The Congenital Heart Collaborative

University Hospitals
Rainbow Babies & Children's
Nationwide Children's Hospital

BETAINE SUPPLEMENTATION REDUCES CONGENITAL DEFECTS INDUCED BY PRENATAL ALCOHOL EXPOSURE

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FASD Research: Results and Relevance

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SCHOOL OF MEDICINE
CASE WESTERN RESERVE UNIVERSITY

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Fetal Alcohol Syndrome

- Craniofacial features
- Neurobehavioral deficits

- Cardiac defects
  - **Conotruncal defects**
  - **Ventricular septal defects**
  - **Cardiac valve defects**

Goal: To prevent as many prenatal alcohol exposure induced congenital defects as possible

- Education – uphill battle!
  - 50% of pregnancies are unplanned
  - Some binge drink alcohol while pregnant
- Prenatal Supplements
  - methyl donors
  - anti-oxidants

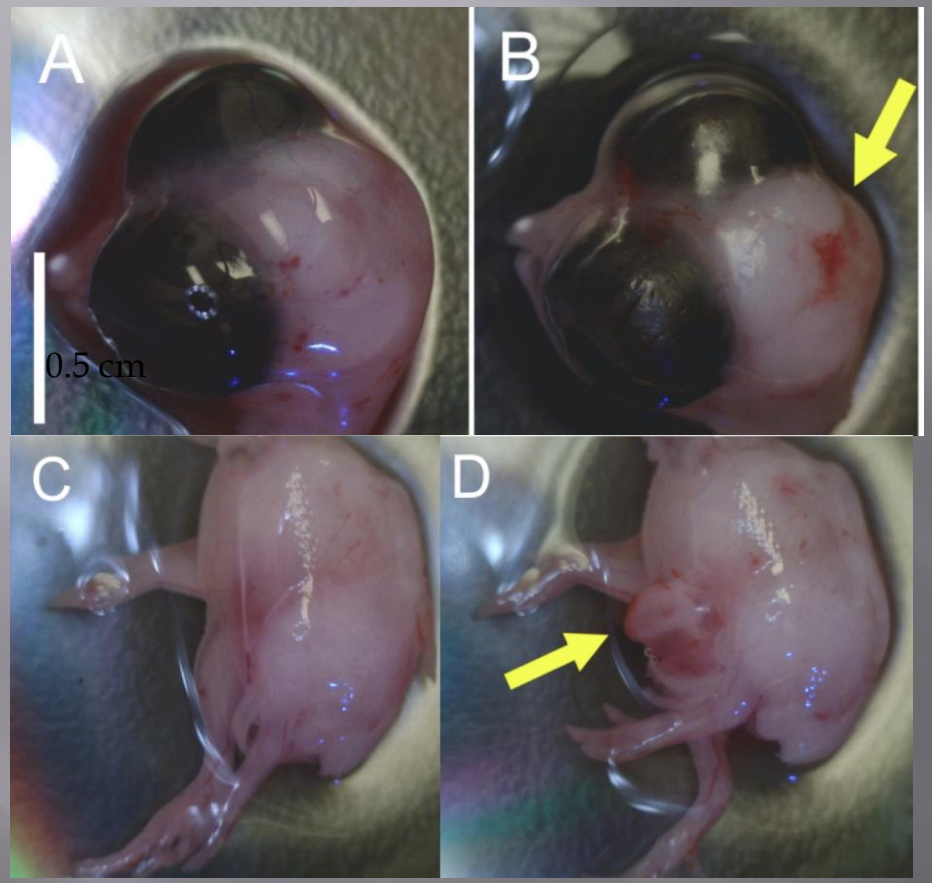
Models & Assays

- Quail eggs
- Ethanol Exposure
  - 40µl of 50% ethanol in saline (BAC=0.179% g/dL)
  - Injected at HH stage 4-5

- Optical Coherence Tomography (OCT)
  - Image at HH stage 19 & 34
  - Non-destructive, non-invasive
  - Axial/Lateral resolution = 8 µm/10 µm
  - Doppler OCT capability
  - Environmental control

