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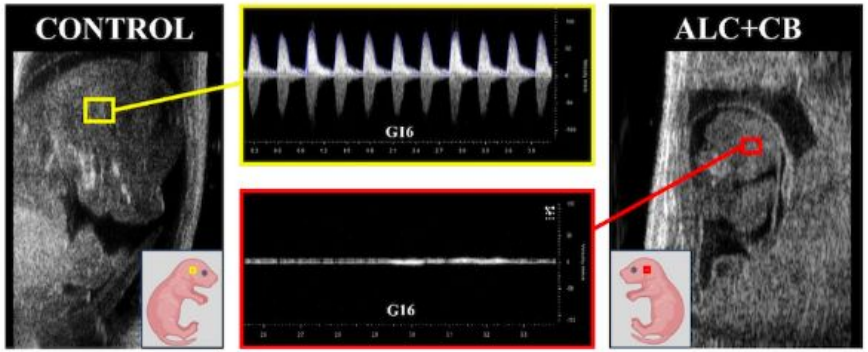
Acute Effects of Prenatal Alcohol and Cannabinoid Exposure on Fetal Development

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INTRODUCTION

- **Prenatal alcohol exposure** can lead to the development of Fetal Alcohol Spectrum Disorders, the most common neurodevelopmental disorder worldwide.
- **Prenatal cannabinoid exposure often co-occurs with alcohol exposure** and independently dysregulates inflammatory responses in exposed offspring.
- Prenatal alcohol exposure increases inflammation, triggering a maternal immune response that can result in fetal resorption (Drews, 2020).
- Maternal and fetal distress is associated with increased fetal resorptions, fetal growth restriction or cessation, edema, hemorrhaging, and reduced fetal blood flow (Flores, 2014).

- **Prenatal alcohol and cannabinoid co-exposure** is associated with reduced cerebral blood flow and subsequent mortality in fetuses (Rouzer, 2024).



<u>Research Question:</u> Are there consistent physical signs of fetal distress following drug exposure, and do they differ by the type of exposure?

METHODS

Drug exposure: at 9 am daily between Gestational Days 12-14



I.P Injection: Cannabinoid agonist CP-55940 (750 µg/kg, in 10% DMSO/Saline) or 10% DMSO in saline (control)

Pregnant C57B1/6J mice

30-minute inhalation of 95% Vapor Ethanol or Room Air

Dissection: at 9am on Gestational Day 18, Dams euthanized for Cesarean Section

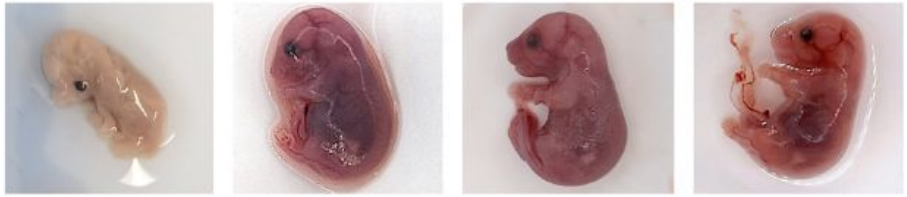


18 days after plug detection

Separate fresh fetuses from placentae, placing them into petri dishes for imaging, dissection, and analysis

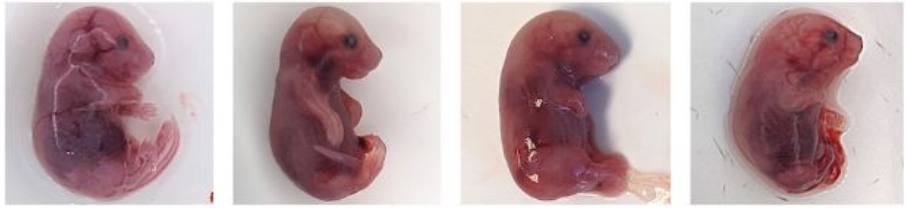
Litter images were reviewed for abnormalities by four lab members blind to prenatal exposure conditions, and given a numerical value. Results were then averaged for statistical analysis.

