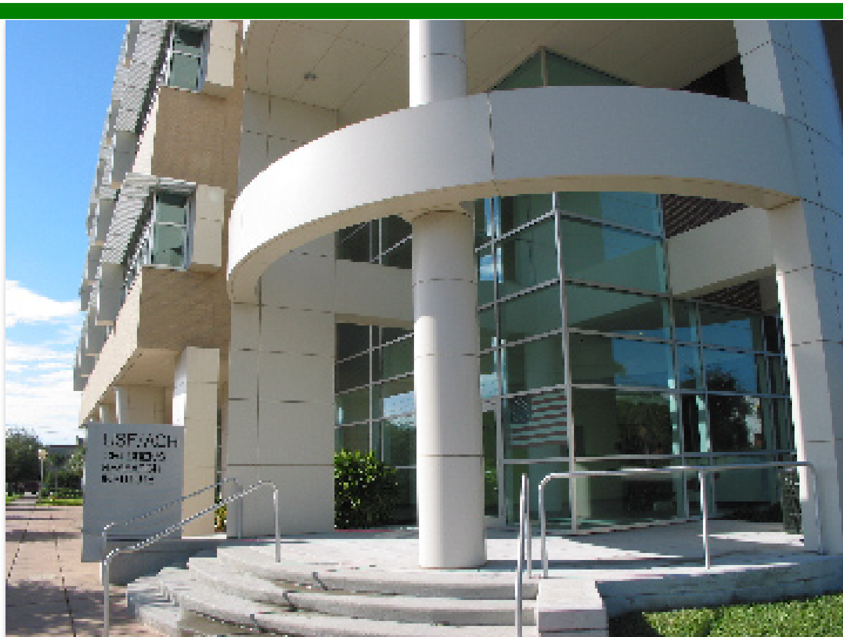
# Prevention of Ethanol-Induced Cardiac Defects by Folate Supplementation in Mouse Model

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# Statistics...

- **In the United States 1 in 5 of over half a million women who report drinking alcohol during pregnancy also admit to *binge* drinking. It is known that even low levels of alcohol (ethanol; EtOH) prenatally can produce human birth defects.**

- **The annual total cost to society for the care of children and adults with Fetal Alcohol Syndrome (FAS) in the US is between 1.4 billion and 9.7 billion.**

- **Epidemiological studies suggest that *54% of live-born children with FAS have some kind of cardiac anomaly*. The malformations include valvuloseptal defects, stenosis of the pulmonary artery, Tetralogy of Fallot, and d-transposition of the great arteries.**

# Congenital Heart Disease

- Congenital heart malformations, the most common of human birth defects, occur in ~ 1% of the population worldwide.

- Valve and associated structures continue to be the most common subtype of CHDs accounting for 25-30% of all cardiovascular malformations.

- Cardiac anomalies appear to arise from abnormal cell lineage decisions of early progenitor cells, disrupting normal patterning of morphogenetic pathways.

## Alcohol (ethanol) consumption during pregnancy is linked to congenital heart defects and to neural-related problems that are associated with Fetal Alcohol Syndrome (FAS).

**A recent recommendation suggested that children born with cardiac defects also be tested for neural-related problems and developmental disabilities (Marino et al., 2012).**

**This underscores <u>the common developmental processes</u> that are involved in both cardiac and neural development occurring at the same time of development during gastrulation.**

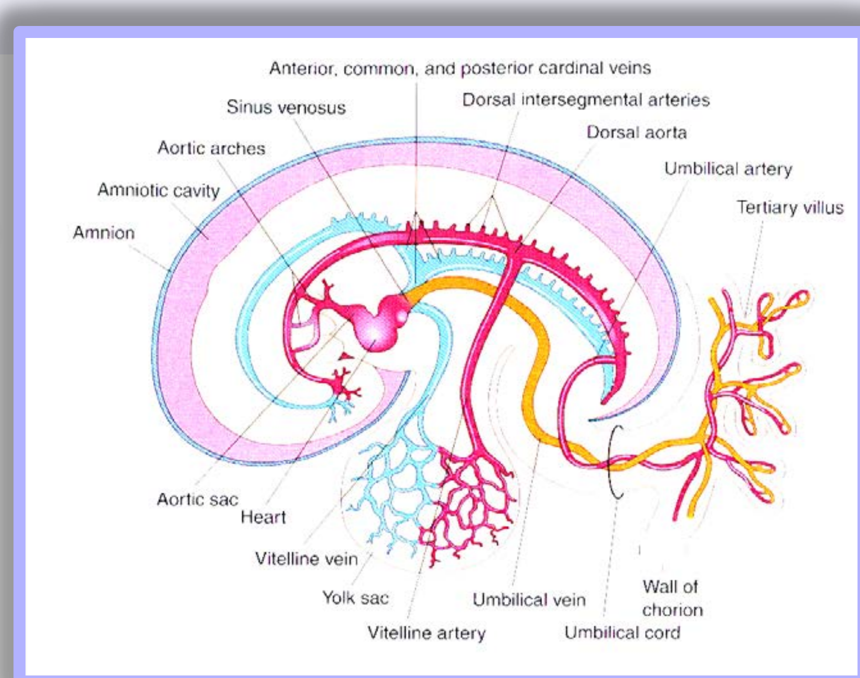
**<u>49% of pregnancies are unintended!</u>** Walker, D., et al., *Fetal Alcohol Spectrum Disorders Prevention: An exploratory study of women's use of, attitudes toward, and knowledge about alcohol.* J Amer Acad Nurse Practitioners, 2005. **17**(5): p. 187-193.

**Gastrulation should be considered a high risk period of pregnancy, as a woman may be yet unaware of her pregnancy and is not taking precautionary measures to reduce exposure risks to the embryo, as for example, by not taking certain drugs, not drinking alcohol, and not smoking.**

**The mother also may not have started folic acid dietary supplementation known to decrease risk of birth defects.**

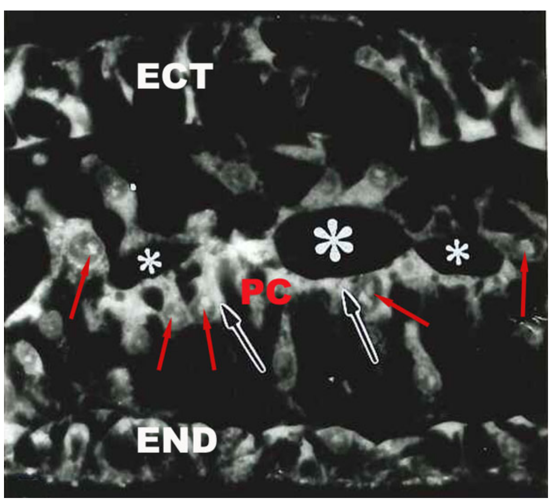
Early cardiac progenitor cells begin to differentiate in the heart fields.

