FASD: Folic acid and Formic Acid - An unholy alliance in the alcohol abusing mother

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Alcohol consumption during pregnancy remains a significant cause of preventable birth defects and developmental disabilities, however, the mechanism of toxicity remains unclear.

Methanol is present as a congener in many alcoholic beverages and is formed endogenously. Since ethanol is preferentially metabolized over methanol, it has been found in the sera and CSF of alcoholics. Toxicity resulting from methanol has been attributed to formic acid. Formic acid is present in significantly higher quantities in biofluids of alcoholic patients. These higher levels can be cytotoxic and cause neuronal cell death. However, the adverse effects can be mitigated by adequate levels of hepatic folic acid, since formic acid elimination depends on folic acid.

During pregnancy, folate concentrations are at least two-fold higher in cord blood than in maternal blood due to increased folate requirements. The reverse has been demonstrated in alcohol abusing pregnancies, suggesting downregulation of folate transporters and low fetal folate levels. Moreover, formic acid can cross the placenta and its adverse effects can be mitigated by folic acid. Thus, the combination of low fetal folate levels and presence of formic acid form a potent cytotoxic combination that may play a significant role in the etiology of fetal alcohol spectrum disorder.

Key words: Formic acid, methanol, folic acid, FASD, neurotoxicity,

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Introduction:

Fetal alcohol spectrum disorder (FASD) is an umbrella term used to describe a range of physical, behavioral, and cognitive effects resulting from *in-utero* exposure to alcohol. The most severe form of FASD is fetal alcohol syndrome (FAS), which is characterized by facial dysmorphism, growth restriction, and CNS neurodevelopmental abnormalities (Sokol *et al.* 2003). A wide spectrum of effects has been described, from the milder form known as alcohol-related neurodevelopmental disorder (ARND), to the extreme manifestation known as FAS. It has been demonstrated that both the pattern and timing of alcohol consumption play a significant role in the development of FASD. Alcohol is one of the most commonly consumed teratogens worldwide among women of reproductive age (Nevin *et al.* 2002), and since roughly half of pregnancies are unplanned (Edwards and Werler 2006; Finer and Zolna 2014), alcohol consumption in this cohort poses a significant public health concern. Light-to-moderate drinking in pregnancy is still controversial. Some studies have found that this level of alcohol consumption may lead to adverse neurodevelopmental effects (D'Onofrio *et al.* 2007; Flak *et al.* 2014b), while other studies have established no such effects (Falgreen Eriksen *et al.* 2012; Skogerbo *et al.* 2012; Underbjerg *et al.* 2012). Due to the demonstrated potential for harm to the fetus, most health organizations advocate abstinence from alcohol throughout pregnancy (Centers for Disease Control and Prevention 2005; Public Health Agency of Canada 2012). Although the total volume of alcohol intake is an important variable, the number of binge drinking episodes may have a greater effect on the risk for adverse effects (Maier and West 2001). Given that the etiology of FASD is not fully understood and that there is significant

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heterogeneity in terms of fetal effects associated with any amount of alcohol consumption, no “safe” level of alcohol intake during gestation has been established (Centers for Disease Control and Prevention 2005; Charness *et al.* 2016; Flak *et al.* 2014a; Sowell *et al.* 2014)

Axelrod and Daly demonstrated the endogenous formation of methanol (MeOH) from S-adenosylmethionine (SAM) in the pituitary glands of humans and various other mammals (Axelrod and Daly 1965b). In an elegant experiment, Majchrowicz and Mendelson (Majchrowicz and Mendelson 1971a) illustrated the rise in MeOH levels among subjects drinking MeOH-free alcohol, which corroborated the finding by Ericksen and Kulkarni that MeOH can be produced endogenously (Eriksen and Kulkarni 1963). Most alcoholic beverages also have a small amount of MeOH as a congener (Roine *et al.* 1989a; Sprung *et al.* 1988a). Both ethanol (EtOH) and MeOH are metabolized by the same enzymes, alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (ALDH), with the final products being acetic acid and formic acid, respectively.

