gene and the Premature Ovarian Insufficiency on Zebrafish (Danio rerio)

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200 million of the human population is affected by infertility—a disease that can develop in both men and women due to various reasons—which causes a decrease in the individuals reproductive capacity. Premature Ovarian Insufficiency (POI) is a type of infertility occurring in women. It may occur due to various external factors as well as a result of genetic variations. After analyzing Whole Exon Sequencing (WES) of a family, a rare homozygous variation was detected in the BIRC6 gene which is an apoptotic inhibitor. This variation was observed in female members only and these individuals have been diagnosed with POI. This has led us to a hypothesis framing a possible connection between POI and BIRC6 gene. In our project, we used the CRISPR/Cas9 method to create a variation in the birc6 gene according to the detected variant. A gRNA targeting the variation region was synthesized and injected to single-cell stage zebrafish embryos along with recombinant Cas9 protein using the microinjection method. For the verification of our knock-out model, HRM Analysis was executed. Subsequently, by Sanger sequencing in F0 generation mutant embryos, crispants, the success of the variation was evaluated. The morphologies of the crispants, observed with a light microscope for 5 days, were examined. The expression of the BIRC6 gene and the anti-apoptotic genes presumed to be connected was evaluated by qPCR analysis. It was observed that the expression of all anti-apoptotic genes was reduced in crispants, strengthening our hypotheses. The success of the knock-out model encouraged us to broaden the scope of our study with a knock-in model. We believe that our model will contribute to therapeutic approaches and drug studies to be developed against infertility.

**Awards Won:**

The Scientific and Technological Research Council of Türkiye TUBITAK: 1st Prize Award