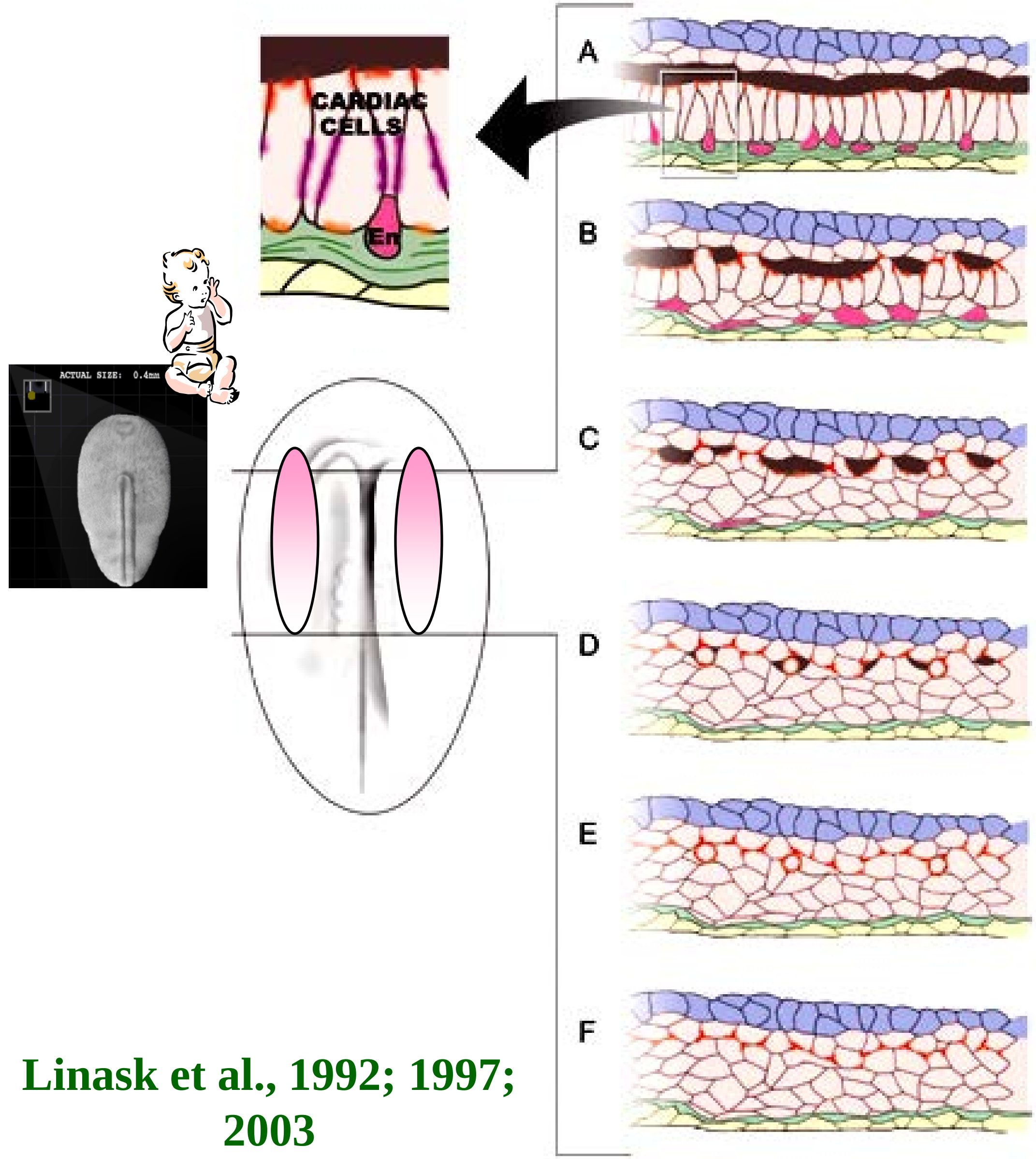

**16 days Human Embryo (**from ***Visual embryo*** website**)**



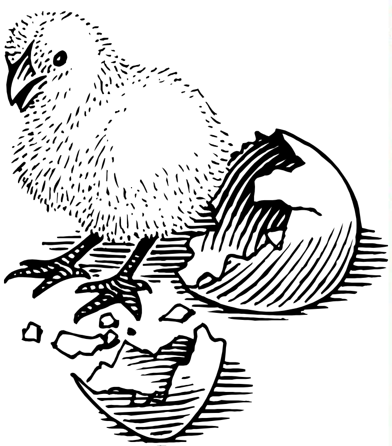
# Heart Organogenesis

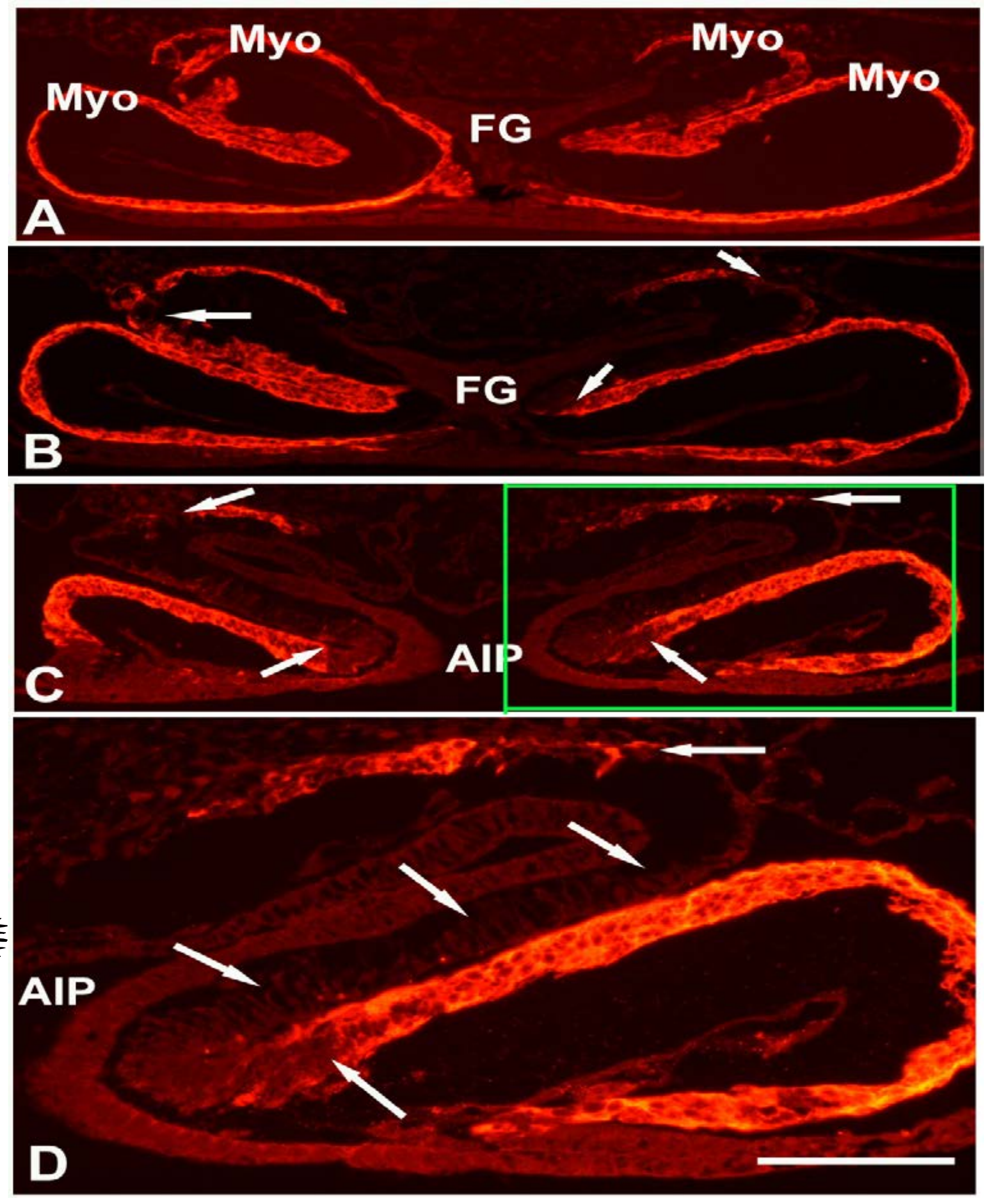
**During *gastrulation*, Wnt/beta-catenin signaling and the induction of the heart compartment occur in an anterior/posterior progression initiating cardiac cell specification and cell sorting across the bilateral regions of the mesoderm layer.**



