Zebrafish retinal stem cell differentiation mechanisms are disrupted by embryonic ethanol exposure

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Prenatal alcohol exposure can lead to a wide range of developmental abnormalities, which are included under the umbrella term fetal alcohol spectrum disorder (FASD). To understand the genesis of FASD defects, the zebrafish is important mechanistic animal model, particularly for retinal development. Previous work from our laboratory showed that ethanol treatment during gastrulation through somitogenesis in zebrafish embryos could recapitulate human ocular defects including microphthalmia, optic nerve hypoplasia, and photoreceptor defects. Ethanol-treated embryos showed increased retinal proliferation in the outer nuclear layer (ONL), inner nuclear layer (INL), and ciliary marginal zone (CMZ). Retinoic acid (RA) and folic acid (FA) co-supplementation rescued most ethanol-induced retinal defects, suggesting that nutrient deficiencies contribute to FASD. To better understand the genesis of ethanol-induced retinal cell differentiation defects, effects of ethanol exposure on retinal stem cell populations in the CMZ and Müller glial cell populations were examined. Ethanol treated retinas had an expanded CMZ, and a reduced expression domain for the cell cycle exit marker, *cdkn1c*. Ethanol treated retinas also showed reduced GFAP-positive Müller glial cells, which are a stem cell population in the central retina. At 72 hpf, the ONL of ethanol exposed fish showed few photoreceptors expressing terminal differentiation markers. Importantly, these poorly differentiated photoreceptors co-expressed the bHLH differentiation factor, *neuroD*, indicating that ethanol exposure produced immature and undifferentiated photoreceptors. Reduced differentiation along with increased progenitor marker expression and proliferation suggest cell cycle exit disruption due to ethanol exposure. Ethanol exposure severely disrupted Wnt and Notch signaling, which are critical for stem cell behavior and differentiation. These defects were rescued by Wnt signaling agonist, RA, and FA treatments. These results suggest ethanol disrupted retinal cell differentiation mechanisms. Further analysis of underlying molecular mechanisms will provide insight into the ethanol-induced retinal defects and potential therapeutic targets.