Since EtOH has a higher affinity for ADH than MeOH, it is preferentially metabolized (Mani *et al.* 1970). As a result, MeOH accumulation from endogenously produced MeOH and/or MeOH consumed as part of an alcoholic beverage has been reported in concentrations of up to 2 mmol/L (Majchrowicz and Mendelson 1971b) and confirmed by others (Iffland and Staak 1990; Jones and Lowinger 1988; Kapur *et al.* 2007; Roine *et al.* 1989; Sprung *et al.* 1988). Altered pharmacokinetic behavior of MeOH in the presence of EtOH has been demonstrated by various authors (Lesch *et al.* 1990; Martensson *et al.* 1988). The presence of MeOH has also been

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suggested as a potential marker for chronic alcohol abuse (Iffland and Staak 1990; Roine et al. 1989)

Neurotoxicity resulting from MeOH consumption is extensively documented in both humans and animals, and has been attributed to the formation of formic acid from the metabolism of MeOH by ADH and ALDH (Jacobsen and McMartin 1986; Roe 1955; Wood and Buller 1904). Formic acid is eliminated through the one-carbon pathway with folic acid acting as a coenzyme. The rate of formic acid (formate) oxidation and elimination is dependent on adequate levels of hepatic folic acid, particularly hepatic tetrahydrofolate (THF) (Johlin *et al.* 1987; Tephly 1991). Indeed, to prevent metabolic acidosis in MeOH poisonings, folic acid is currently part of standard of care to drive formic acid oxidation into carbon dioxide and water. It has been demonstrated that significantly higher formate levels are produced when folate-deficient animals are exposed to MeOH, as compared to folate-sufficient animals (Lee *et al.* 1994; McMartin *et al.* 1975; Noker *et al.* 1980). Sokoro and colleagues determined that the half-life of formic acid was 40 minutes in folate-sufficient minipigs compared to 120 minutes in folate-deficient minipigs (Sokoro *et al.* 2008).

Our group demonstrated the presence of formic acid in the sera of alcoholic patients (Kapur *et al.* 2007), which was significantly higher ($p < 0.001$) compared to non-alcohol controls. In an experiment using rat brain slice cultures, we further showed that formic acid concentrations achieved in alcoholic patients' sera can be cytotoxic and can cause neuronal cell death. Formic acid cytotoxicity was shown to be both time- and dose- dependent and it was

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found that neuronal cell death could be mitigated by folic acid.

There is an abundance of literature implicating acetaldehyde in the pathogenesis of alcohol-related disorders, but to our knowledge there is no literature that has questioned the role of formic acid in chronically alcohol abusing individuals. To better understand the relationship between EtOH toxicity and FASD, it is imperative to consider MeOH and its metabolite, formic acid, as potential contributors to the teratogenic effects associated with EtOH. Accumulation of MeOH suggests higher-than-normal levels of formic acid in alcohol-drinking populations. When folate levels are low, elimination of formic acid is slower and its levels are elevated (Sokoro et al. 2008; Noker *et al.* 1980). Women who are folate-deficient and consume alcohol may have higher levels of formic acid. Since animal studies suggest that formic acid may be toxic to the developing fetus (Brown-Woodman *et al.* 1995), should these women become pregnant, their fetuses are at greater risk. In this paper, we review the role of formic acid and folic acid in the pathogenesis of FASD.

Formate Metabolism:

Two non-free radical pathways have been proposed for the conversion of formate to carbon dioxide: oxidation through the catalase-peroxidative system (Chance 1950), and via the one-carbon pool. Formate enters the one-carbon pool by combining with THF to form 10-formyl-THF, a reaction catalyzed by formyl-THF synthetase (Johlin et al. 1987). This is followed by oxidation of 10-formyl-THF to carbon dioxide, mediated by formyl THF dehydrogenase (10-

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FTHFDH) (Figure 2).