Color



White *Blue/Purple* *Dark Red* *Pink*

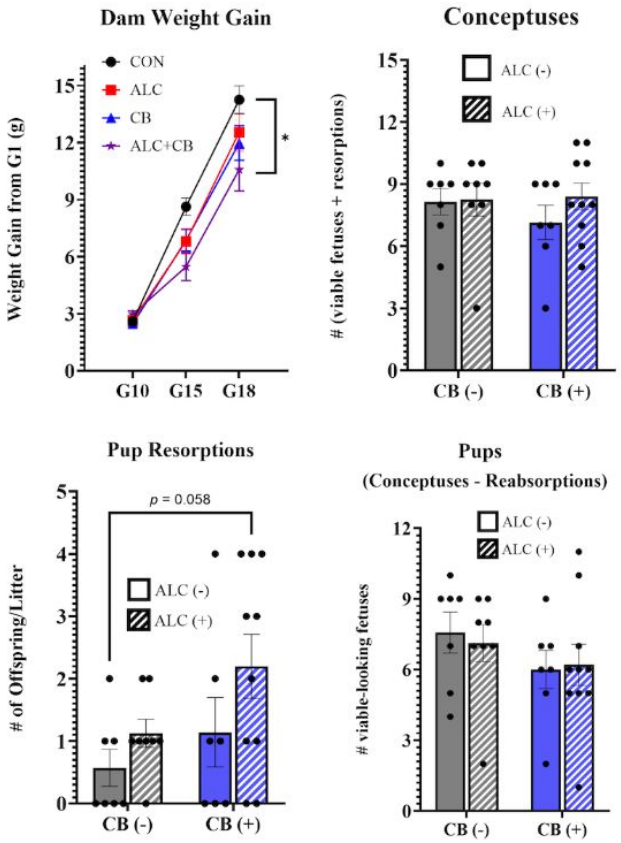
Physiological Complications



Missing Eye *Bleeding* *Swelling* *Limb Difference*

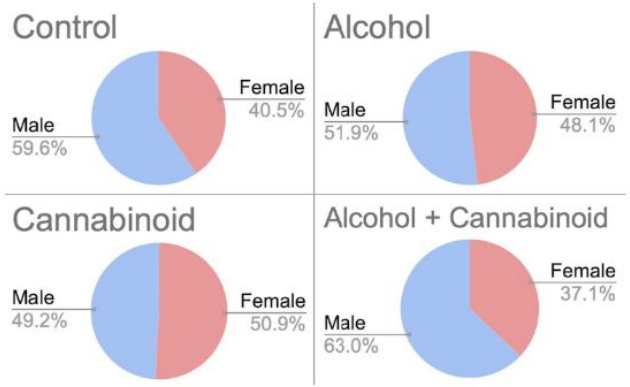
RESULTS - LITTER FEATURES

Litter Characterization (*N* = 32)

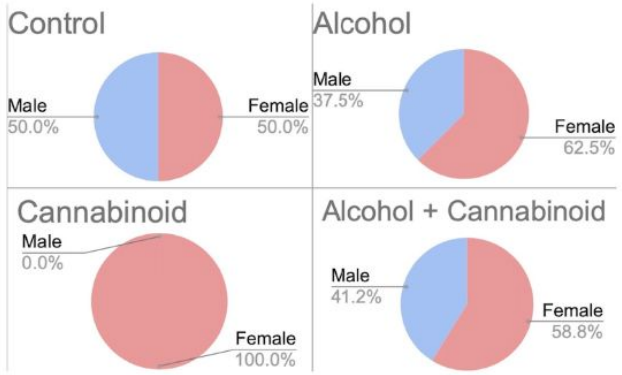


RESULTS - SEX RATIOS

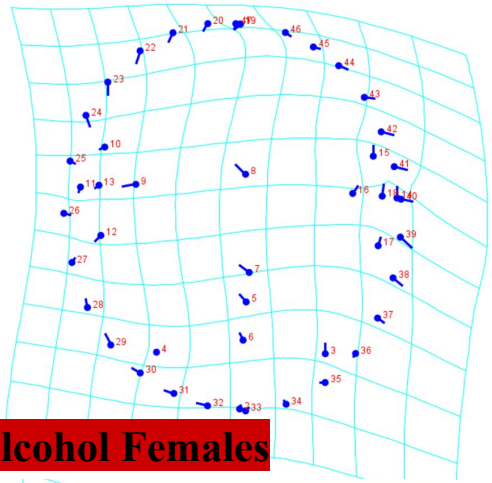
Viable-Appearing Fetal Sex Ratios (*N* = 208)



