Developmental Toxicity of Maternal, Paternal, and Dual Parental Alcohol Consumption and the Long-term Effects on Offspring Growth, Craniofacial, and Neurological Development

Kara Thomas, Kelly Thomas, Nimisha Srikanth, Alexis Roach, Alison Basel, Katherine Zimmel, Yudi Bedi, Nicole Mehta, Luke Dotson, Hayden Reitz, Rachel Toler, Tyler Brown, Alan Nguyen Pham, Aidan Slagter, Morgan Stovall, Pia Valenzuela, Shunmeng Cui, Michael Golding

BBC Behavior, Biology, and Chemistry — Translational Research in Addiction

Texas A&M University — Veterinary Medicine & Biomedical Sciences

I. INTRODUCTION

According to the CDC, *Fetal Alcohol Spectrum Disorders (**FASDs**) are a group of conditions that can occur in a person whose **mother drank alcohol during pregnancy***. This definition places the fault for FASD-associated mental and physical defects exclusively on the mother. Despite the efforts to recognize **paternal epigenetic contributions**, research examining congenital disabilities is limited and remains exclusively focused on **maternal exposures**. ***We hypothesized that FASD-associated developmental defects would become exacerbated in a dual parental model of alcohol consumption.***

II. METHODOLOGY

FIGURE 1. Experimental design to examine the impact of paternal, maternal, and dual parental patterns of alcohol exposure on offspring growth, craniofacial, and neurological development

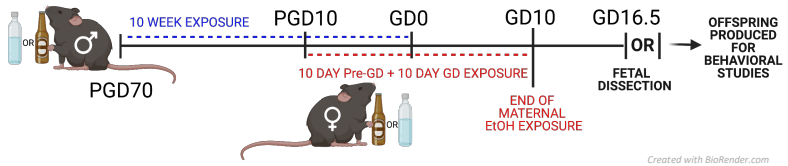

FIGURE 1. We exposed both male and female adult [postnatal day (PND) 90] C57BL/6J mice to either control or alcohol (10% EtOH) preconception treatment groups using the Drinking in the Dark (DID) method of voluntary alcohol consumption. Males were exposed for two spermatogenic cycles (70 days), while females were exposed to treatment for 10 days prior to conception and continued exposure until pregnancy confirmation on gestational day (GD) 10. Once pregnancies were confirmed, females were removed from treatment and pregnancies were either terminated at GD16.5 or allowed to continue to birth. Offspring underwent behavioral study assays (open field maze, nestlet shredding test, marble burying test, and novel object recognition) at week 6 (early adolescence), 9 (late adolescence), 12 (young adult), and 15 (mature adult).

III. RESULTS

1 Fetal Growth Restriction

FIGURE 2. Offspring growth is uniquely altered by paternal, maternal, and dual parental ethanol consumption

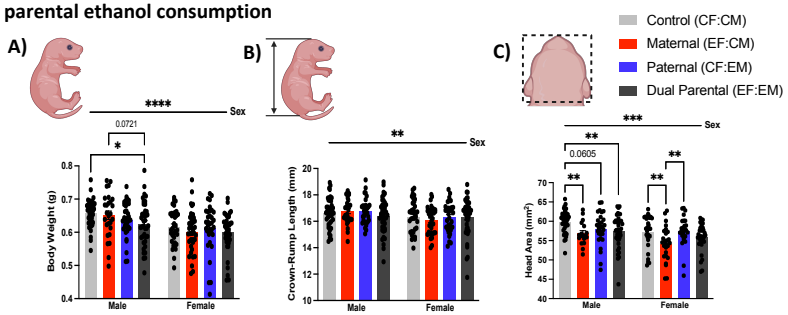

FIGURE 2. Male and female offspring average body weight **(A)**, crown-rump length **(B)**, and head area **(C)** at GD16.5; *Two-way ANOVA, Uncorrected Fisher's LSD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

2 Facial Morphometrics

FIGURE 3. Facial morphometric analysis reveals different craniofacial phenotypes depending on parental ethanol exposure

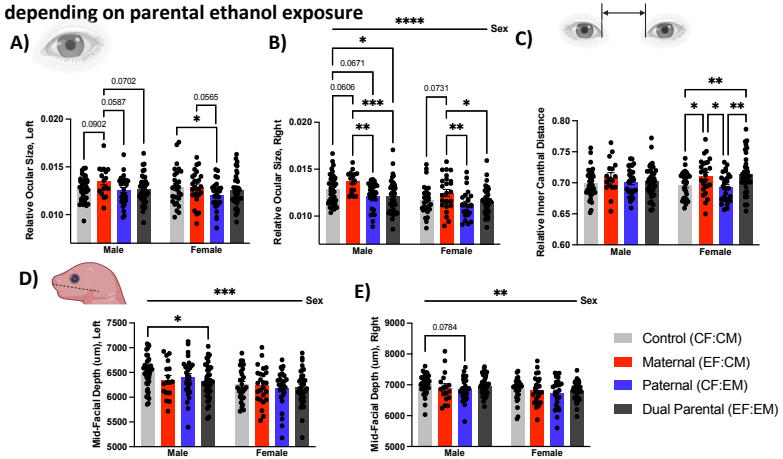

FIGURE 3. Male and female offspring average left **(A)** and right **(B)** relative ocular size, relative inner canthal distance **(C)**, and left **(D)** and right **(E)** mid-facial depth at GD16.5; *Two-way ANOVA, Uncorrected Fisher's LSD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