Studies have shown that this is the major route of formate metabolism (Chiao and Stokstad 1977;Johlin *et al.* 1987;Makar and Tephly 1976;Palese and Tephly 1975) and the predominant one in primates (McMartin *et al.* 1977). Folic acid plays a major role as a coenzyme in one-carbon metabolism and is a key participant in the biosynthesis of DNA, RNA, and certain amino acids. Formate oxidation to carbon dioxide is dependent upon folic acid in rats, monkeys (McMartin *et al.* 1977;Noker *et al.* 1980), and humans (Johlin *et al.* 1989). Although the liver is the main source for folate, Neymeyer *et al.* showed the presence of folate and 10-FTHFDH in the retina, optic nerve, and various regions of the rat brain (Neymeyer *et al.* 1997;Neymeyer and Tephly 1994). In the brain, folate levels were found to be present at concentrations between 3% and 14% of those found in the liver. The presence of folate and 10-FTHFDH in the brain suggests that formic acid can be metabolized in this tissue.

Formate can cause oxidative stress by producing free radicals through the Fenton-like reaction (Dikalova *et al.* 2001;Walling 1975). In this reaction, a hydroxyl radical ($\cdot\text{OH}$) is formed, which in turn oxidizes formate (HCO_2^-), forming the carbon dioxide anion radical ($\cdot\text{CO}_2^-$). The $\cdot\text{CO}_2^-$ radical then reacts with molecular oxygen, forming carbon dioxide and the cytotoxic reactive oxygen species (ROS) $\cdot\text{O}_2^-$ superoxide radical.

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Experiments by Chance have shown that formate can be metabolized by the catalase-
peroxidative system (Chance 1950). When anti-oxidants are depleted, increased ROS are
formed (Treichel *et al.* 2004). Formic acid-induced cell damage has been attributed to the
generation of the cytotoxic ROS species. Formic acid disrupts mitochondrial electron transport
and energy production by inhibiting cytochrome oxidase activity (Nicholls 1975; Nicholls
1976; Sharpe *et al.* 1982), and causes cell death through the increased production of cytotoxic
ROS, secondary to the blockade of the electron transport chain.

The rate of formate oxidation and elimination is dependent on adequate levels of hepatic
folic acid, particularly hepatic THF (Johlin *et al.* 1987; Tephly and McMartin 1974; Tephly 1991).
Thus, significantly higher formate levels are achieved when the folate-deficient animals are
exposed to MeOH (Dorman *et al.* 1994; Lee *et al.* 1994; McMartin *et al.* 1975; Noker *et al.* 1980).
Tephly has suggested that people with inadequate folate metabolism would be prone to the
MeOH toxicity, since they would have difficulty metabolizing one-carbon units (Tephly 1991).

While there is a large body of literature relating maternal folic acid deficiency to fetal
neural tube defects and mitochondrial dysfunctions, there are no references relating low dose
formic acid to cytotoxicity, and only a few references relating formic acid to mitochondrial
inhibition. The latter has been shown in the context of MeOH intoxication and retinal
dysfunctions (Seme *et al.* 1999; Seme *et al.* 2001). The toxic effects of formate have also been
demonstrated in dissociated primary mouse neural cell cultures (Dorman *et al.* 1993). The
concentration of formate that resulted in 50% LDH leakage after 8-hour incubation was

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estimated to be 45 mM. The total intracellular ATP concentration was significantly reduced following exposure to either 20 mM or 40 mM of formate for 8 hours, and is consistent with the hypothesis that formate may inhibit mitochondrial function, resulting in decreased intracellular ATP and neurotoxicity.

