Placental formic acid and folic acid after alcohol exposure: two sides to the story

Fetal Alcohol Canadian Expertise (FACE) Research Roundtable

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September 14, 2010

Canadian Foundation on Fetal Alcohol Research

Background: More Than Just Ethanol

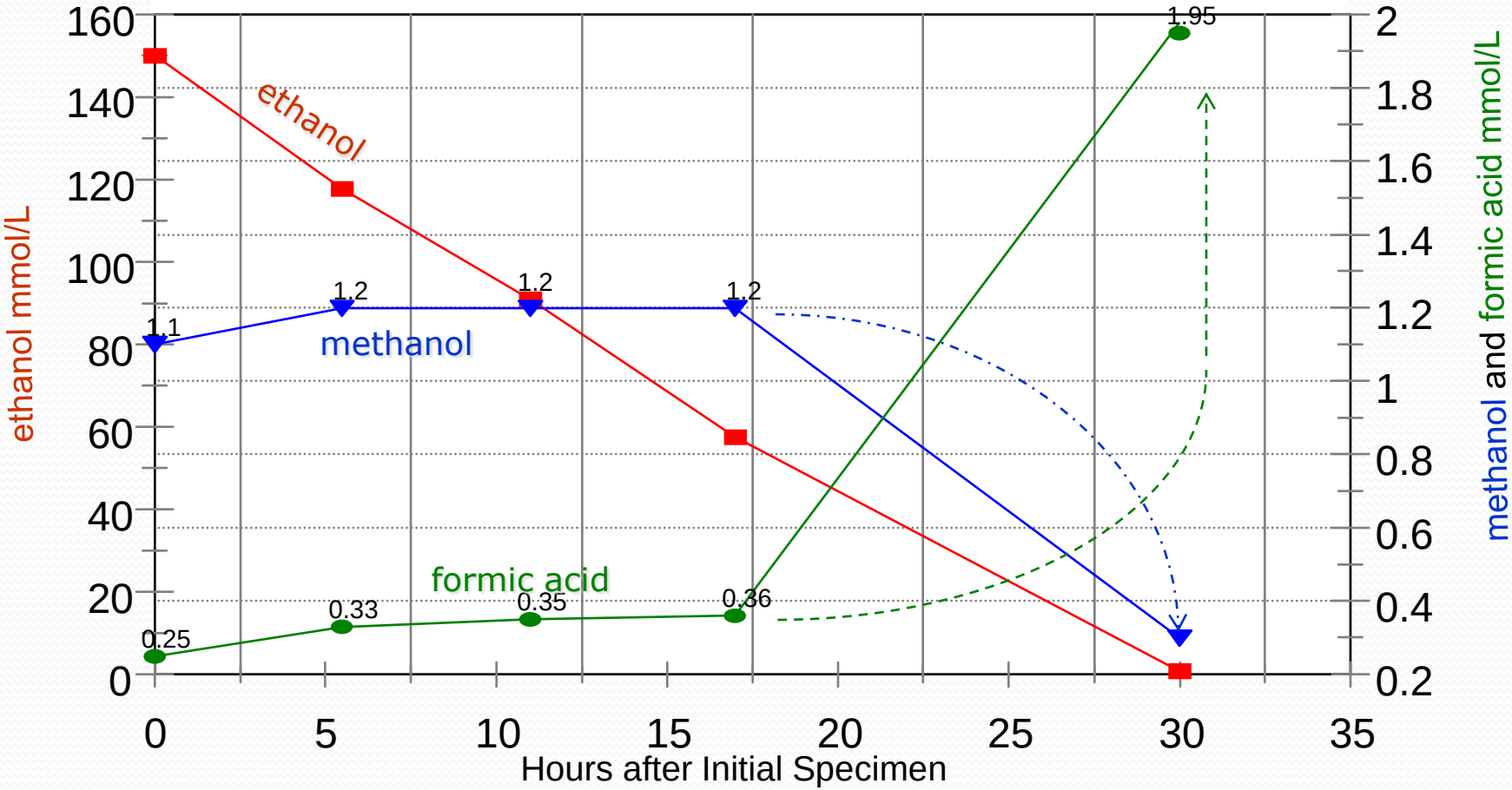
- Methanol is found in alcoholic beverages and also formed endogenously[1,2]
- Methanol is metabolized to formic acid
- Formic acid detected in heavy drinkers[2]

	mg/L		mg/L
Beer	4-50	Rum	6-70
White wine	15-45	Scotch whisky	100-130
Red wine	70-130	Irish whisky	10-110
Cognac	180-370	US-Whisky	200-330
Calvados	310-640	Cornwine	5-100
Kirsh	1900-2500	Aquavit	5-650
Plum	3000-4500	Gin	10-1350
Slivovitz	1500-4000	Vodka	5-170
Liqour	10-560	Bitter	10-340

[1] Sprung et al. *Wiener Klinische Wochenschrift* 1988;100:282-8.

[2] Kapur et al. *Alcohol Clin Exp Res* 2007;31:2114-20.