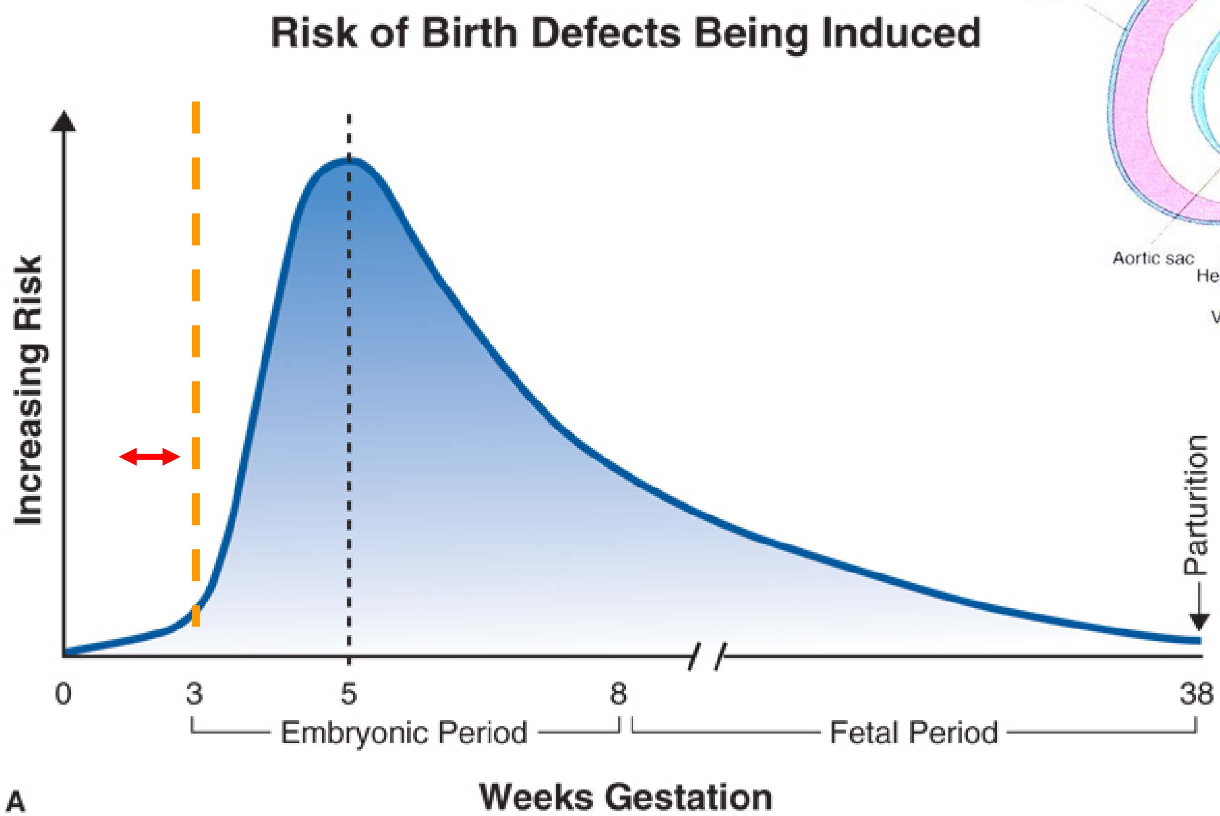




Linask et al., 1992; 1997; 2003



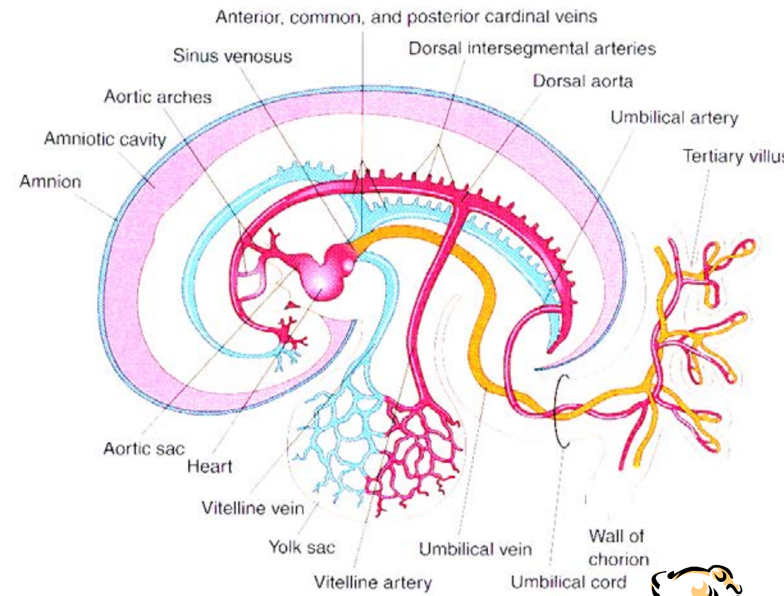

Myo
Myo
FG
Myo
Myo
A
FG
B
AIP
C
AIP
D

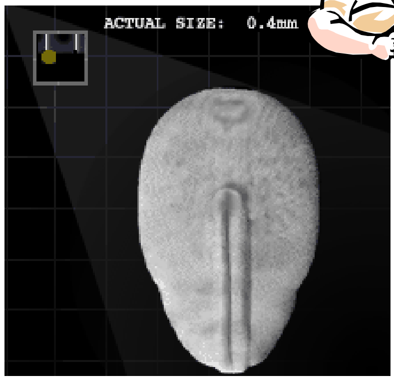
Moore and Persaud. 2003




11-5A Risk of birth defects graph





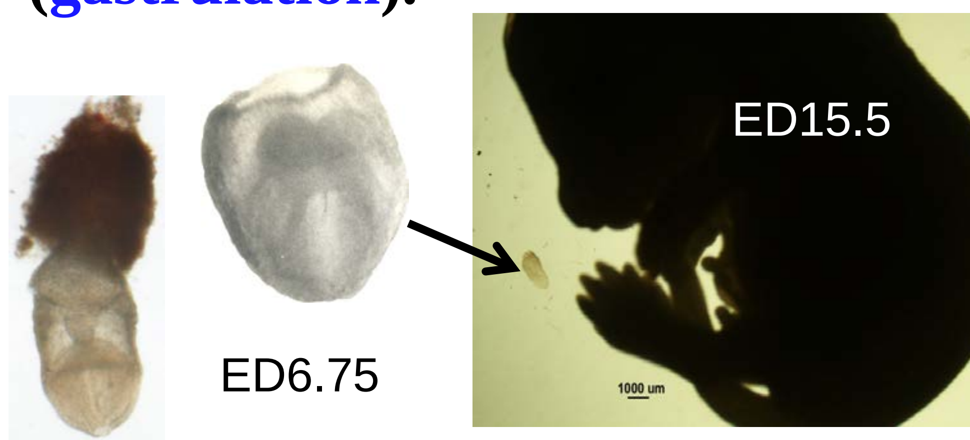

**16 days Human Embryo (**from ***Visual embryo*** website**)**

**From: Langman's Essential Medical Embryology by T.W.Sadler, Lippincott, Williams, and Wilkins, 2005**

# Defining the effects of alcohol on heart development in the mouse model.

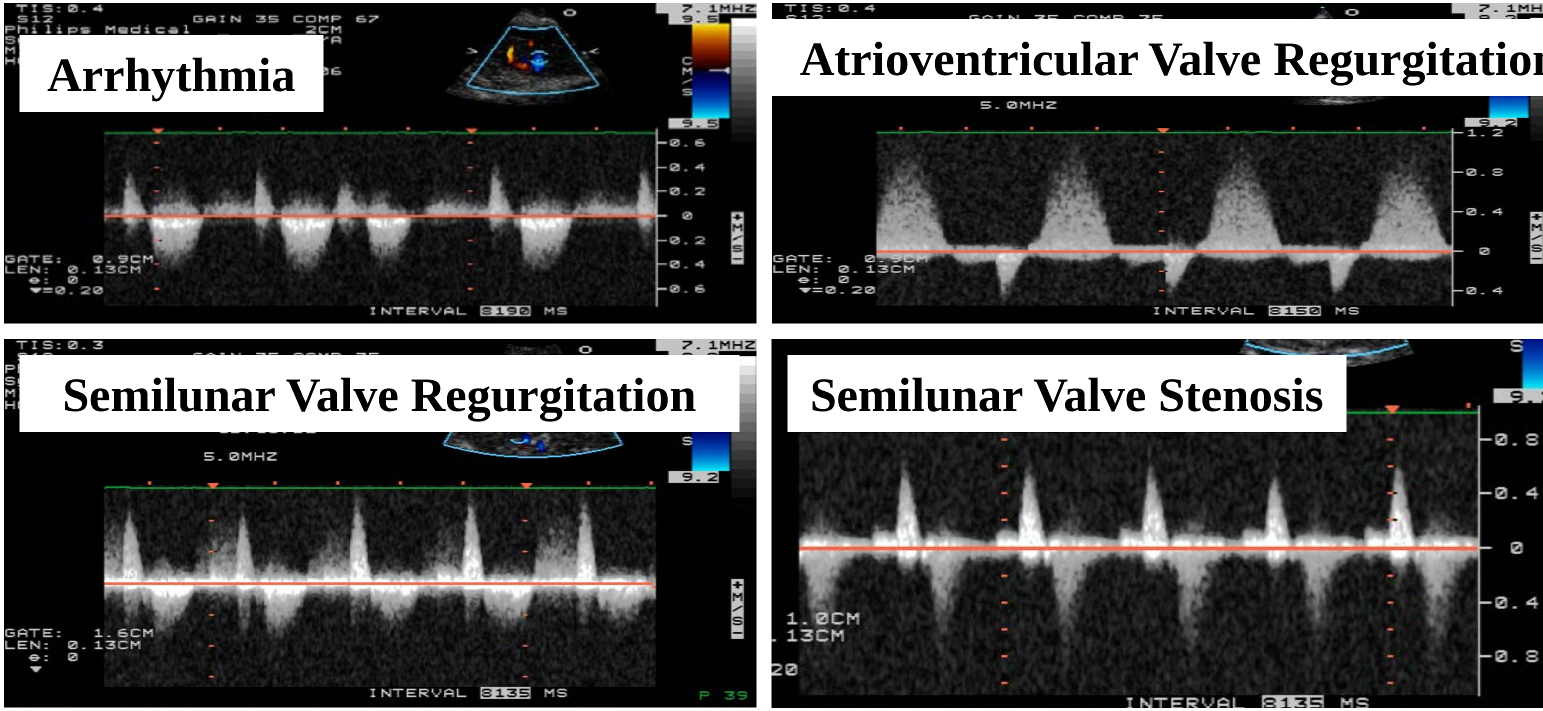
**METHODOLOGY:**

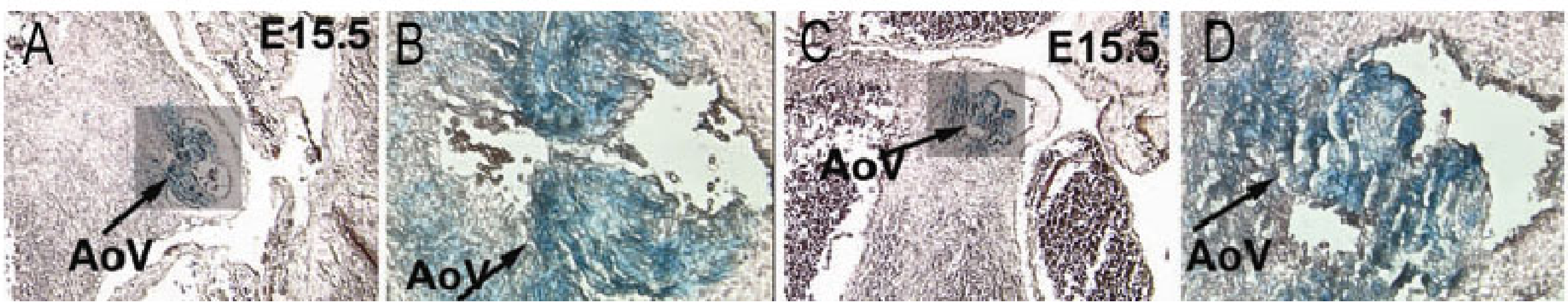
- **<u>Pregnant mice</u> were exposed acutely to an accepted binge drinking level of EtOH (two i.p. injections of 2.9 g EtOH/kg maternal weight, given 3 hrs apart).**