Folic Acid and Alcohol (EtOH):

Human beings are fully dependent on dietary sources or supplements for their folate supply. Folic acid is an essential coenzyme in normal purine, thymidylate, and methionine synthesis, and in many other biochemical reactions involving single-carbon transfers. Chronic alcohol ingestion has been shown to reduce the intestinal absorption of dietary folic acid leading to a decrease in the folate metabolic pool (Halsted *et al.* 2002b). As a result, folate deficiency is a common finding in chronic alcoholics (Axelrod and Daly 1965a; Eells *et al.* 2000a; Halsted *et al.* 2002b; Halsted *et al.* 2010). This malabsorption has been attributed to the downregulation of folate transporters (Halsted 1980a; Halsted *et al.* 2002b; Hamid *et al.* 2007b; Hamid and Kaur 2007a; Thakur and Kaur 2015b). Folate deficiency can lead to a decrease in SAM levels (Miller *et al.* 1994). The overall status of the one-carbon pathway is also dependent on the levels of methionine and vitamin B6 and B12 (Bailey and Gregory 1999; Barak *et al.* 1991; Barber *et al.* 1999; Halsted *et al.* 2002a; Lucock 2000; McPartlin *et al.* 1993). A decrease in the folate pool leads to prolonged formate blood levels, since the rate at which formate combines with THF is reduced, which is the first step in its metabolism to carbon dioxide (McMartin *et al.* 1977). This ultimately leads to formate-mediated cytotoxicity.

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During pregnancy, folate requirements increase, as it is needed for DNA synthesis and cell division. Fetal folate levels (cord blood levels) are 2- to 4- fold higher relative to maternal blood levels (Economides *et al.* 1992; Guerra-Shinohara *et al.* 2002; Stark *et al.* 2007). Folate deficiency during pregnancy is associated with abnormalities in the fetus (Greenberg *et al.* 2011; Safi *et al.* 2012). The extent to which folic acid is transferred across the placenta and into the fetal circulation is mediated by placental folate transporters folate receptor- α at the microvillous membrane of the syncytiotrophoblast (Bisseling *et al.* 2004; Solanky *et al.* 2010), the reduced folate carrier, and the proton-coupled folate transporter (Prasad *et al.* 1995; Yasuda *et al.* 2008b; Yasuda *et al.* 2008a). Hutson and colleagues measured folate in both maternal and umbilical cord blood at the time of delivery in pregnancies with chronic and heavy alcohol exposure and in non-drinking controls. They found that among alcohol-exposed pairs, the fetal to maternal folate ratio was reversed with maternal levels being higher (Hutson *et al.* 2012). This suggests that chronic and heavy alcohol use in pregnancy impairs folate transport across the placental wall. Folate transporters in the alcohol using/abusing pregnant woman must be downregulated at the placental level. The mechanism is perhaps similar to the downregulation of folate transporters in the context of intestinal absorption of dietary folic acid (Halsted 1980b; Halsted *et al.* 2002b; Hamid *et al.* 2007a; Hamid and Kaur 2007b; Thakur and Kaur 2015a).

It is well established that ethanol can readily cross the placenta, and acetaldehyde can be been detected in fetal tissues (Karl *et al.* 1988). Interestingly, our group found formic acid in the CSF of three of four alcoholic patients, and in all four of the corresponding serum samples

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(Kapur et al. 2007). The presence of formic acid in the CSF suggests that either it crossed the blood-brain barrier or is formed in-situ from the metabolism of water-soluble MeOH that crossed the blood-brain barrier. Thus, it is conceivable that formic acid has the physicochemical properties to cross the placental barrier. Using a human placental perfusion model, Hutson and colleagues showed that formic acid does indeed cross the placenta(Hutson *et al.* 2013) leading to decreased hCG secretion. A decrease in hCG suggests that formic acid may alter steroidogenesis and differentiation of cytotrophoblasts, and has the potential to be toxic to the developing fetus. The authors also showed that this adverse effect is mitigated by folic acid when it was added to their perfusion model (Hutson et al. 2013). Thus, altered folate concentrations within the placenta and the fetus, combined with increased formic acid, may in part contribute to the deficits observed in FASD. We hypothesize that low folate and high formate contributes to the development of alcohol related brain damage, and that the neuronal damage is caused by oxidative stress and mitochondrial dysfunction.