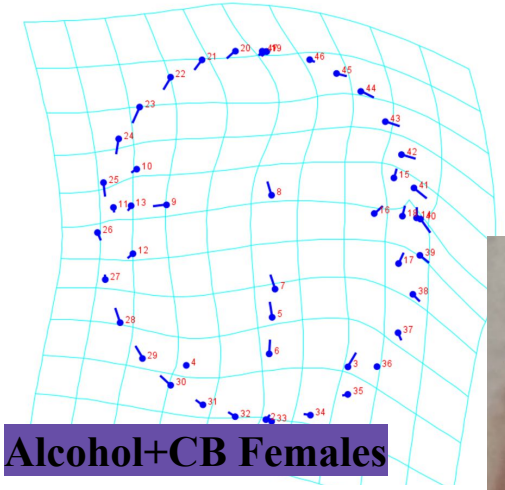
Resorption Sex Ratios (*N* = 35)



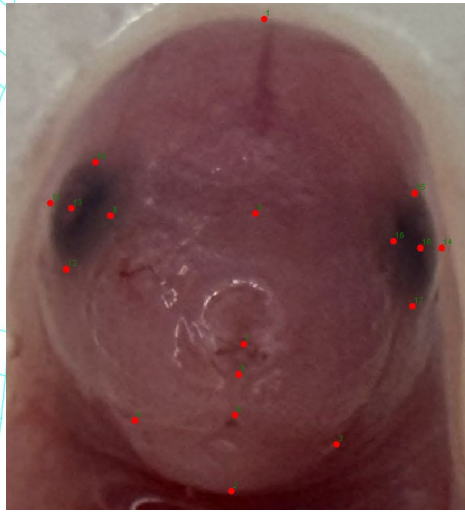
RESULTS: Craniofacial

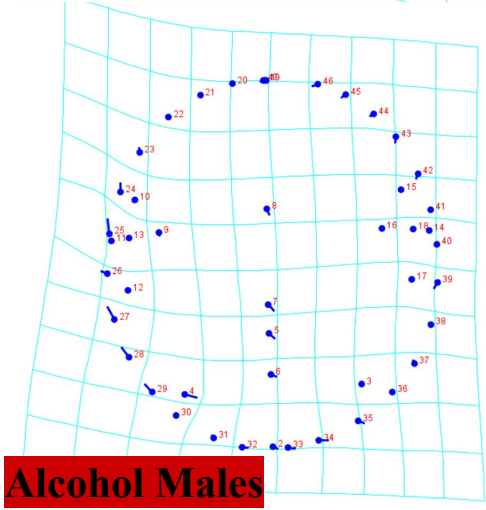


Alcohol Females

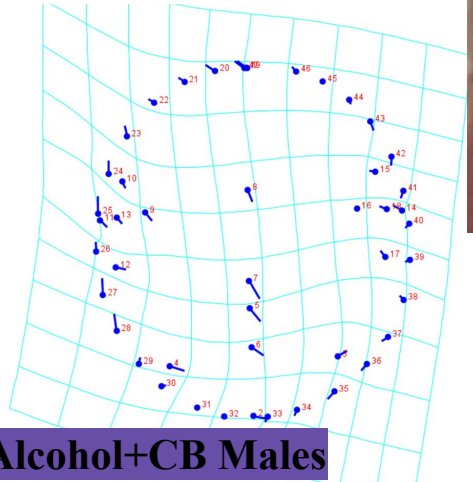


Alcohol+CB Females





Alcohol Males



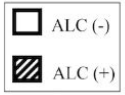
Alcohol+CB Males

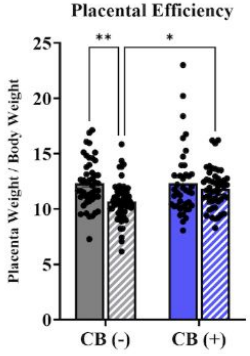
RESULTS - FETAL ABNORMALITIES

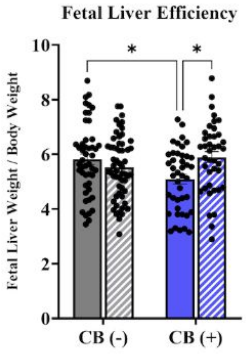
Outcome	Model	Omnibus LR χ^2 (df)	*p*	ALC Effect (OR/RR)	ALC *p*	CB Effect (OR/RR)	CB *p*	Interaction *p*
Conceptuses / litter	Poisson GLM (changes in rate)	1.07 (3)	0.784	RR = 1.01	0.449	RR = 0.88	0.712	0.513
Reabsorptions	Binomial GLM (changes in odds)	**9.40 (3)**	**0.024**	*OR = 2.09*	*0.076*	**OR = 2.52**	**0.023**	0.871
Total abnormalities	Binomial GLM (changes in odds)	**13.50 (3)**	**0.004**	**OR = 15.91**	**0.009**	**OR = 10.17**	**0.035**	**0.021**
Color abnormalities	Binomial GLM (changes in odds)	*7.16 (3)*	*0.067*	**OR = 2.65**	**0.034**	*OR = 2.36*	*0.084*	0.237

RESULTS - TISSUE EFFICIENCY

Tissue weight was divided by fetal body weight for evaluation of changes in organ growth proportions.







CONCLUSION

- Prenatal drug exposure reduced maternal weight gain and viable pups numbers per litter.
- All exposures increased fetal resorptions, with a pronounced vulnerability in female fetuses.
- Drug-exposed fetuses exhibited increased physiological signs of distress, including discoloration (consistent with hypoxia), bleeding, eye malformations, swelling, and limb abnormalities.
  - These phenotypes are consistent with pathways leading to fetal resorption/death.