- **Exposure targeted ED 6.75 (gastrulation).**



**On ED 15.5 of gestation doppler echocardiography was used to determine effects of alcohol on embryonic mouse heart function in control and ethanol exposed embryos.**


# A binge drinking exposure of ethanol on ED 6.75 resulted in abnormal fetal cardiac function and valve defects.




**Blue signal is active Wnt/β-catenin signaling using the TopGal transgenic line.**

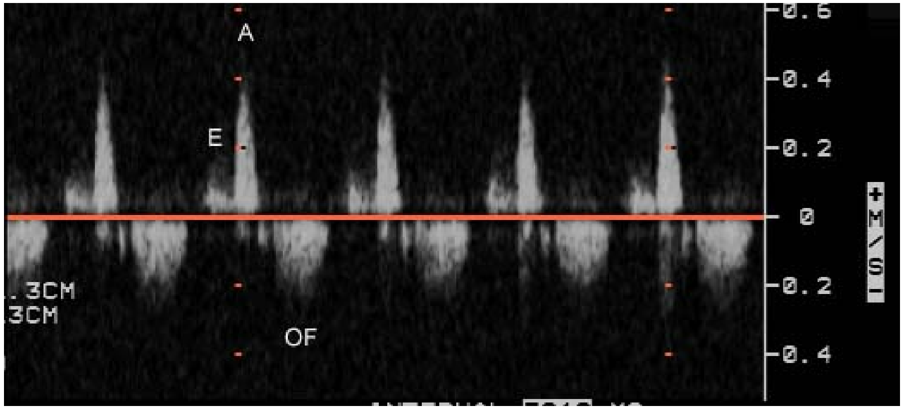
# Folic Acid Supplementation

**<u>Question</u>: Does <u>folic acid</u> when provided early in gestation on morning after conception reverse the adverse effects of EtOH on mouse heart development,?**

**Serrano, M., Brinez, P., Han, M., Lastra-Vicente, R., and K.K. Linask. 2010. Fetal Alcohol Syndrome: Cardiac birth defects and prevention with folate. Amer. J. Obst. Gyn. 203(1):75.e7-75.e15.**