Previous Work: Late Stages

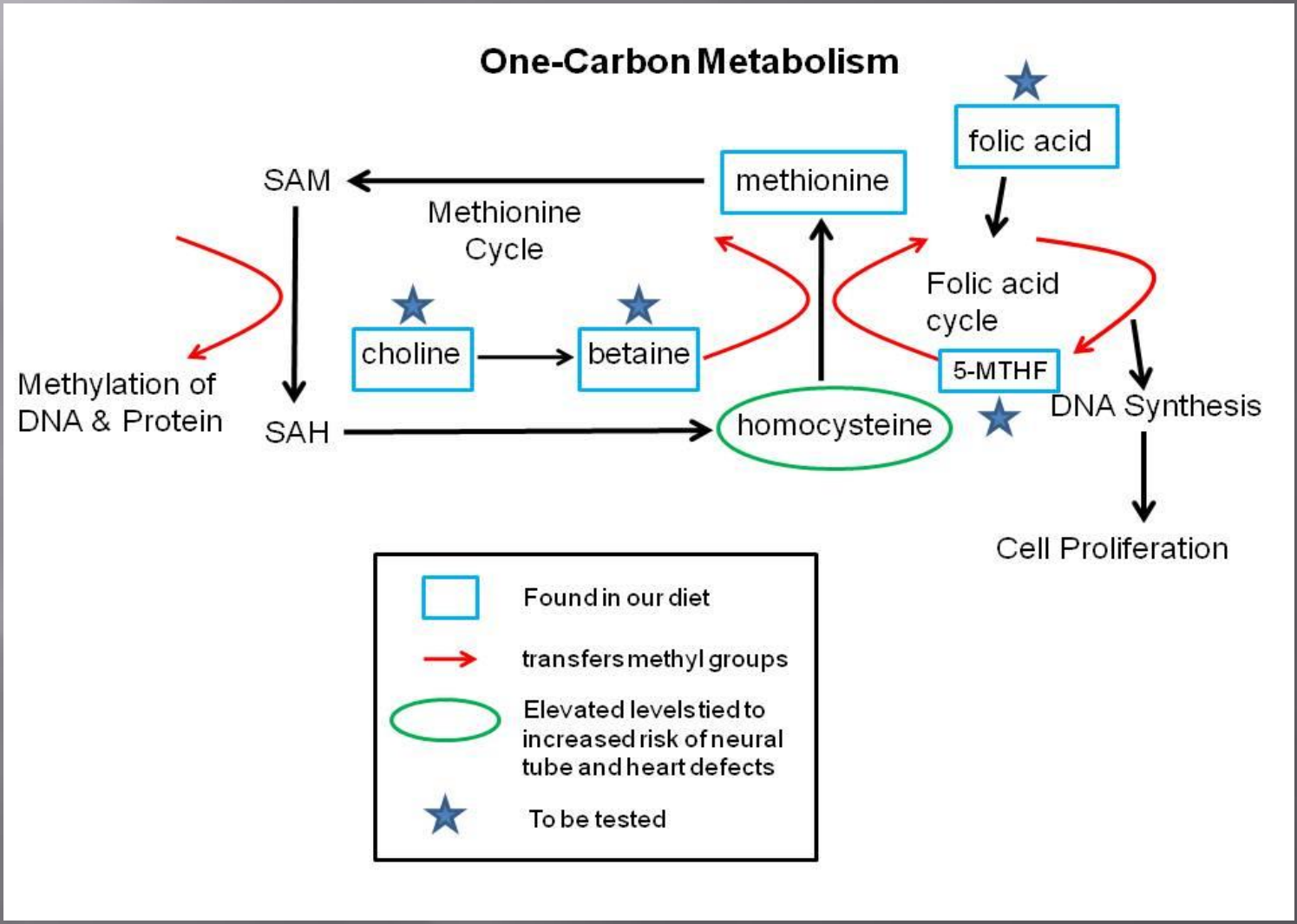
Control Ethanol



G. Karunamuni, AJP Heart and Circulatory Physiology, 2014

	Control	Ethanol Exposed
Survival	82%	52% ↓
Normal body/head	14 (100%)	7 (58%) ↓
Normal heart	14 (100%)	5 (42%) ↓
VSD	0 (0%)	5 (42%) ↑
Missing great vessel	0 (0%)	3 (25%) ↑
Abnormal valves	0 (0%)	3 (25%) ↑