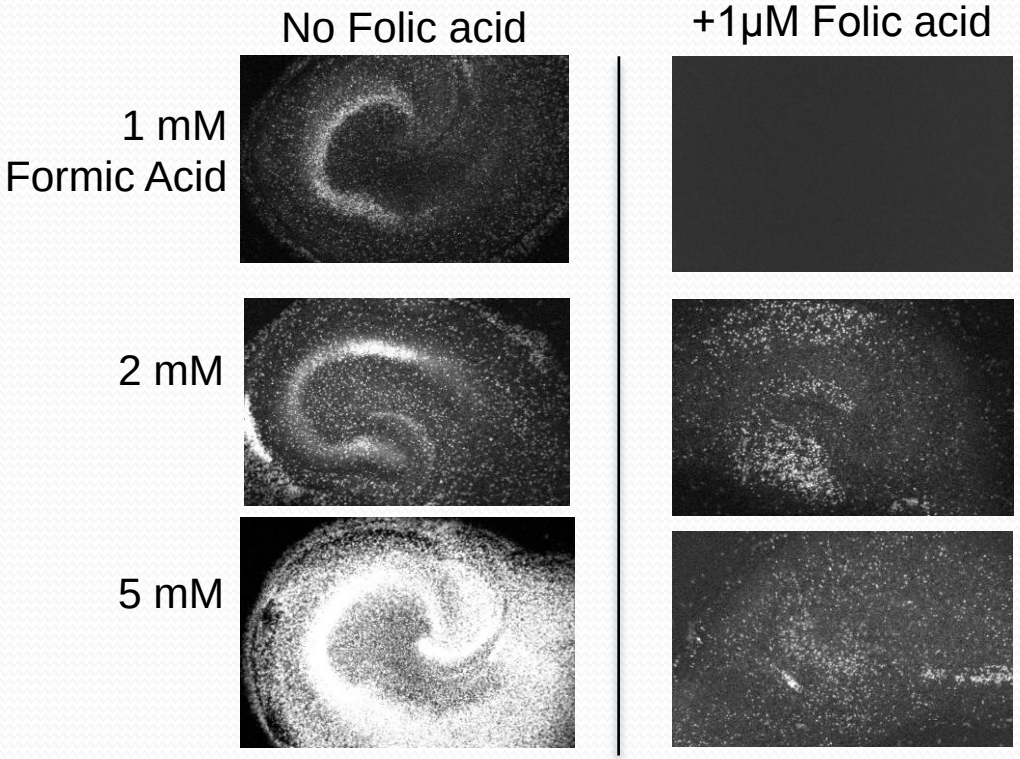
EtOH, MeOH and Formic Acid Profile



Kapur et al. (2007) Alcohol Clin Exp Res 31:2114-20.

Background: Formic Acid in FASD?

- **In alcohol-exposed pregnancies:[2]**
  - **Formic acid measured in maternal and cord blood**
  - **Formic acid & cognitive function negatively correlated**
- **Animal and *in vitro* studies suggest that placental folic acid transport may be compromised**



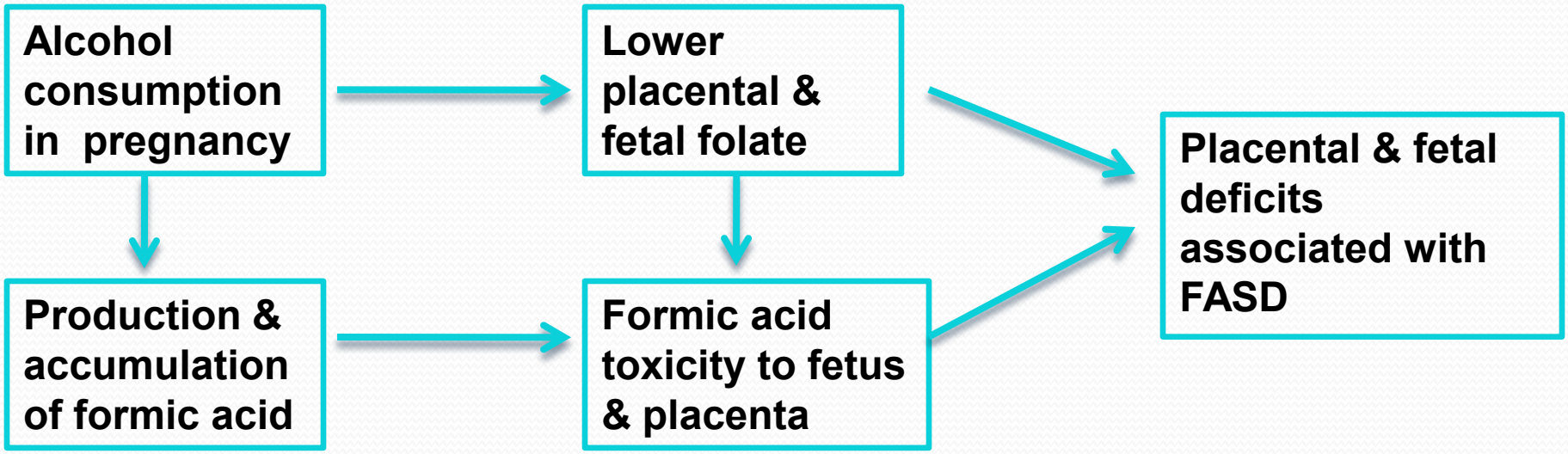
Propidium iodide staining (dead cells stain white)[1]

[1]Kapur et al. *Alcohol Clin Exp Res* 2007;31:2114-20. [2]Kapur et.al. *Alcohol Clin Exp Res 2009;*33:134A.