Ethanol and acetaldehyde can be metabolized in the brain, since the activities of ethanol-metabolizing enzymes – catalase, CYP2E1, ADH, and ALDH – are shown to be present in this tissue (Brzezinski *et al.* 1999;Kapoor *et al.* 2006;Roberto *et al.* 2006;Sun and Sun 2001;Upadhyia *et al.* 2000;Vasiliou *et al.* 2006;Yadav *et al.* 2006;Zimatkin *et al.* 2006). However, placental CYP2E1 is inducible and its activity has been demonstrated to be variable among heavy drinking women (Rasheed *et al.* 1997). Using an animal model, Yadav and colleagues showed that CYP2E1 activity is present in different regions of the brain (Yadav et al. 2006). Vasiliou and colleagues suggest in the conclusion of their animal work that “*although the*

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249 *contribution and CYP2E1 and catalase in ethanol oxidation may be of little significance, these*
 250 *enzymes appear to play a significant role in ethanol metabolism in the brain”*(Vasiliou et
 251 al.2006). Using brain tissues from human abortuses, Brzezinski and co-workers found CYP2E1
 252 activity in various regions of the developing fetal brain (Brzezinski et al.1999). They stated that
 253 *“relatively low levels of the P-450 isoform present in conceptual brain may be sufficient to*
 254 *generate reactive intermediates that elicit neuroembryotoxicity following maternal alcohol*
 255 *consumption”*. Thus, in the context of alcohol consumption in pregnancy, both formic acid and
 256 CYP2E1 activity can mediate toxicity in the developing fetus.

258 In 1985, Stromland followed 30 children of nine mothers who had used alcohol or drugs
 259 during pregnancy and found defects of the outer eye region and/or intraocular abnormalities in
 260 90% of these children (Stromland 1985;Stromland 1987). In a later publication Stromland and
 261 Sundelin,(Stromland and Sundelin 1996) described the abnormalities of these same children
 262 and found that the children born to mothers who abstained from alcohol during some of their
 263 pregnancies were born without birth defects. Anomalies of the outer eye region, like ptosis and
 264 strabismus, were frequently found in children with FAS, but were not common in controls.
 265 Malformations of the fundus were the most frequently occurring abnormalities in the children
 266 with FAS. Forty-eight percent of children showed hypoplasia of the optic nerve head and 49%
 267 had abnormal tortuosity of the retinal arteries.

269 Visual impairment is one of the most common ophthalmic abnormalities found in
 270 children who have been identified as having FAS (Hug *et al.* 2000;Pinazo-Duran *et al.*

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1993;Stromland and Pinazo-Duran 2002). The severity of toxic lesions varies among children and can be correlated to the degree of alcohol abuse during pregnancy (Eells *et al.* 2000b;Pinazo-Duran *et al.* 1997;Stromland and Pinazo-Duran 1994;Stromland and Sundelin 1996). Formic acid is known to be toxic to the optic nerve. It has been suggested that formate inhibits retinal mitochondrial function and increases oxidative stress (Seme *et al.* 2001). Although formic acid and its association with visual impairment has been reported in both animal and human studies, to our knowledge, this association has not been made in children with visual impairment born to mothers who used alcohol during pregnancy.

Brown-Woodman *et al.* (Brown-Woodman *et al.* 1995) assessed the embryonic toxicity of formic acid in an *in-vitro* explanted rat embryo cultured in rat serum and showed that formic acid can be toxic to the embryo. Eells developed a non-primate (rat) model to study MeOH-induced visual toxicity (Eells 1991). The author stated, “*relatively brief exposures to formate appear to produce reversible retinotoxic actions detectable by electroretinography (ERG). Furthermore these ERG alterations occur at formate levels lower than those required to affect flash-evoked cortical potential and lower than those associated with retinal and optic disc edema in humans and monkeys.*” There are numerous animal studies that report on similar ophthalmologic abnormalities following MeOH administration (Ashwell and Zhang 1994;Eells *et al.* 1996;Murray *et al.* 1991;Pinazo-Duran *et al.* 1993).