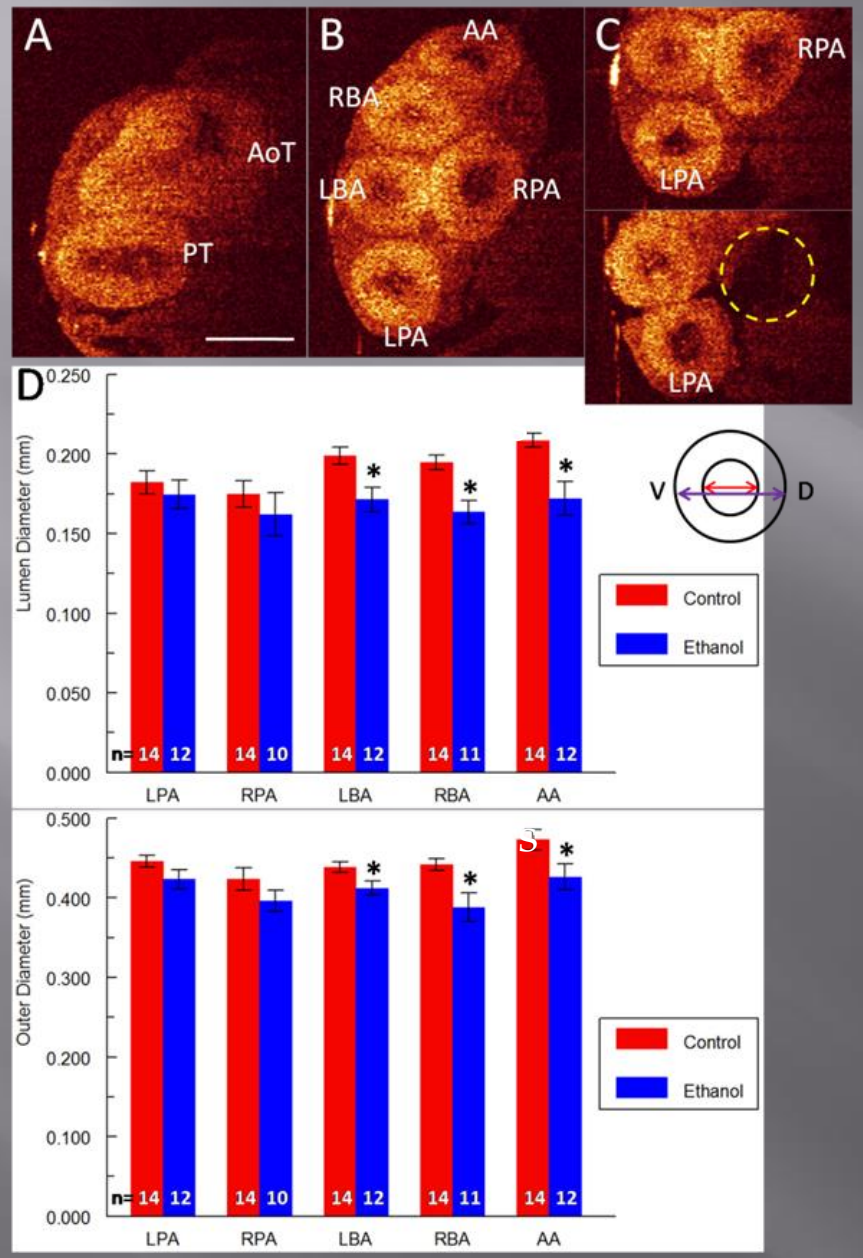
Preventing PAE induced CHDs by providing methyl donors