Background: Folic acid

- **Folic acid needed for proper development**
- **Lower maternal folate associated with hyperactivity and peer problems** ***(Schlotz, 2009)***
- **More severe abnormalities in folate deficient pregnancies after alcohol exposure** ***(Lin, 1991; Gutierrez et al., 2007)***
- **Lower folate levels in fetal brain after prenatal alcohol exposure** ***(Lin et al., 1992)***
- **Alcohol consumption during pregnancy creates oxidative stress to both the placenta and fetus and this can be mitigated by folic acid** ***(Gundogan, 2010; Cano, 2001)***

Hypotheses



Objectives

- To determine if folate transport to the human fetus is altered in pregnancies with chronic alcohol exposure
- To determine if formic acid produced by the mother crosses the placenta and if this transfer can be mitigated by folic acid.
- To determine if formic acid is toxic to the placenta and if this toxicity can be mitigated by folic acid.

1 - Methods

- Subjects part of a larger study led by Drs. Bhushan Kapur and Brenda Stade and supported by a CIHR-NET grant

- Serum folate was measured in maternal & cord blood at from alcohol-abusing mothers (daily consumption or >8 drinks/week, n=23) and controls (n=8).

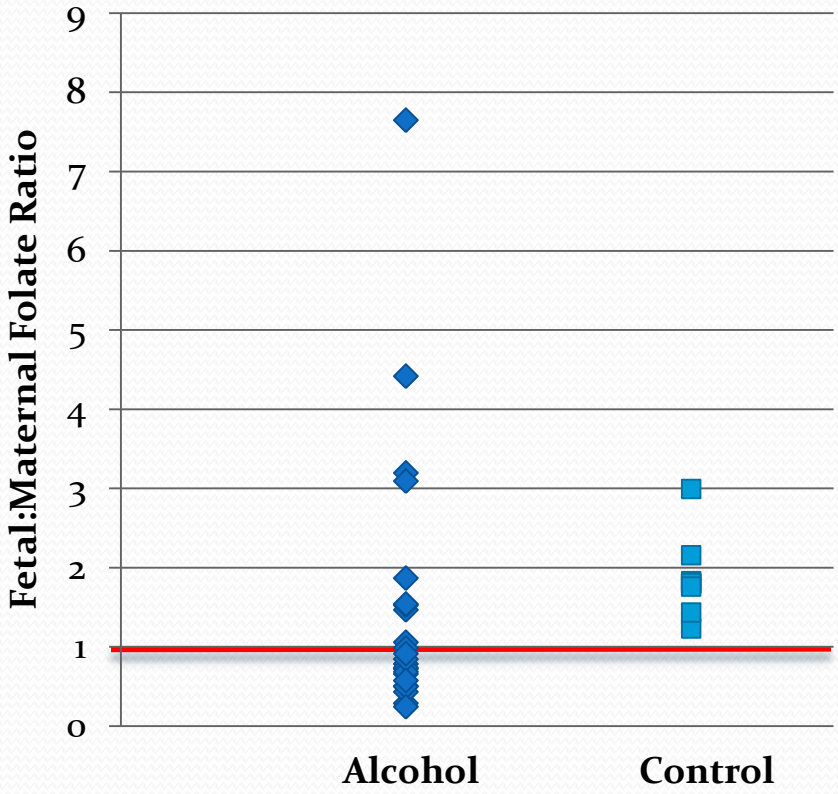
- In the alcohol group,
  - Cigarette use was common
  - Cocaine, THC, and opiate use also reported

1 - Results

Parameter	Median (Range)
Maternal Age	29 (16-44) years
Gravidity	3 (1-10)