3 Brain Development

FIGURE 4. Parental alcohol consumption impacts fetal brain development

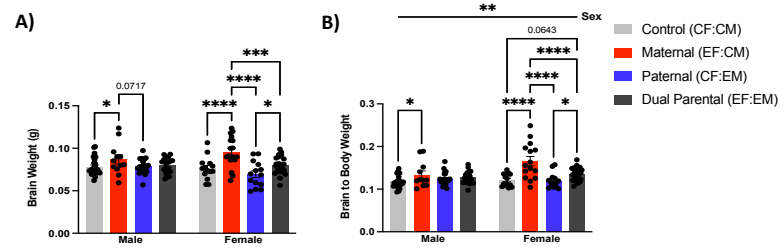

FIGURE 4. GD16.5 male and female offspring average brain weight **(A)** and brain weight relative to fetal body weight **(B)**; *Two-way ANOVA, Uncorrected Fisher's LSD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

4 Behavior

FIGURE 5. Offspring behavioral phenotypes from early adolescence through mature adulthood

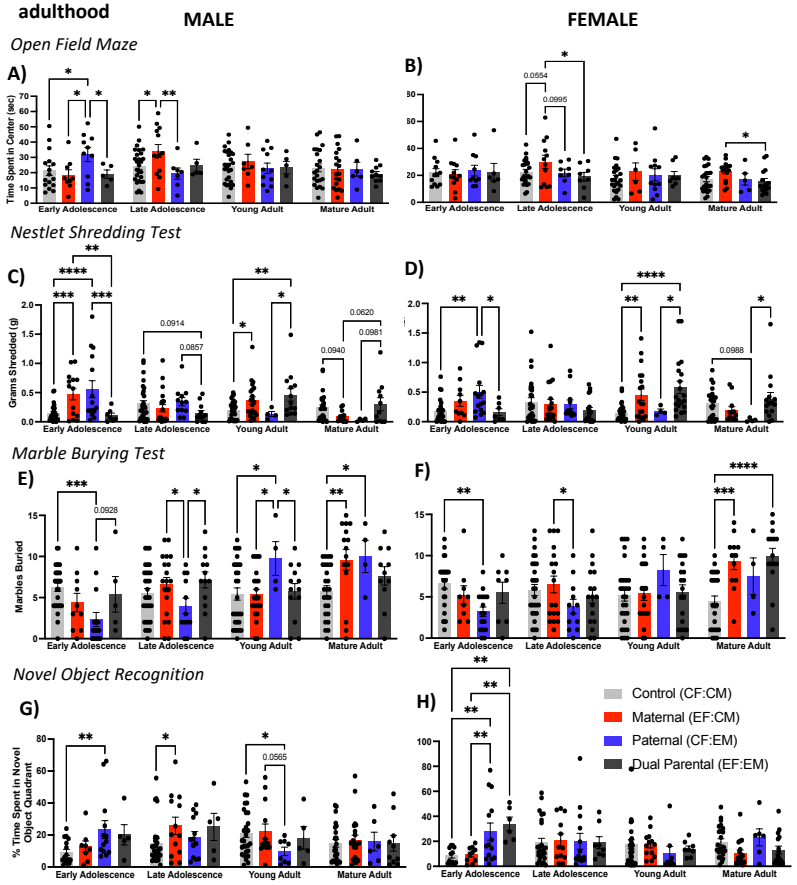

FIGURE 5. Time spent in the center of the open field maze for male **(A)** and female **(B)** offspring, grams of nestlet shredded for male **(C)** and female **(D)** offspring, marbles buried by male **(E)** and female **(F)** offspring, and percent time spent in novel object quadrant for male **(G)** and female **(H)** offspring; *Two-way ANOVA, Uncorrected Fisher's LSD; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

IV. CONCLUSIONS

- ☑ **Paternal alcohol consumption induces offspring fetal growth restriction, facial dysmorphology, and altered neurological development.**
- ☑ **When compared to maternal exposure alone, dual parental alcohol exposure presents a unique offspring phenotype.**
- ☑ **Parental alcohol consumption affects offspring behavioral phenotypes from early adolescence through adulthood.**
- ☑ **Male and female offspring are affected differently by parental alcohol consumption.**

V. ACKNOWLEDGEMENTS

This work was funded by the NIH – National Institute of Alcoholism and Alcohol Abuse, R01AA028219, *Heritable epigenetic effects of Paternal alcohol use on FASD phenotypes* (2020 – 2025) and a medical research grant from the W.M. Keck Foundation, *Paternal contributions to fetal alcohol spectrum disorders: questioning the prevailing paradigm* (2019 – 2023).