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Retinoic Acid Deficiency Induces FASD-Like Craniofacial and Neurodevelopmental Malformations: A New Molecular Etiology of FASD

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Background:

Prenatal alcohol exposure (PAE) resulting in Fetal Alcohol Spectrum Disorder (FASD) is the most common cause of neurodevelopmental impairments in the western world, with a prevalence of 1% in Canada. It is well established in *Xenopus* models that a single exposure to alcohol during gastrulation is sufficient to induce the developmental defects associated with FASD.

Objective:

Acute ethanol exposure overwhelms the aldehyde metabolic enzymes that would normally convert retinol (Vitamin A) to retinoic acid (RA). We hypothesize that PAE reduces RA levels during critical developmental stages in early gastrulation and this aberration drives the later craniofacial malformations associated with FASD.

Methods:

To biochemically mimic the alcohol-induced RA deficiency *in vivo,* we genetically engineered a mouse expressing Cyp26A1 from the endogenous *Goosecoid* (*Gsc*) promoter. The *Gsc* promoter dictates spatial-temporal expression to the Spemann Mangold Organizer at gastrulation. Cyp26A1 degrades endogenous RA in these cells, mimicking the reduced RA levels induced by acute alcohol exposure and dysregulating neural crest cells induction.

Results:

Gsc:Cyp26A1 mice were derived by germline transmission; F1 mice are born with a Mendelian ratio of 0.66:1 (het:wt, n=351). This loss of mutant embryo viability may reflect the prevalent miscarriages observed in human PAE pregnancies. *Gsc:Cyp26A1*x*RARE-LacZ* embryos show reduced RA activity in the frontonasal prominence region (early face and forebrain). Mutant embryos also demonstrate body-axis developmental variation, indicating developmental perturbation of retinoic acid pathways in FASD relevant tissues (n=48). E18.5 embryos examined using scanning electron microscopy demonstrate mutant embryos have FASD sentinel craniofacial malformations (n=66). P60 *Gsc:Cyp26A1* mice also develop craniofacial malocclusions at significantly higher rates than WT littermates (12.5% vs 0.04%; n=208 and 3711).

Conclusion:

Our data provides *in vivo* evidence that strongly supports retinoic acid deficiency as a major molecular etiology of craniofacial malformations associated with FASD. The finding suggests Vitamin A supplementation may significantly reduce or prevent FASD outcomes in children.