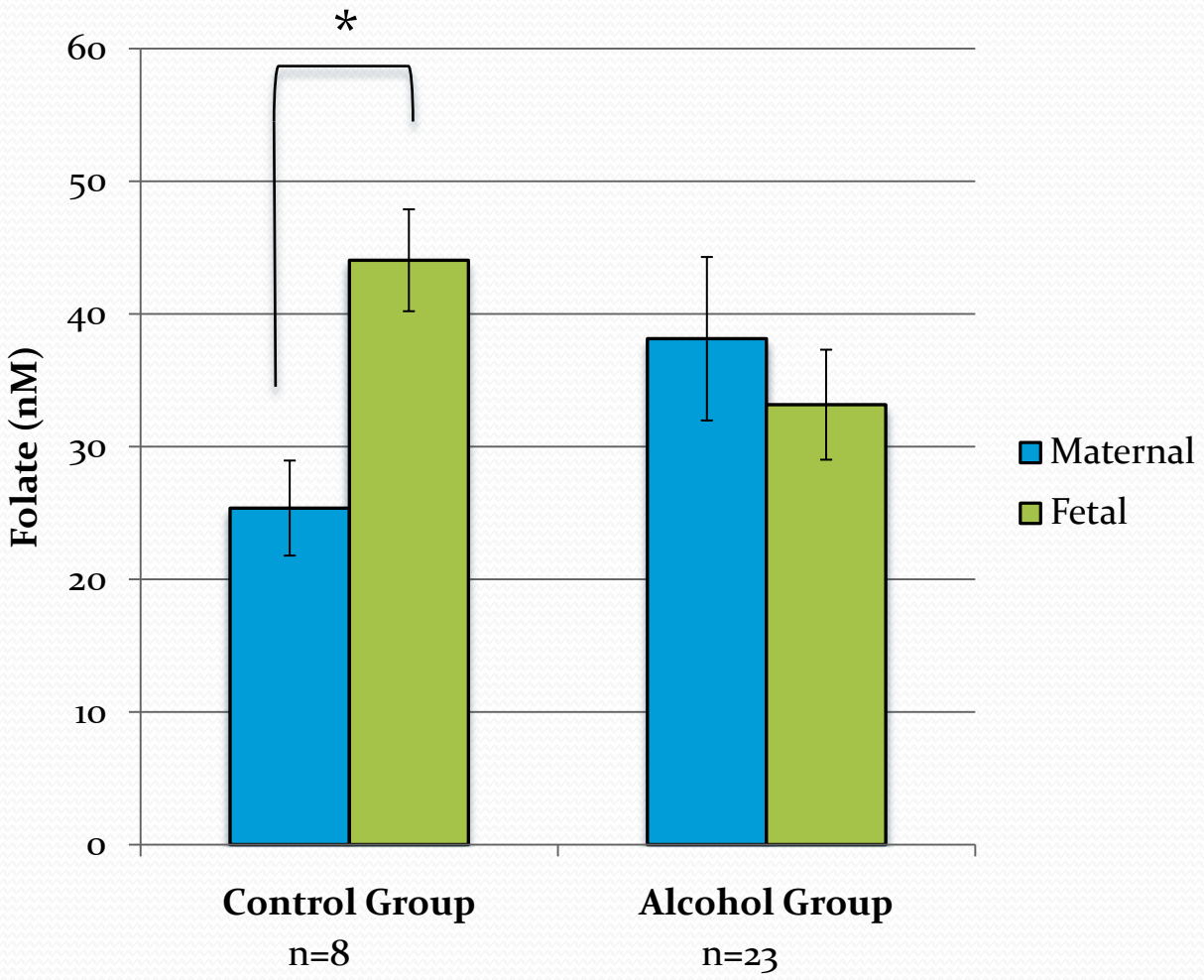
Parameter	Mean (SD)
Gestational Age	37.5 (2.6) weeks
Fetal Length	49.7 (4.2) cm
Fetal Head Circumference	33.3 (2.0) cm
Fetal Birth Weight	3039 (623) g

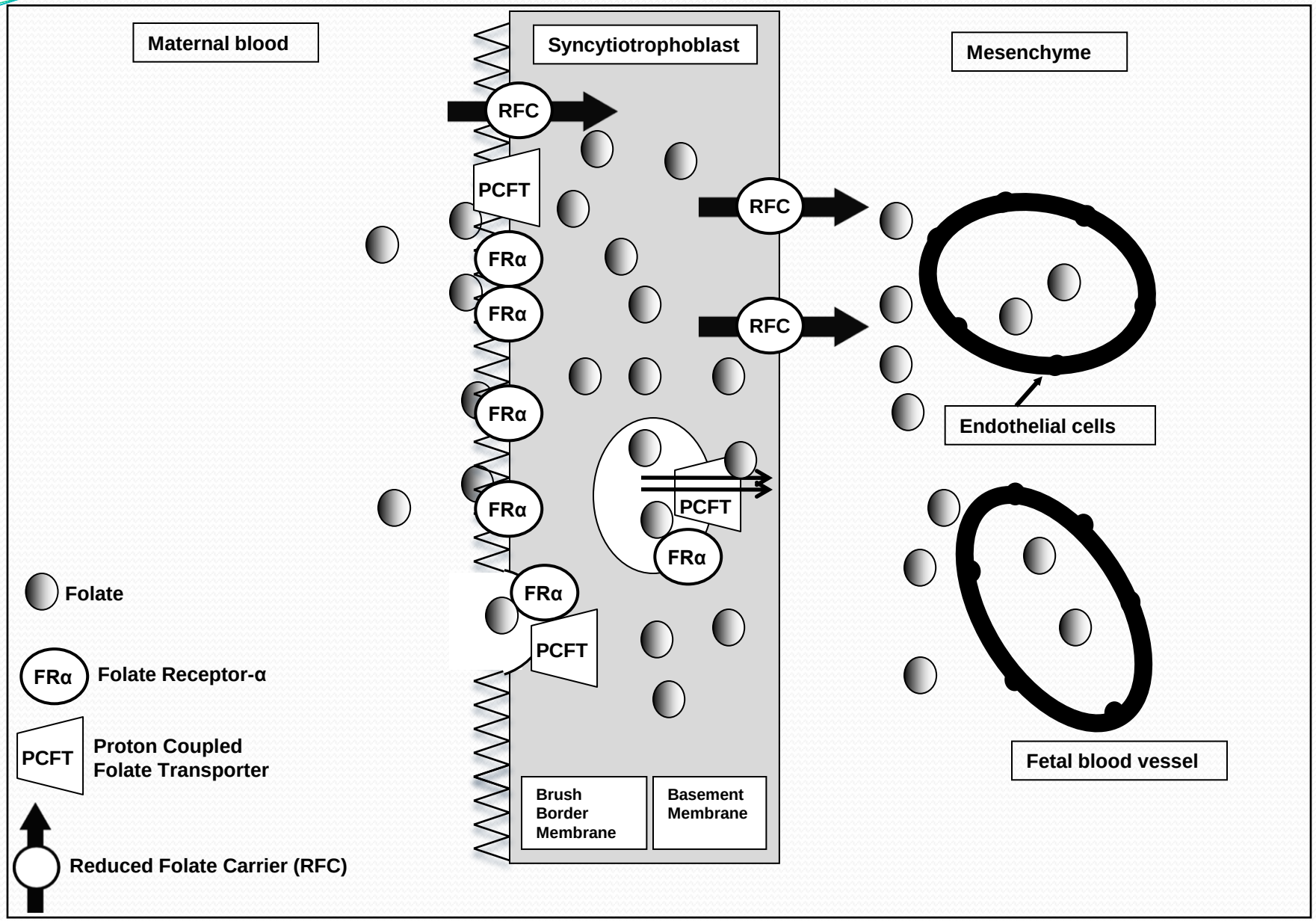
1 - Results



F:M Folate ratio significantly lower in the alcohol group (*p<0.05), suggesting altered transfer/regulation by the placenta