Studies on non-rodent *Xenopus* (frogs) (Yelin *et al.* 2007) and zebrafish (Marrs *et al.* 2010) models show that ethanol exposure during early development causes a decrease in the

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expression of eye marker morphogenetic genes *Pax6* and *Tbx3*, which are involved in the formation of the eye (Kozmik 2005; Li *et al.* 1997). A reduction in the expression of these genes could be the result of overexpression of sonic hedgehog (*shh*) signaling pathway (Ahlgren *et al.* 2002; Yelin *et al.* 2007). To elucidate the mechanism, Kashyap and colleagues (Kashyap *et al.* 2011) designed a rescue experiment to target both sonic hedgehog (*shh*) and retinoic acid (RA) signaling pathways that may be affected by ethanol. Ethanol is suggested to act as a competitive inhibitor of the enzyme retinol dehydrogenase, which converts retinol (Vitamin A) to RA, a morphogen for vertebrate limb and nervous system morphogenesis (Duester *et al.* 1991). Their studies show that ethanol-mediated defects were independent of these pathways suggesting alternate pathways (Ahlgren *et al.* 2002; Yelin *et al.* 2007). Perhaps the effects of ethanol on these pathways are both tissue and stage specific (Kiecker 2016). In the zebrafish model, Muralidharan and colleagues (Muralidharan *et al.* 2015), studied ethanol-mediated retinal tissue defects and folic acid's potential for rescue. They showed that folic acid co-supplementation with ethanol significantly rescued optic nerve and photoreceptor defects.

Human visual functions are also impaired with chronic low-level exposure to MeOH (Frederick *et al.* 1984). Toxicity to the visual system produced by either acute, subacute, or chronic MeOH exposure share a common mechanism, which is most likely mediated by formic acid (Eells *et al.* 1996; Murray *et al.* 1991). Formic acid toxicity is likely determined by folic acid and 10-formyltetrahydrofolate dehydrogenase concentrations, which have been shown to be present in both human and rat retinas (Martinasevic *et al.* 1996).

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The pattern and timing of maternal alcohol consumption play a significant role in the etiology of FASD. Although the total volume of alcohol intake is an important variable, the number of binge drinking episodes, four or more drinks in about two hours for women, may have a greater impact on the risk of adverse effects (Maier and West 2001). Binge drinking leads to high blood EtOH concentration and perhaps even higher levels of formic acid to which the fetus is exposed. Similarly, chronic drinking likely leads to sustained levels of formic acid in the fetus. Since brain development is continuous during the gestational period, chronic or episodic high-level exposure to formic acid may be a significant contributing factor to the development of FASD (Figure 3).

Several studies have shown that there is enzymatic activity in the brain, which can metabolize both EtOH and acetaldehyde (Brzezinski et al. 1999; Kapoor et al. 2006; Roberto et al. 2006; Sun and Sun 2001; Upadhyay et al. 2000; Vasiliou et al. 2006; Yadav et al. 2006; Zimatkin et al. 2006). Yadav et al. measured NDMA-*d* activity in the presence and absence of alcohol (Yadav et al. 2006). They showed a regional difference in CYP2E1-dependent NDMA-*d* activity that was inducible when there was pre-treatment with EtOH. Significant increase in enzymatic activity was observed in the olfactory lobe, mid brain, hippocampus, hypothalamus, and cerebellum. Bell and co-workers reported a high prevalence of epilepsy and seizure history in children with FASD (Bell *et al.* 2010a). Impairments, such as cognitive dysfunctions and seizures are associated with the above mentioned regions of the brain (Matthews and Silvers 2004). Thus, it is conceivable that formic acid that crosses the placental barrier and/or is locally produced from MeOH can significantly impair brain function (Figure 4).