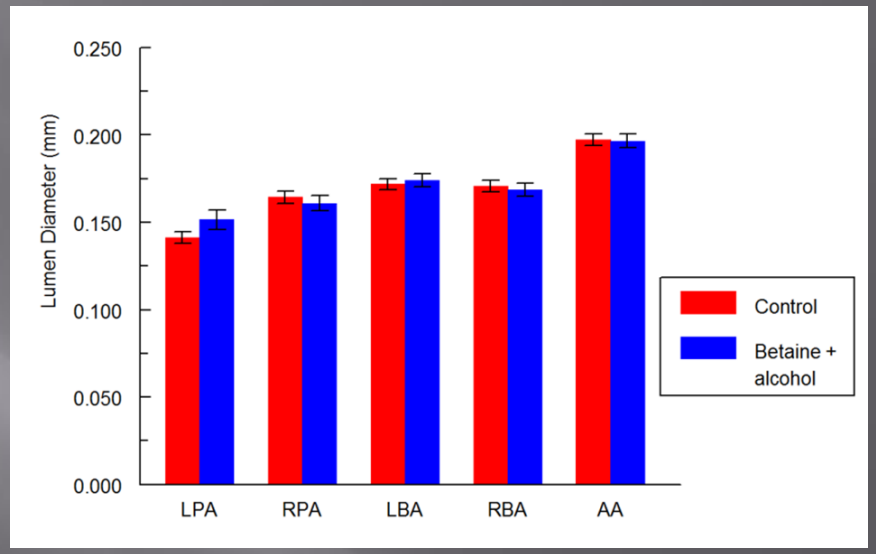


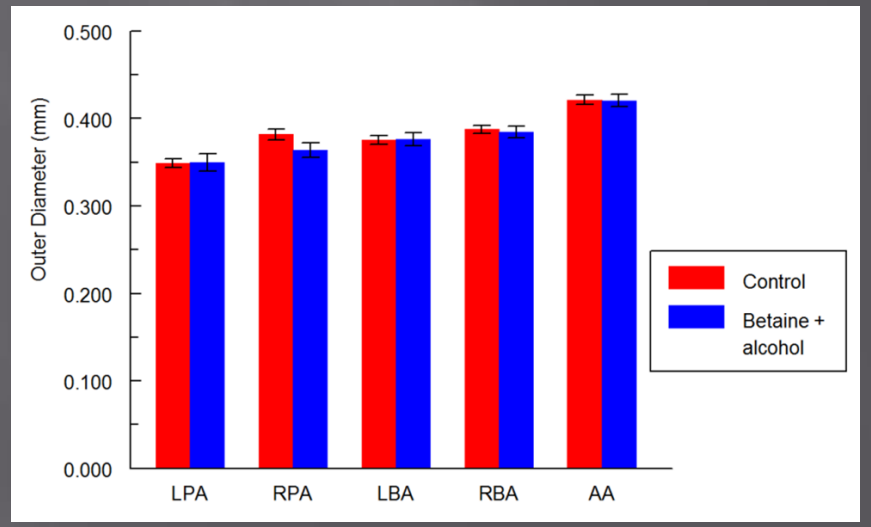
Betaine improves survival and reduced head and body wall defects

	Number of survivors	Survivors with gross morphological defects
Uninjected (n=47)	42 (89%)	0 (0%)
Saline (n=35)	31 (89%)	0 (0%)
Ethanol (n= 48)	22 (46%)	11 (50%)
5 uM betaine w/ethanol (n=62)	45 (73%)	12 (27%)