## Mouse Model: Dietary folate supplementation (10 mg/kg or 6.2 mg/kg) was started on morning of vaginal plug detection.

**Only the high dose of folate provided complete protection of cardiac defects.**




**A combination of folate and myo-inositol is more effective in embryonic protection than folate alone.**

# Microarray Analysis of Cardiac Gene Expression after Hcys or Li Environmental Exposure and Folate Protection:

## 46,000 genes analyzed- partial list shown

| Probe Set ID | MGI ID | Gene Symbol | Gene name | cont_f1 | cont_m1 | fol_f1 | fol_m1 | hcys_f1 | hcys_m1 | hcys_fol_n | hcys_fol_n |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1415670_at | MGI:1858696 | *Copg* | coatomer protein complex | 4381.894 | 4049.183 | 4048.528 | 4553.339 | 3572.049 | 4224.668 | 4177.908 | 3817.761 |
| 1415671_at | MGI:1201778 | *Atp6v0d1* | ATPase, H+ transporting, ly | 1764.673 | 1976.29 | 2320.816 | 1823.785 | 1349.806 | 1398.403 | 1425.568 | 1307.183 |
| 1415672_at | MGI:1931029 | *Golga7* | golgi autoantigen, golgin s | 1948.241 | 3758.104 | 3848.113 | 3239.172 | 2262.918 | 2360.037 | 1922.55 | 2670.27 |
| 1415673_at | MGI:97788 | *Psph* | phosphoserine phosphatas | 618.5833 | 505.0409 | 553.3615 | 509.2196 | 349.7614 | 505.1182 | 541.353 | 526.3654 |
| 1415674_a_at | MGI:1926211 | *Trappc4* | trafficking protein particle | 496.6929 | 694.7563 | 1089.279 | 742.8884 | 450.3383 | 282.4902 | 387.5005 | 304.3258 |
| 1415675_at | MGI:1330238 | *Dpm2* | dolichol-phosphate (beta-I | 383.3334 | 448.2151 | 497.1434 | 280.0154 | 290.23 | 420.5334 | 359.4152 | 354.0223 |
| 1415676_a_at | MGI:1194513 | *Psmb5* | proteasome (prosome, ma | 1499.129 | 1386.078 | 1453.692 | 1717.444 | 1192.992 | 1220.144 | 1012.892 | 1748.755 |
| 1415677_at | MGI:1196314 | *Dhrs1* | dehydrogenase/reductase | 1136.014 | 1244.641 | 1454.54 | 1331.653 | 923.0054 | 560.6948 | 972.069 | 805.2863 |
| 1415678_at | MGI:99878 | *Ppm1a* | protein phosphatase 1A, m | 2657.436 | 2711.358 | 2963.827 | 2415.589 | 1570.609 | 2853.237 | 2096.704 | 2768.292 |
| 1415679_at | MGI:1913590 | *Psenen* | presenilin enhancer 2 hom | 1510.746 | 1949.031 | 2056.696 | 2146.628 | 1023.225 | 1496.061 | 1052.053 | 1755.721 |
| 1415680_at | MGI:103097 | *Anapc1* | anaphase promoting comp | 432.6488 | 500.3459 | 518.5495 | 445.2057 | 461.4906 | 415.4733 | 345.8844 | 488.6902 |
| 1415681_at | MGI:2137229 | *Mrpl43* | mitochondrial ribosomal p | 575.8404 | 763.6791 | 728.1792 | 841.1053 | 874.0128 | 505.4614 | 470.8344 | 812.7607 |
| 1415682_at | MGI:1929705 | *Xpo7* | exportin 7 | 1540.643 | 1087.316 | 1259.561 | 1147.983 | 1253.236 | 1364.663 | 1234.937 | 1223.098 |
| 1415683_at | MGI:102579 | *Nmt1* | N-myristoyltransferase 1 | 1954.37 | 1552.287 | 1419.278 | 1525.768 | 1953.782 | 1784.272 | 1678.184 | 1917.643 |
| 1415684_at | MGI:1277186 | *Atg5* | autophagy-related 5 (yeast | 175.0484 | 125.1713 | 106.6942 | 166.6456 | 103.6443 | 133.7943 | 83.76933 | 83.23336 |
| 1415685_at | MGI:1924034 | *Mtif2* | mitochondrial translationa | 1029.952 | 957.166 | 1211.991 | 869.0511 | 1016.741 | 852.2298 | 923.4233 | 1319.663 |
| 1415686_at | MGI:1915615 | *Rab14* | RAB14, member RAS oncog | 4394.826 | 5038.791 | 5949.819 | 4853.312 | 5657.124 | 2099.307 | 4270.967 | 3103.666 |
| 1415687_a_at | MGI:97783 | *Psap* | prosaposin | 1769.432 | 2682.05 | 3061.212 | 3122.189 | 3023.867 | 951.5561 | 2342.256 | 2293.545 |
| 1415688_at | MGI:1914378 | *Ube2g1* | ubiquitin-conjugating enzy | 1532.697 | 1708.67 | 1466.205 | 1423.635 | 1834.254 | 1220.537 | 1467.764 | 1801.445 |
| 1415689_s_at | MGI:1919989 | *Zkscan3* | zinc finger with KRAB and S | 1029.743 | 1751.251 | 1476.208 | 1414.746 | 1736.538 | 857.3862 | 1377.533 | 1270.622 |
| 1415690_at | MGI:2137224 | *Mrpl27* | mitochondrial ribosomal p | 245.3592 | 569.4192 | 771.1092 | 635.697 | 283.609 | 255.0949 | 590.2464 | 348.7104 |
| 1415691_at | MGI:107231 | *Dlg1* | discs, large homolog 1 (Drc | 1486.687 | 1728.542 | 1665.818 | 1528.437 | 1245.248 | 1382.072 | 1187.482 | 1488.407 |
| 1415692_s_at | MGI:88261 | *Canx* | calnexin | 2963.536 | 2887.495 | 2973.107 | 2362.416 | 2142.342 | 2128.076 | 2030.749 | 1788.563 |
| 1415693_at | MGI:1915069 | *Derl1* | Der1-like domain family, n | 1704.223 | 1531.252 | 1181.956 | 1220.054 | 1500.627 | 1178.364 | 1014.351 | 1196.8 |
| 1415694_at | MGI:104630 | *Wars* | tryptophanyl-tRNA synthet | 76.75212 | 144.1621 | 140.4248 | 138.1436 | 26.49555 | 88.80827 | 106.4045 | 117.0879 |
| 1415695_at | MGI:1347005 | *Psma1* | proteasome (prosome, ma | 2291.929 | 3236.799 | 3652.097 | 3196.399 | 1995.114 | 2566.91 | 3341.725 | 3635.205 |
| 1415696_at | MGI:98230 | *Sar1a* | SAR1 gene homolog A (S. c | 5528.373 | 6803.128 | 6628.831 | 7948.772 | 5872.595 | 5226.385 | 6864.207 | 7825.054 |
| 1415697_at | MGI:2442040 | *G3bp2* | GTPase activating protein ( | 1712.134 | 1797.804 | 2299.286 | 1563.901 | 2355.497 | 1507.226 | 2064.454 | 1969.053 |
| 1415698_at | MGI:1917329 | *Golm1* | golgi membrane protein 1 | 1591.254 | 1502.734 | 1345.283 | 1762.217 | 956.4868 | 1489.529 | 1349.136 | 1888.793 |
| 1415699_a_at | MGI:2384801 | *Gps1* | G protein pathway suppres | 801.5099 | 664.5256 | 623.386 | 755.8324 | 625.7438 | 694.2394 | 454.5206 | 732.7302 |
| 1415700_a_at | MGI:1914687 | *Ssr3* | signal sequence receptor, | 12621.73 | 10972.25 | 9355.132 | 13481.27 | 14362.36 | 13294.61 | 14600.53 | 14106.93 |
| 1415701_x_at | MGI:1929455 | *Rpl23* | ribosomal protein L23 | 17189.58 | 12166.4 | 11366.39 | 21292.34 | 27892.49 | 18064.55 | 29430.91 | 23213.85 |