- Alcohol exposure reduced placental efficiency, whereas cannabinoid exposure reduced fetal liver efficiency, indicating tissue-specific effects.
- Exposed females exhibited a significant superior shift in craniofacial landmarks, while exposed males exhibited a significant inferior shift in craniofacial landmarks.

ACKNOWLEDGEMENTS

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LINKEDIN & SOURCES

www.linkedin.com/in/mckay-domen-629260297

Higgins SL, Bhadsavle SS, Gaytan MN, Thomas KN, Golding MC. *Chronic paternal alcohol exposures induce dose-dependent changes in offspring craniofacial shape and symmetry*. Front Cell Dev Biol. 2024 Jul 1;12:1415653. https://doi.org/10.3389/fcell.2024.1415653 . PMID: 39011393; PMCID: PMC11246915.

Flores, L.E., Hildebrandt, T.B., Kühl, A.A. *et al. Early detection and staging of spontaneous embryo resorption by ultrasound biomicroscopy in murine pregnancy.* Reprod Biol Endocrinol 12, 38 (2014). https://doi.org/10.1186/1477-7827-12-38

Rouzer SK, Sreeram A, Miranda RC. *Reduced fetal cerebral blood flow predicts perinatal mortality in a mouse model of prenatal alcohol and cannabinoid exposure*. BMC Pregnancy Childbirth. 2024 Apr 11;24(1):263. https://doi.org/10.1186/s12884-024-06436-9 . PMID: 38605299; PMCID: PMC11007973.

Drews B, Landaverde LF, Kühl A, Drews U. *Spontaneous embryo resorption in the mouse is triggered by embryonic apoptosis followed by rapid removal via maternal sterile purulent inflammation*. BMC Dev Biol. 2020 Jan 9;20(1):1. https://doi.org/10.1186/s12861-019-0201-0 PMID: 31918653; PMCID: PMC6953269.