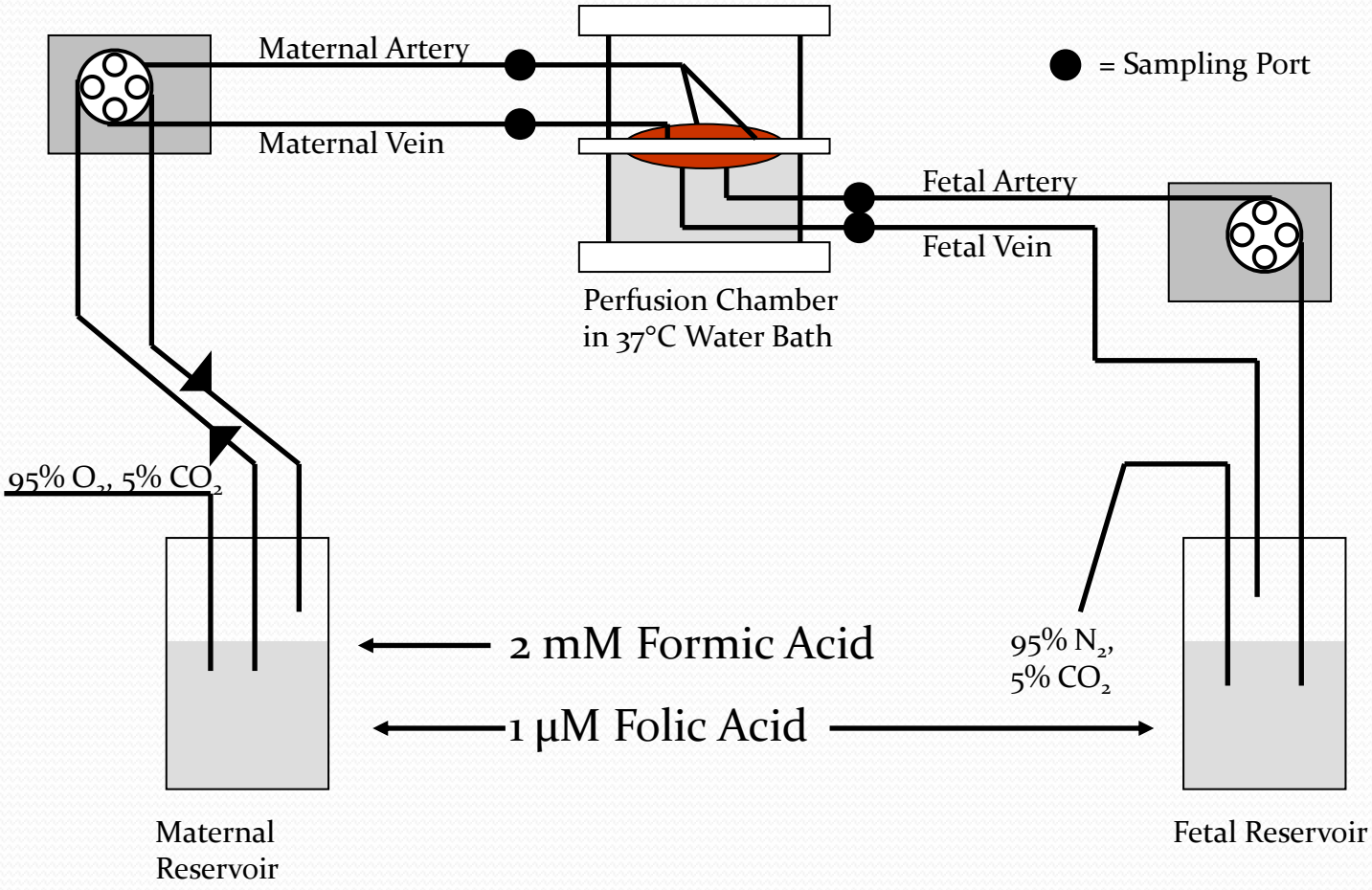
1 - Results

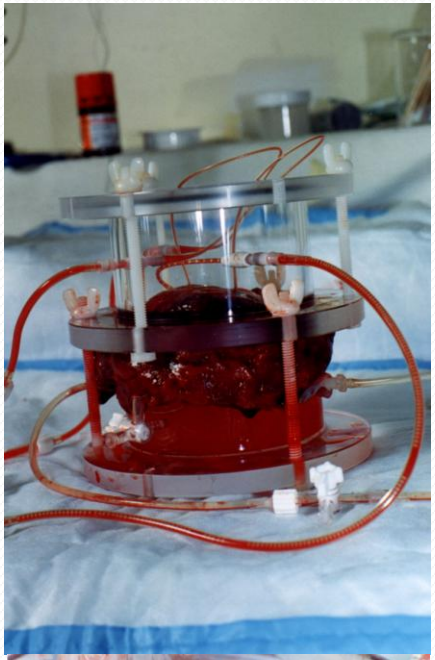

*
60
50
40
30
20
10
0
Folate (nM)
Maternal
Fetal
Control Group
n=8
Alcohol Group
n=23