# Gender-related Changes in Fetal Heart of Wnt-Associated Gene Expression

**Table 1 . Gender-Related Changes in Fetal Heart of Wnt- Associated Gene Expression with Lithium, Homocysteine or Folate Exposure**

| MGI ID | Symbol | Name | lith_f1_vs cont_f1 | lith_m1_vs cont_m1 | lith_f1_vs fol_f1 | lith_m1_vs fol_m1 | lith_f1_vs lith_fol_f1 | lith_f1_vs lith_fol_f2 | hcys_f1_vs cont_f1 | hcys_m1_vs cont_m1 | hcys_f1_vs fol_f1 | hcys_m1_vs fol_m1 | hcys_m1_vs hcys_fol_m1 | hcys_m1_vs hcys_fol_m2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MGI:101926 | *Dlx5* | distal-less homeobox 5 | DOWN | UP | UP | DOWN | UP | DOWN | UP | UP | UP | DOWN | UP | UP |
| MGI:109247 | *Ddit3* | DNA-damage inducible transcript 3 | UP | DOWN | UP | DOWN | UP | UP | DOWN | UP | DOWN | DOWN | UP | UP |
| MGI:109340 | *Pitx2* | paired-like homeodomain transcripti | UP | UP | UP | DOWN | UP | UP | DOWN | INCONSISTENT | DOWN | DOWN | DOWN | DOWN |
| MGI:1344337 | *Grem1* | gremlin 1 | UP | DOWN | UP | DOWN | DOWN | UP | UP | UP | UP | DOWN | DOWN | DOWN |
| MGI:1352498 | *Ndrg2* | N-myc DOWNstream regulated gene 2 | UP | UP | UP | DOWN | DOWN | DOWN | UP | DOWN | UP | DOWN | DOWN | DOWN |
| MGI:97845 | *Rac1* | RAS-related C3 botulinum substrate 1 | UP | INCONSISTEN | UP | DOWN | INCONSISTEN | UP | UP | UP | UP | DOWN | INCONSISTENT | INCONSISTENT |
| MGI:1921749 | *Sost* | sclerostin | UP | DOWN | UP | DOWN | UP | DOWN | DOWN | UP | DOWN | DOWN | UP | UP |
| MGI:1923988 | *Paf1* | Paf1, RNA polymerase II associated fa | DOWN | DOWN | UP | DOWN | DOWN | DOWN | DOWN | DOWN | UP | DOWN | DOWN | DOWN |
| MGI:2442252 | *Lrp4* | low density lipoprotein receptor-rela | UP | DOWN | UP | DOWN | DOWN | UP | UP | DOWN | UP | DOWN | DOWN | DOWN |
| MGI:2442609 | *Rnf43* | ring finger protein 43 | UP | UP | UP | DOWN | UP | UP | UP | UP | UP | DOWN | UP | UP |
| MGI:102784 | *Tgfb1i1* | transforming growth factor beta 1 ind | DOWN | UP | DOWN | UP | DOWN | DOWN | DOWN | UP | DOWN | UP | UP | UP |
| MGI:105100 | *Ctnnd1* | catenin (cadherin associated protein) | DOWN | UP | DOWN | UP | DOWN | DOWN | DOWN | UP | INCONSISTENT | UP | UP | UP |
| MGI:108117 | *Emd* | emerin | DOWN | UP | DOWN | UP | UP | UP | UP | DOWN | DOWN | DOWN | DOWN | DOWN |
| MGI:1270862 | *Axin2* | axin2 | DOWN | UP | DOWN | UP | DOWN | UP | DOWN | UP | DOWN | UP | UP | UP |
| MGI:1278315 | *Lrp5* | low density lipoprotein receptor-rela | UP | DOWN | DOWN | UP | UP | UP | DOWN | UP | DOWN | UP | UP | UP |
| MGI:1344332 | *Wif1* | Wnt inhibitory factor 1 | UP | DOWN | DOWN | UP | DOWN | DOWN | UP | DOWN | UP | DOWN | DOWN | DOWN |
| MGI:1347469 | *Foxl1* | forkhead box L1 | DOWN | DOWN | DOWN | UP | DOWN | DOWN | UP | DOWN | UP | UP | DOWN | DOWN |

**Abbreviations**: lith, lithium exposure; f1 or f2 are female embryos #1 or #2; cont, control embryo; m1 or m2, male embryos #1 or #2; fol, folate only exposure; lith_fol, lithium exposure with folate supplementation; hcys, homocysteine exposure; hcys_fol, homocysteine exposure with folate supplementation

# Gender-Associated Changes: Gene Ontology of Specific Biological Processes

Fatty acid β-oxidation
Organic acid catabolic proc
Carboxylic acid catab proc
ATP biosynthesis
Fatty acid catab processes
Lipid oxidation
Fatty acid metabolic proc
Coenzyme metabolic proc
Energy coupled proton trans
Electron transport chain
Cofactor metabolic proc

**The bioinformatic analyses indicate the misexpression can have a *gender bias* for specific genes.**

Table 5. Gender-Associated Changes: Gene Ontology (GO) Specific Biological Processes

| GO ID | Description | hcys_f1_vs_cont_f1 | hcys_f1_vs_fol_f1 | lith_f1_vs_cont_f1 | lith_f1_vs_fol_f1 | lith_f1_vs_lith_fol_f1 | lith_f1_vs_lith_fol_f2 | hcys_m1_vs_cont_m1 | hcys_m1_vs_fol_m1 | hcys_m1_vs_hcys_fol_m1 | hcys_m1_vs_hcys_fol_m2 | lith_m1_vs_cont_m1 | lith_m1_vs_fol_m1 | Number of Instances |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| GO:0007186 | G-protein coupled receptor sign | NS | INCONSIS | UP | UP | NS | NS | UP | UP | NS | UP | UP | UP | 8 |
| GO:0006635 | fatty acid beta-oxidation | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 7 |
| GO:0016054 | organic acid catabolic process | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 7 |
| GO:0046395 | carboxylic acid catabolic proc | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 7 |
| GO:0055114 | oxidation-reduction process | UP | UP | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 7 |
| GO:0006082 | organic acid metabolic process | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0006457 | protein folding | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 6 |
| GO:0006754 | ATP biosynthetic process | UP | UP | NS | UP | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 6 |
| GO:0007155 | cell adhesion | NS | DOWN | NS | NS | DOWN | NS | UP | NS | UP | UP | NS | DOWN | 6 |
| GO:0009062 | fatty acid catabolic process | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 6 |
| GO:0019395 | fatty acid oxidation | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 6 |
| GO:0019752 | carboxylic acid metabolic proc | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0022610 | biological adhesion | NS | DOWN | NS | NS | DOWN | NS | UP | NS | UP | UP | NS | DOWN | 6 |
| GO:0032787 | monocarboxylic acid metaboli | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0034440 | lipid oxidation | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 6 |
| GO:0043436 | oxoacid metabolic process | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0044281 | small molecule metabolic proc | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0044282 | small molecule catabolic proce | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0044712 | single-organism catabolic proc | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | DOWN | 6 |
| GO:0061053 | somite development | NS | DOWN | NS | NS | NS | NS | UP | UP | UP | UP | NS | UP | 6 |
| GO:0071804 | cellular potassium ion transpo | NS | UP | UP | UP | NS | NS | UP | UP | NS | NS | UP | NS | 6 |
| GO:0071805 | potassium ion transmembrane | NS | UP | UP | UP | NS | NS | UP | UP | NS | NS | UP | NS | 6 |
| GO:0072329 | monocarboxylic acid catabolic | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | DOWN | 6 |
| GO:0003012 | muscle system process | UP | UP | UP | UP | NS | NS | NS | NS | NS | DOWN | NS | NS | 5 |
| GO:0006091 | generation of precursor metabo | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0006310 | DNA recombination | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | NS | 5 |
| GO:0006412 | translation | UP | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | DOWN | 5 |
| GO:0006631 | fatty acid metabolic process | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0006732 | coenzyme metabolic process | NS | NS | DOWN | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0006818 | hydrogen transport | UP | UP | NS | UP | NS | NS | NS | NS | DOWN | DOWN | NS | NS | 5 |
| GO:0007218 | neuropeptide signaling pathwa | UP | DOWN | UP | NS | NS | NS | NS | NS | NS | UP | NS | UP | 5 |
| GO:0009108 | coenzyme biosynthetic process | DOWN | NS | DOWN | DOWN | DOWN | NS | NS | NS | NS | DOWN | NS | NS | 5 |
| GO:0009142 | nucleoside triphosphate biosyn | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0009145 | purine nucleoside triphosphate | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0009201 | ribonucleoside triphosphate bi | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0009206 | purine ribonucleoside triphosp | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0015985 | energy coupled proton transpo | UP | UP | NS | UP | NS | NS | NS | NS | DOWN | DOWN | NS | NS | 5 |
| GO:0015986 | ATP synthesis coupled proton t | UP | UP | NS | UP | NS | NS | NS | NS | DOWN | DOWN | NS | NS | 5 |
| GO:0021545 | cranial nerve development | NS | DOWN | NS | NS | NS | DOWN | UP | NS | UP | UP | NS | NS | 5 |
| GO:0022900 | electron transport chain | UP | UP | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0033539 | fatty acid beta-oxidation using | NS | NS | NS | NS | NS | NS | DOWN | DOWN | DOWN | DOWN | DOWN | NS | 5 |
| GO:0035282 | segmentation | NS | DOWN | NS | NS | NS | NS | UP | UP | UP | UP | NS | NS | 5 |
| GO:0044710 | single-organism metabolic pro | NS | NS | NS | DOWN | NS | NS | NS | DOWN | DOWN | DOWN | NS | DOWN | 5 |
| GO:0051186 | cofactor metabolic process | NS | NS | NS | DOWN | NS | NS | DOWN | DOWN | DOWN | DOWN | NS | NS | 5 |
| GO:0060349 | bone morphogenesis | NS | DOWN | UP | NS | NS | NS | UP | UP | NS | UP | NS | NS | 5 |