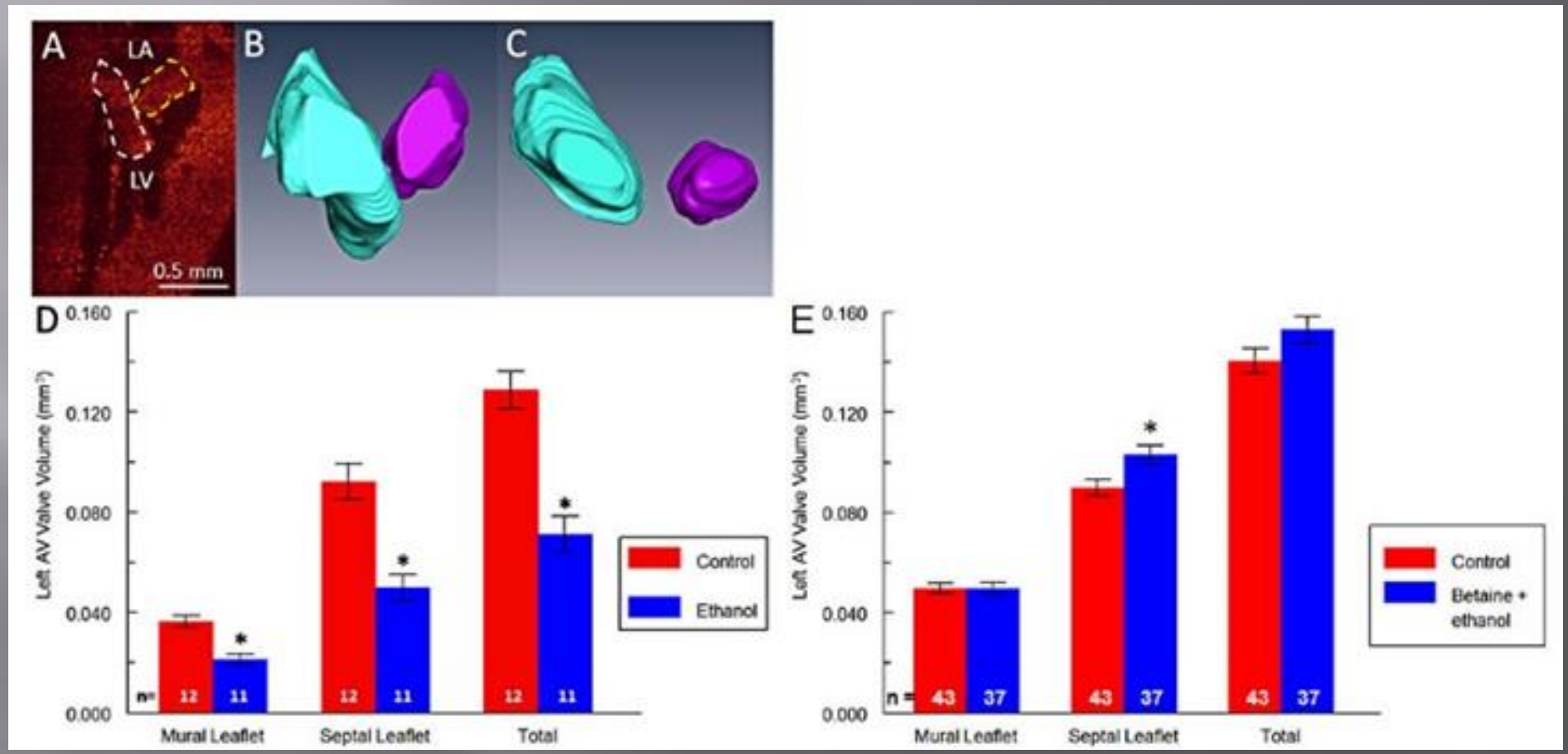
Betaine prevented great artery defects







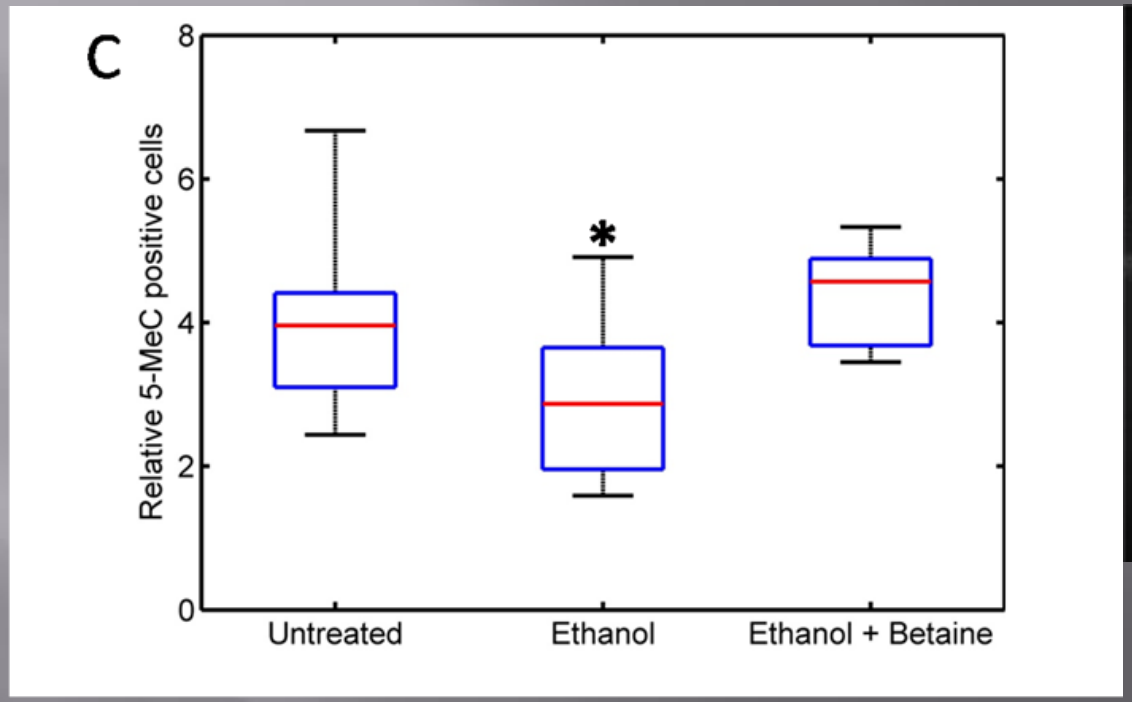
Betaine prevented valve defects

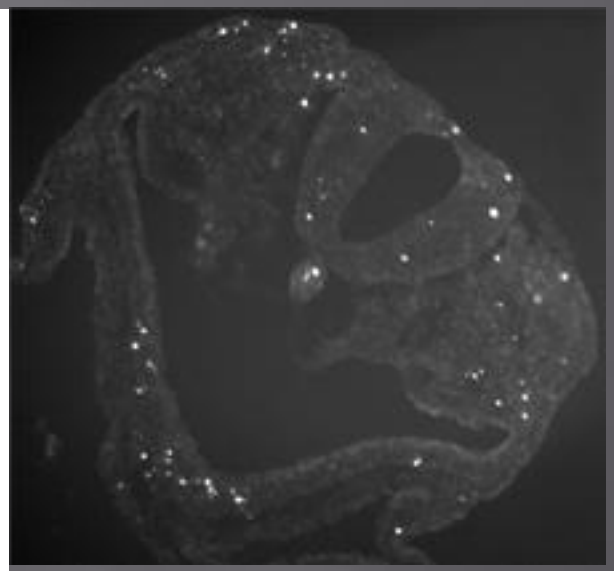


Betaine prevents ethanol-induced cardiac defects

	Control	Ethanol	Betaine/ EtOH
# of embryos	43	12	36
Normal heart	42 (98%)	5 (42%)	31 (86%)
VSDs	0 (0%)	5 (42%)	0 (0%)
Missing vessel	0 (0%)	3 (25%)	2 (6%)
DORV	0 (0%)	1 (8%)	1 (3%)
Misaligned aorta	0 (0%)	1 (8%)	1 (3%)
Hypoplastic right ventricle	0 (0%)	1 (8%)	0 (0%)
Abnormal rotation of ventricle	1 (2%)	1 (8%)	1 (3%)
Abnormal valve morphology	0 (0%)	3 (25%)	0 (0%)
Collateral artery	0 (0%)	0 (0%)	1 (3%)