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Other Mechanisms of Alcohol Teratogenesis

A variety of other mechanisms have been proposed to explain the toxic effects of ethanol on the growing fetus. In addition to the link between folate deficiency, oxidative stress, and their respective associations with FASD discussed in this paper, ethanol consumption has also been associated with the augmented production of prostaglandins, which can adversely impact fetal development (Ylikorkala *et al.* 1988). Interestingly, it has been demonstrated that the administration of a prostaglandin synthesis inhibitor in mice prior to alcohol consumption is associated with a 50% reduction in malformations relative to mice that receive the inhibitor subsequent to alcohol intake (Randall and Anton 1984).

Oxidative metabolism – the primary mechanism by which ethanol is eliminated in adults – plays a minor role in the metabolism of ethanol in the fetus, especially earlier in gestation (McCarver and Hines 2002; Smith *et al.* 1971). Conversely, the enzymes involved in the non-oxidative metabolism of ethanol are present early in gestation (Bearer *et al.* 1992; Bearer *et al.* 1995; Krekels *et al.* 2012; Stanley *et al.* 2005). Consequently, the non-oxidative pathways of ethanol metabolism may play a compensatory role in early gestation, in the context of alcohol exposure (Zelner and Koren 2013). Fatty acid ethyl esters (FAEE) are non-oxidative metabolites formed via the breakdown of ethanol. These metabolites have been established as biomarkers of chronic excessive alcohol use in adults (Auwarter *et al.* 2001; Pragst *et al.* 2001) and in utero exposure to alcohol (Bearer *et al.* 1992; Bearer *et al.* 1999; Caprara *et al.* 2005; Hungund and

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Gokhale 1994;Klein *et al.* 1999). FAEE have also been established as mediators of toxicity, since they have been demonstrated to reduce cell proliferation, uncouple oxidative phosphorylation, increase the fragility of lysosomes, decrease protein synthesis, increase the formation of lipid droplets, and incorporate into organic bilayers leading to their disordering (Best and Laposata 2003;Pragst *et al.* 2001). FAEE have been shown to accumulate in tissues susceptible to the toxic effects of ethanol, such as the liver, heart, brain, and adipose tissue (Laposata and Lange 1986). The presence of FAEE is associated with liver, pancreatic, myocardial, mitochondrial, and CNS damage (Bora *et al.* 1996;Gubitosi-Klug and Gross 1996;Haber *et al.* 1993;Hungund *et al.* 1988;Lange and Sobel 1983;Ponnappa *et al.* 1994;Szczepiorkowski *et al.* 1995;Werner *et al.* 1997). Elevated levels of FAEE in the meconium of ethanol-exposed neonates have been linked to deficits in various CNS domains, such as executive functioning and cognition (Min *et al.* 2015;Noland *et al.* 2003;Peterson *et al.* 2008).

Ethanol has also been associated with the disruption of the endocrine system (Haley *et al.* 2006), impairment of neurogenesis (Cartwright and Smith 1995;Heaton *et al.* 2002), a rise in cellular apoptosis (Cartwright and Smith 1995), and alterations in gene expression (Vangipuram *et al.* 2008). There are a number of risks factors associated with development of FASD, including but not limited to improper nutrition, deficient prenatal care, higher maternal age, paternal drinking, history of substance abuse, having a previous child with FASD, and low education level and socioeconomic status (Esper and Furtado 2014;May and Gossage 2011). Genetic factors may also play a role in the development of FASD (Ramsay 2010). Genetic variants that lead to altered ethanol metabolism can function to modulate the risk of adverse effects (Chernoff 1980). For

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instance, variants at the *ADH1B* (*ADH1B*2* and *ADH1B*3*) in the mother or child are associated with more efficient ethanol metabolism, which in turn has been associated with a mild protective effect relative to those that do not possess these variants (Arfsten *et al.* 2004; Jacobson *et al.* 2006). Ultimately, it is likely that the interplay of various proposed mechanisms give rise to FASD (Cohen-Kerem and Koren 2003).