Maternal blood
Syncytiotrophoblast
Mesenchyme
RFC
PCFT
FRα
FRα
FRα
FRα
FRα
FRα
PCFT
RFC
RFC
PCFT
FRα
PCFT
Endothelial cells
Fetal blood vessel
Brush Border Membrane
Basement Membrane
Folate
FRα
Folate Receptor-α
PCFT
Proton Coupled Folate Transporter
Reduced Folate Carrier (RFC)

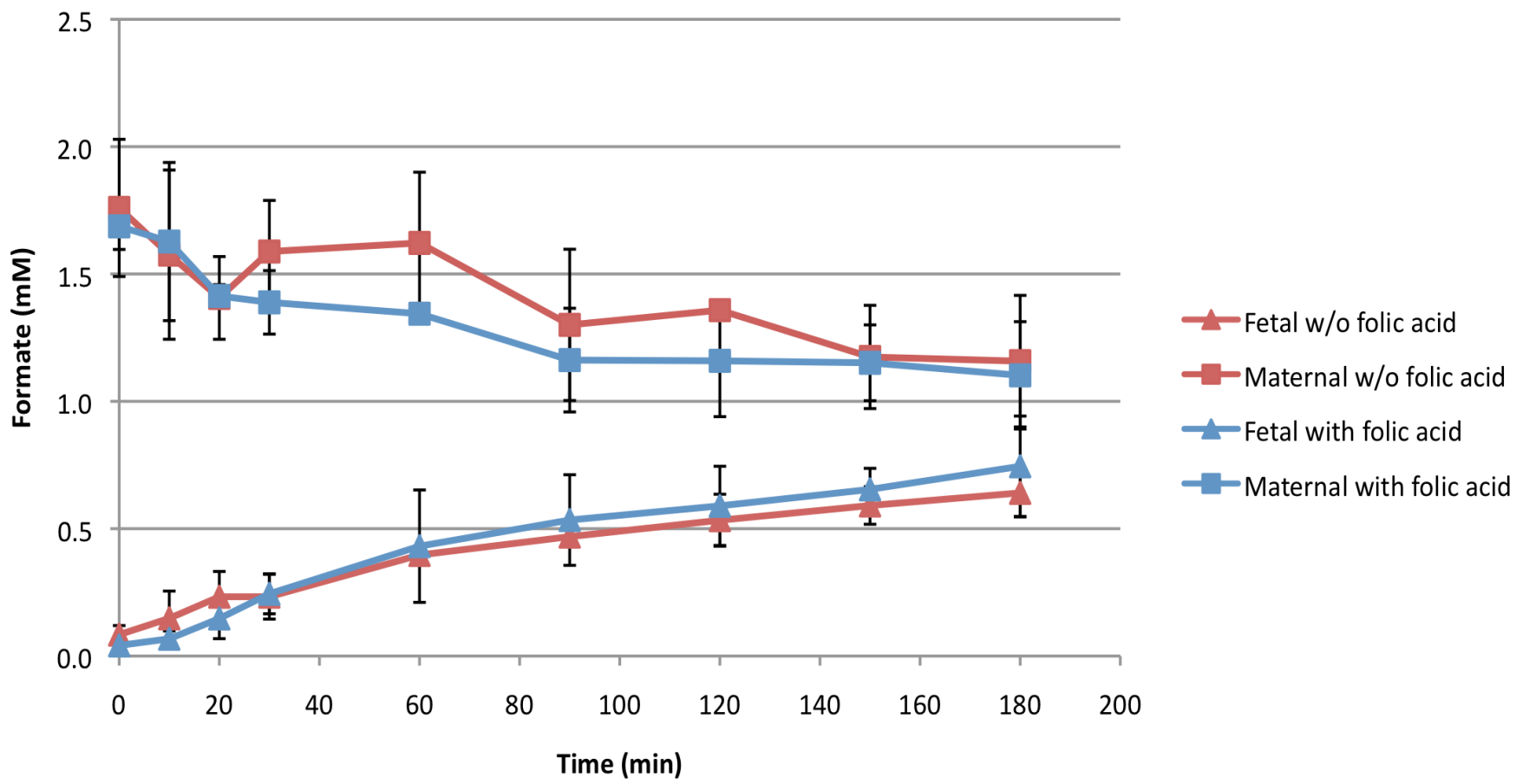
2 & 3 - Methods

- **Dual perfusion of a single lobule from a term placenta**

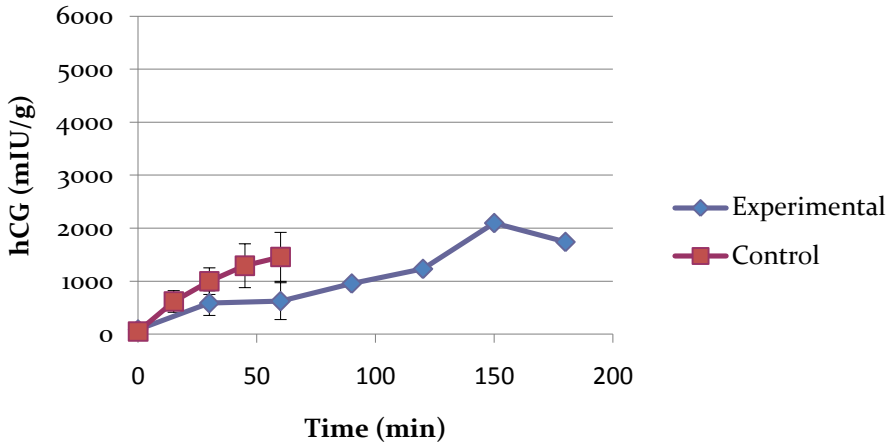

Maternal Artery
Maternal Vein
= Sampling Port
Fetal Artery
Fetal Vein
Perfusion Chamber
in 37°C Water Bath
95% O2, 5% CO2
2 mM Formic Acid
1 µM Folic Acid
95% N2,
5% CO2
Maternal
Reservoir
Fetal Reservoir