DNA Methylation

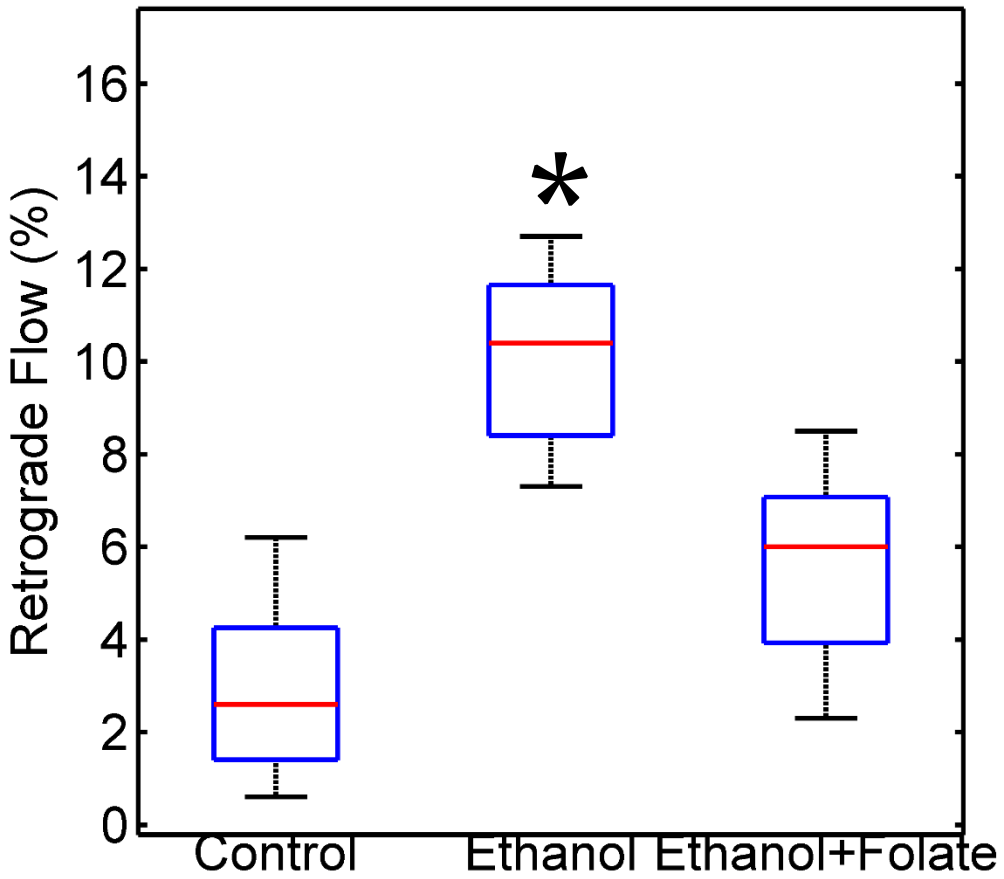




Anti- 5 methyl cytosine

HH Stage 12 transverse sections at the level of the CNCC *P≤0.05

Folate normalizes early heart structure and function



Valve leaflet volumes were also normalized

Summary

- The methyl donor betaine ….
  - improves survival and reduced head and body defects due to ethanol exposure
  - prevents late cardiac valve and great artery defects
  - normalizes DNA methylation in embryo tissues
- The methyl donor folic acid…
  - Known to reduce congenital defects including CHDs
  - prevents early abnormalities that could lead to CHDs

PROGRESS TOWARDS OUR GOAL

http://dietpill-reviews.co.uk/wp-content/uploads/2013/07/0527-diet-pill_vg-300x1991.jpg

FUTURE STUDIES

- Test other compounds & combinations
  - Other methyl donors
  - Antioxidants (Glutathione, D3T)
- Test efficacy/safety in the mouse model
- Test timing and mode of supplementation/fortification
- Clinical trials