Conclusions

Studies show that methanol and formic acid levels are elevated in human subjects consuming alcoholic beverages. Alcoholics have low folate levels due to reduced absorption, and following alcohol consumption, formic acid levels rise and can be neurotoxic. FASD is associated with significant neurological problems such as behavioural, cognitive and motor impairment (Chokroborty-Hoque *et al.* 2014) depression (Pei *et al.* 2011), and epileptic seizures (Bell *et al.* 2010b; Nicita *et al.* 2014). Women who consume alcohol during pregnancy may also have low folic acid and higher formic acid levels that can be teratogenic to the fetus. (Iffland and Staak 1990; Jones and Lowinger 1988; Kapur *et al.* 2007; Roine *et al.* 1989). The role of folate in the pathogenesis of neural tube defects (Kim 1999) and cardiovascular disease (Stolzenberg-Solomon *et al.* 1999) is well established. Moreover, epidemiological studies have shown that folate supplements can significantly reduce the risk of pancreatic cancer (Fenech 2001) and risk for cardiovascular diseases (Jiang *et al.* 2003). Folate requirements increase during gestation and folate deficiency during pregnancy is associated with abnormalities in the fetus. Alcohol downregulates folate transport across the placenta resulting in low fetal folate levels. Sustained

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levels of formic acid during chronic drinking, and perhaps even higher formate levels during binge drinking, may explain why binge drinking is particularly harmful to the fetus (Figure 3). This supports our hypothesis that low folic acid and high formic acid levels are a toxic combination in pregnant women who drink.

Pregnant women who consume alcohol and are folate-deficient may accumulate formic acid and produce a high-risk constellation for the fetus. We hypothesize that FASD and associated neuronal damage occurs through formic acid-induced oxidative stress and mitochondrial dysfunction. Folic acid and formic acid play a significant part in the etiology of the very complex syndrome that is FASD.

To our knowledge there is no published literature linking folic acid deficiency and formic acid to FASD, although numerous studies suggest alcohol-mediated oxidative stress as the possible mechanism (Brocardo *et al.* 2011; Dreosti 1993; Gupta *et al.* 2016; Muralidharan *et al.* 2015; Sarmah *et al.* 2016; Sogut *et al.* 2017). Thus, treatment using nutrients that have anti-oxidant properties such as folic acid appear promising.

Our hypothesis raises a number of questions that need to be addressed including: what could be considered clinically relevant concentrations of folic acid and formic acid that cause teratogenicity? What duration of formic acid exposure will result in fetal cytotoxicity? What role might formaldehyde and ethanol-induced folic acid deficiency be on the neurodevelopmental process? Studies in genomic, genetic, and epigenetic mechanism may help in elucidating the

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molecular mechanisms of this very complex disorder. It is clear that further research is required to answer these and other questions.

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Titles

Figure 1: Methanol metabolism

Figure 2: Metabolism of formate by folate-dependent pathway

Figure 3: FASD – Gestation continuum

Figure 4: Implications of different studi

Draft

Figure 1 Methanol metabolism
152x16mm (300 x 300 DPI)

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Figure 2: Metabolism of formate by folate-dependent pathway

139x139mm (300 x 300 DPI)

Figure 3: FASD – Gestation continuum

403x79mm (96 x 96 DPI)

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**Formic acid is produced locally in the fetal brain,
and/or it crosses the placenta from mother to fetus**

**Increased formic acid concentration will
lead to formation of lesions at the local site**

**Lesions can have significant functional
impact in these brain tissues/organs**

Figure 4: Implications of different studies

156x84mm (300 x 300 DPI)