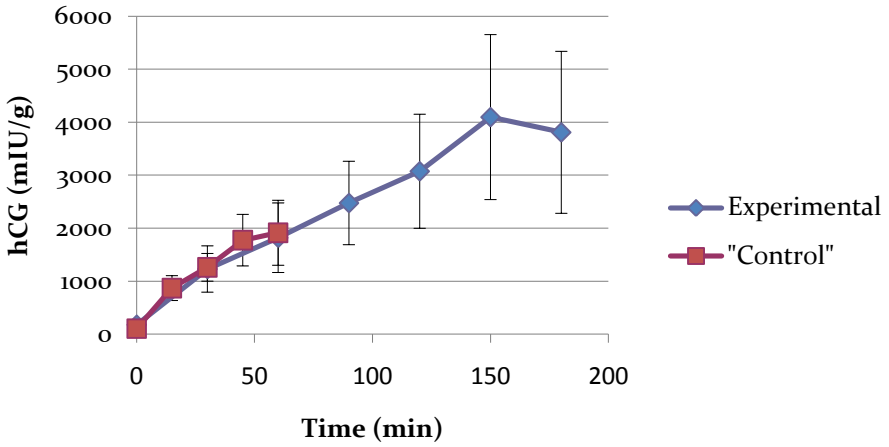
2 & 3 - Results


2.5
2.0
1.5
1.0
0.5
0.0
Formate (mM)
0
20
40
60
80
100
120
140
160
180
200
Time (min)
Fetal w/o folic acid
Maternal w/o folic acid
Fetal with folic acid
Maternal with folic acid

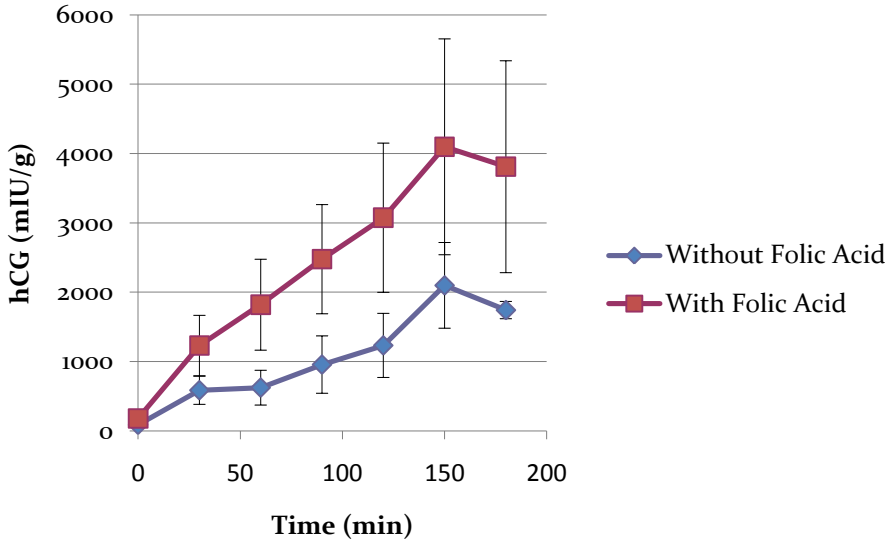
Without Folic Acid



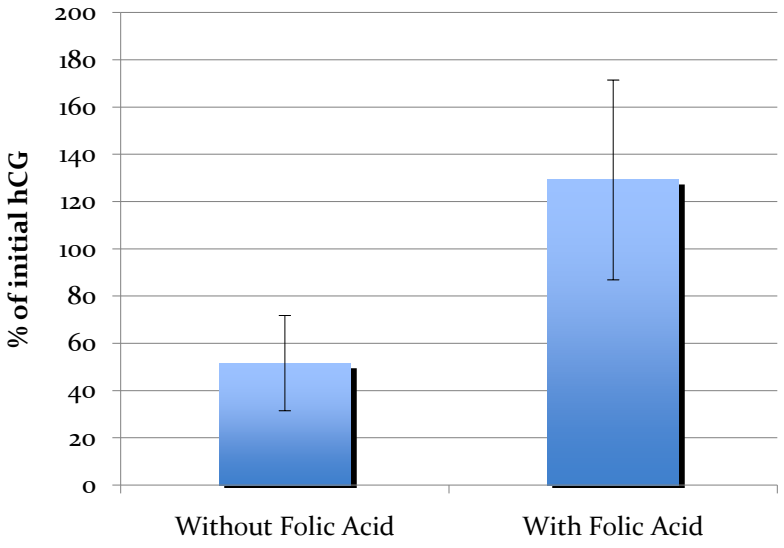
With Folic Acid



Experimental Period



Tissue hCG



2 & 3 - Results

- **Formic acid rapidly crosses the placenta (within 10 minutes) in perfusions**

- **Preliminary data suggest the placental secretion and tissue levels of hCG are decreased after addition of formic acid and that this decrease is mitigated by folic acid**

