



In Ovo Cannabidiol Exposure Alters Bone Morphogenetic Protein 4 (BMP4) Signaling and Forebrain Organization in Chick Embryos

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Abstract

The increasing prevalence of cannabidiol (CBD) use during pregnancy raises concerns regarding its impact on fetal neurodevelopment. This study examined how the administration of CBD *in ovo* influences mortality rates, physical development, and the structural arrangement of the forebrain in avian embryos. Fertilized eggs were injected with 10 μ M CBD or vehicle control into the yolk sac at Hamburger–Hamilton (HH) stages 7–8 (24 hours of incubation). Embryos were evaluated at Days 3, 6, and 13 using morphological, histological, and molecular analyses. Early exposure significantly reduced survival at Day 3 (79.3% vs. 93.8%, $p=0.041$) and accelerated neural tube differentiation, coinciding with a 2.3-fold upregulation of Bone Morphogenetic Protein 4 (BMP-4) ($p=0.009$). While survival rates normalized by Day 6, transient growth retardation was observed. By Day 13, CBD-treated embryos exhibited distinct morphological alterations, including increased beak length ($p=0.023$) and brain weight ($p=0.007$). Histological examination of the forebrain revealed narrowed ependymal zones, aberrant mesenchymal-like cell infiltration, and significantly reduced neurite extension. These findings suggest that early CBD exposure disrupts neurodevelopmental timing and forebrain architecture via dysregulated BMP-4 signaling, underscoring the potential for persistent neurodevelopmental abnormalities. Although embryos demonstrated somatic resilience, the persistent cellular and structural anomalies highlight the potential neuroteratogenic risks of CBD exposure during early embryogenesis.

Keywords: Cannabidiol, Forebrain development, In ovo model, Chick embryo