# Genes Appearing in Multiple Gene Ontology Categories Pertaining to Lipid Metabolism

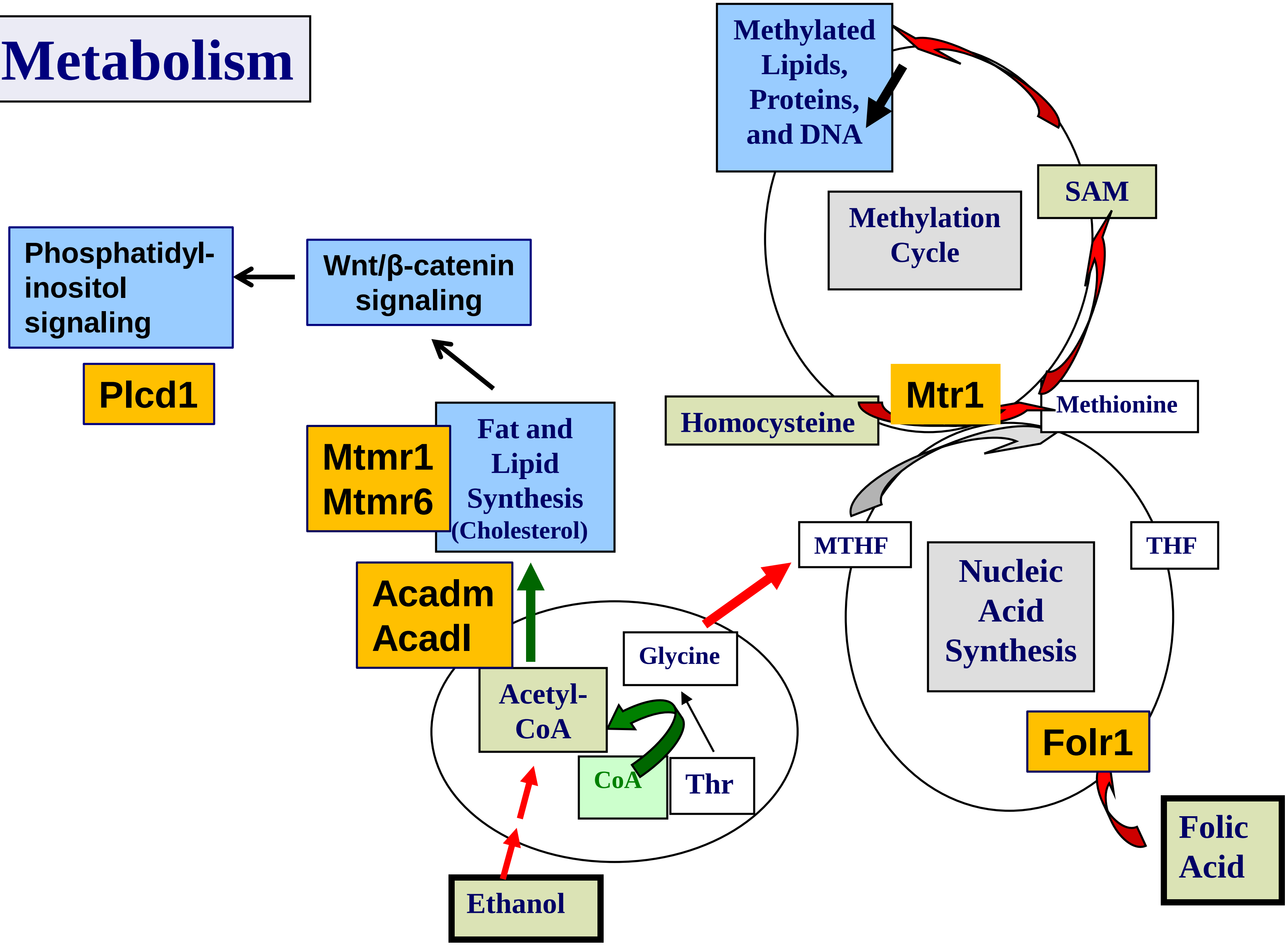
Analyzing genes that appear in all of the classes as (i) cellular processes of **fatty acid oxidation**, (ii) **fatty acid beta oxidation**, (iii) **lipid oxidation** or (iv) **fatty acid beta oxidation using acyl CoA dehydrogenase**, in both Li- and HCy-exposed heart tissue, the genes appearing in all classes included:

*acyl CoA dehydrogenase* 11 (*Acad11*)
*acyl CoA dehydrogenase long chain* (*Acadl*)
*acyl CoA dehydrogenase* medium chain (*Acadm*)
*acyl CoA dehydrogenase short chain* (*Acads*)
*acyl CoA dehydrogenase very long chain* (*Acadvl*) and
*electron transferring flavoprotein dehydrogenase* (*Etfdh*)

Notably all of the protein products of these genes localize to the mitochondria.

**Lipids essentially serve three critical cell functions: energy storage, structural components of membranes, and lipid modifications for activity of cell signaling factors**.