Case: F:M ratio of folate = 0.24, Cord formic acid = 0.2 mM

Conclusions

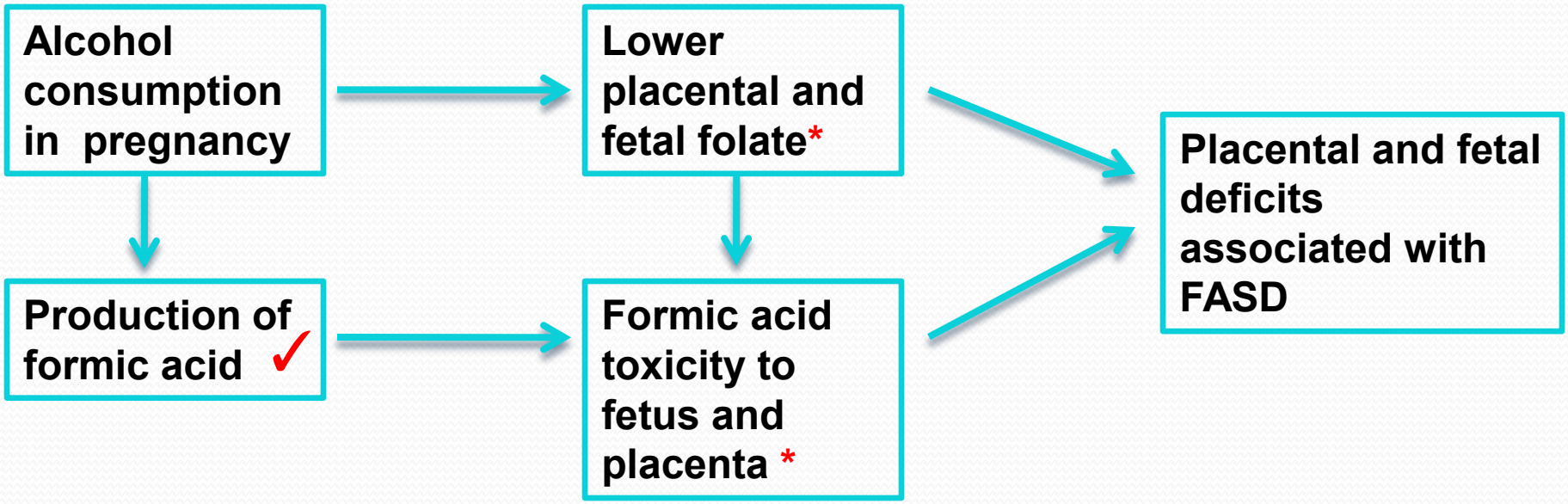
- We show, *for the first time*, placental transfer of folic acid is decreased in human pregnancies with chronic and heavy alcohol exposure
- Maternal-derived formic acid can rapidly cross the placenta and may reduce hCG secretion

Significance

- Together, formic acid and the decrease in folic acid may contribute to deficits associated with FASD

- Possible pathways for fetotoxic effects
  - Formic acid leads to oxidative stress
  - Folic acid needed for DNA synthesis and cell proliferation
  - Folic acid is an antioxidant and is also important for DNA methylation

Back to our hypotheses…



* Studies underway to better characterize the placental and fetal toxicity of formic acid and to determine the molecular mechanisms for the decreased folate transport across the placenta

Can we simply give more folic acid?

- Wang et al. 2009
  - 1mM folic acid (1mM) in mouse whole embryo culture blocked alcohol-induced defects
- Xu et al., 2008
  - 60 mg/kg intragastrically mitigated alcohol-induced microcephaly in mice
- Yanaguita et al., 2008
  - 2 mg/kg diet normalized fetal weight and length in mice after alcohol exposure
- Garcia-Rodriguez et al, 2003
  - 8mg/kg diet - normalized protein expression in rats after alcohol exposure
- Cano et al., 2001
  - 152 µg/day in rats decreased oxidative stress markers in fetal tissues after alcohol exposure
- Wentzel & Eriksson, 2008
  - 10 mg/kg sc folic acid decreased fetal resorption and malformations in rats after alcohol exposure

But for now…

- **Clinical practice should continue to council women for proper folic acid supplementation (5mg/d for at-risk women)**

Acknowledgements

Dr. Bhushan Kapur
Dr. Brenda Stade
Dr. Denis Lehotay
Daniel Bennett
Sofia Tran

Dr. Gideon Koren
Angelika Lubetsky
Jeff Eichhorst
Rinat Hackmon
Asnat Walfisch

Funding

Canadian Foundation on Fetal Alcohol Research

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada

Vanier Canada Graduate Scholarships

McLaughlin Centre for Molecular Medicine

VELUT ARBOR ÆVO