# Metabolism

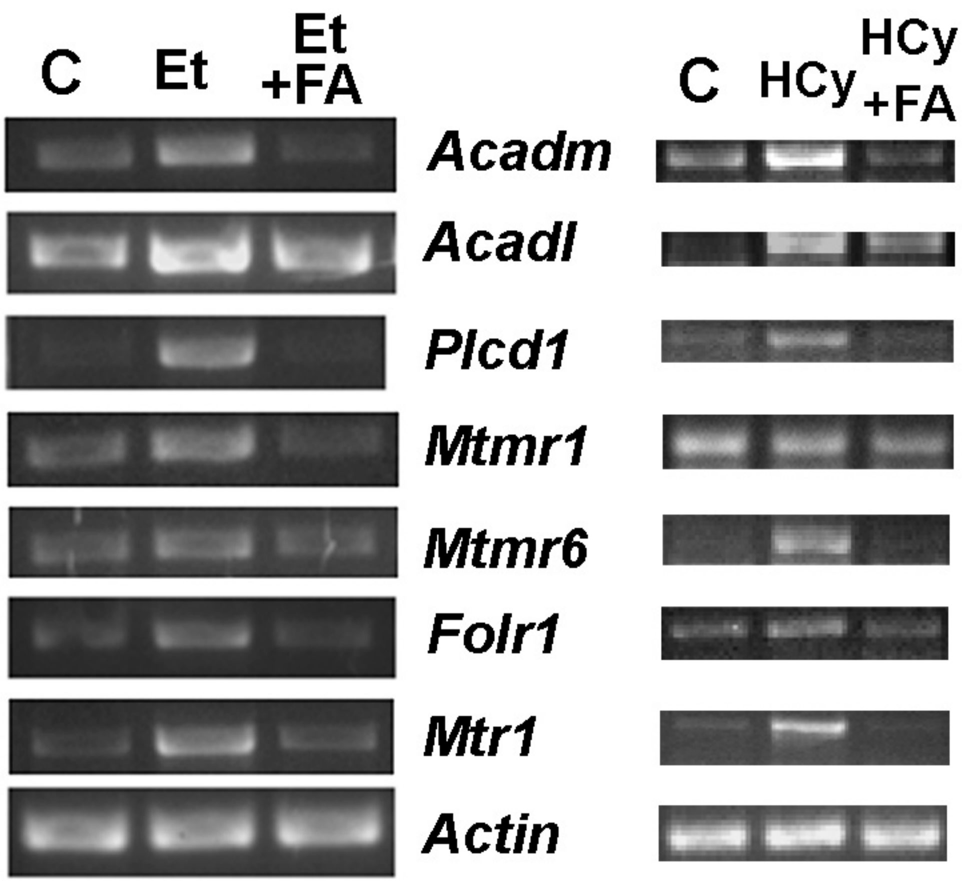


Methylated Lipids, Proteins, and DNA
SAM
Methylation Cycle
Phosphatidyl-inositol signaling
Wnt/β-catenin signaling
Plcd1
Mtr1
Homocysteine
Methionine
Mtmr1
Mtmr6
Fat and Lipid Synthesis (Cholesterol)
MTHF
THF
Nucleic Acid Synthesis
Acadm
Acadl
Glycine
Acetyl-CoA
CoA
Thr
Folr1
Folic Acid
Ethanol

# Alcohol alters gene expression similarly to that of elevated HCy exposure: RT-PCR Analyses of the ED 15.5 mouse heart

**Alcohol Exposure**

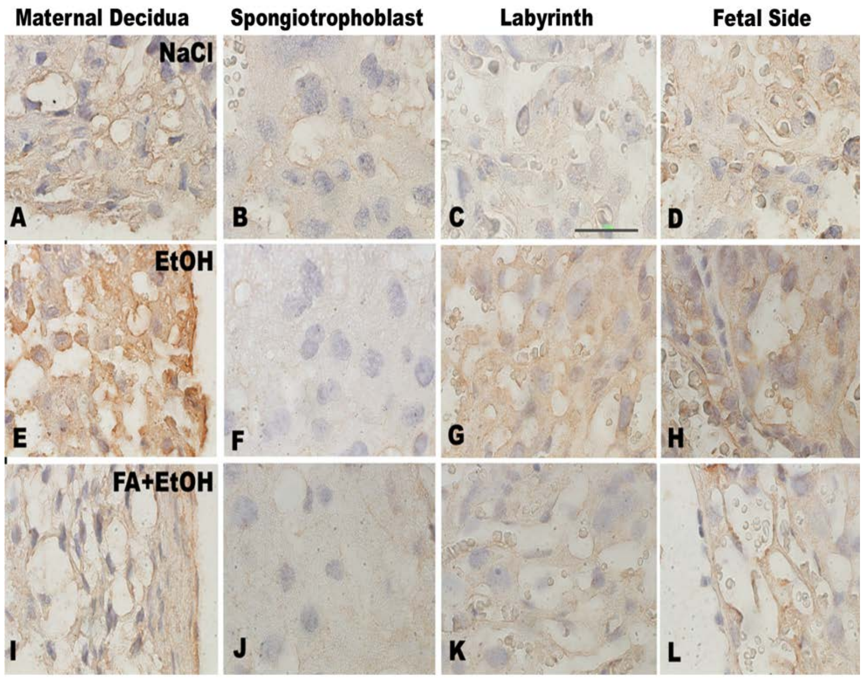
**Homocysteine Exposure**

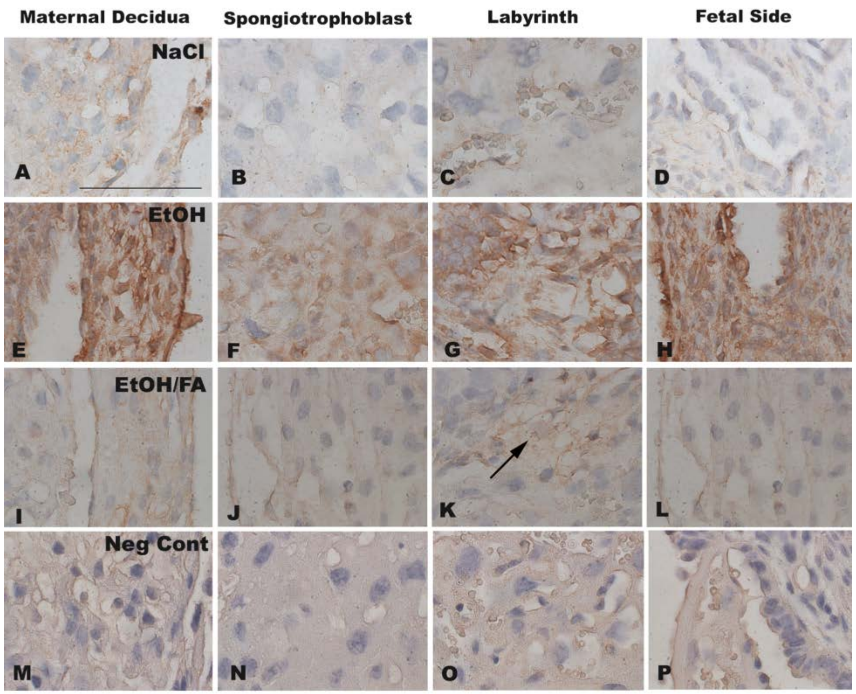



Importantly, our RT-PCR data analyzing the alcohol-exposed fetal heart indicate the fatty acid metabolism- and folate-related genes are **upregulated *within the embryonic and fetal heart tissues***, *seemingly to overcome folate metabolism-related deficiency.*

**Folate protects gene expression by maintaining expression close to control levels.**

# Heart-Placenta Axis: IUGR and Ethanol Effects




**NMHC-IIA** has a unique, but necessary role in placentation. Function is unknown. If knocked out, embryonic lethality occurs.

**NMHC-IIB** appears to be involved in cell motility. If knocked out, embryos develop, but die from cardiac defects at mid-gestation.

**Han, et al., 2012. Am J Obstet Gynecol 207, 140 e147-119.**

# Changes in Fatty Acid Metabolism *within the Embryo*: An emerging common theme in...

- **Alcohol abuse**
- **Diabetes**
- **Obesity**
- **Lithium exposure**
- **Elevated homocysteine (folate deficiency)**
- **...and they all relate to a higher risk of CHD**

**Genes involved in fatty acid metabolism can be epigenetically regulated by specific methylation patterns within promoter and intragenic regions.**

**These patterns can be transmitted from one generation to another.**

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