Alcohol and its effect on fetal development: what do we know?

"...combining fields of miRNAs and epigenetics unlocks a Pandora's box of variations/combinations of events that ethanol can usurp to significantly contribute to the various forms of FASD."

Pediatric HEALTH

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If there is one simple premise linking alcohol to early mammalian development it is that combining the two can have devastating consequences. Labeled a teratogen, alcohol is capable of inducing a broad range of adverse effects on a developing fetus [1]. Specifically ethyl alcohol, when imbibed during pregnancy, can induce a cascade of events that alter cellular behavior, including cell–cell interactions and cell viability. These changes, in turn, disrupt tissue and, eventually, organ function(s). The potential result is a cluster of symptoms collectively known as fetal alcohol spectrum disorder (FASD) or fetal alcohol effects. The research group at the Lucile Packard Children's Hospital at Stanford University (CA, USA) [101] concisely distinguishes FASD into three categories.

Fetal alcohol syndrome

Fetal alcohol syndrome (FAS) Consists of the most severe effects including fetal death. Infants born with FAS have abnormal facial features and growth and CNS problems, including mental deficiencies.

Alcohol-related neurodevelopmental disorder

Children with alcohol-related neurodevelopmental disorder do not have the full range of symptoms constituting FAS; instead they have a spectrum of behavioral and learning problems, which may include mathematical difficulties, impaired memory or attention, impulse control with or without judgment problems, and poor school performance.

Alcohol-related birth defects

Birth defects related to prenatal alcohol exposure include abnormalities in the heart, kidneys, bones and/or hearing.

Interestingly, there has been a recent literary uptick in primary articles and reviews concerning aspects of FASD, many of which have focused on two fronts of research: diagnosing distinct types of FASD and basic research focusing on understanding alcohol-induced perturbations of genotypic events. Arguably, leading the way in research is the CDC, which has been heavily involved in FASD intervention and diagnosis. The CDC has invested substantial multi-year funding in areas where they have identified new FASD factors, such as vulnerable populations and intervention techniques.

A number of other preeminent institutions are also delving into FASD prevention and research, such as the Mayo Clinic (MN, USA), the Universities involved in the Midwest Regional Fetal Alcohol Syndrome Training Center, and the University of Washington (WA, USA) to name a few. Of these, the University of Washington FASD (UW FASD) Center stands out at having made substantial progress especially on the diagnostic front. This FASD Center is funded by the Substance Abuse and Mental Health Service Administration (SAMHSA) and is part of the State Systems Center for Excellence [102].

Researchers at the UW FASD Center created a four-digit diagnostic code, providing a comprehensive method for diagnosing the full spectrum of outcomes for patients, simultaneously enhancing ideas in both clinical and preventative research [2,3]. A brief communiqué I received from D Dubovsky at the UW FASD Center was rather impressive. In essence, the center is in their second year of funding 23 subcontract initiatives that provide prevention or diagnosis and intervention services across state, local and tribal settings [Dubovsky D, Pers. Comm.]. Of the prevention initiatives, six are implementing Project Choices (targeting women at risk of having an alcohol-exposed pregnancy before they become pregnant), seven are implementing Screening and Brief Intervention, and two are implementing the Parent-Child Assistance Program. A further eight programs are implementing diagnosis and intervention services designed to improve the functioning and quality of life of children with

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FASD and their families. SAMHSA touches all 50 states at varying levels of commitment and funding, which is enabling many institutions to make strides in FASD outcomes.

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Until recently, FASD research has focused more on describing the effects of ethanol exposure on fetuses, which, while important, has not resulted in fully elucidating the mechanistic aspects of altered development that leads to congenital defects. Much of the work that has established the link between ethanol and FASD has emanated from a number of different laboratories, most of which have demonstrated in both *in vivo* and *in vitro* models that although ethanol can affect most, if not all, neural cell types, it especially preys upon neural crest (NC) cells. The vulnerability of NC cells is due to their lack of important catalases and other enzymes, such as super oxide dismutase that combat free radicals. Since alcohol produces free oxygen species (e.g., hydrogen peroxide, superoxide and hydroxyl anions) within these cells, their lack of an effective coping mechanism results in a range of adverse effects from impaired migration to cell death. This explains a common phenotype found in FASD patients – facial deformities – given that NC cells make up the majority of facial bones [4].

Advances in FASD basic research have been the subject of three recent reviews [4–6], each of which provides a unique perspective. They not only delineate the development of distinct categories of FASD research, they also provide details explaining the effects of alcohol during development on cell migration, apoptosis, cell growth and differentiation, cell adhesion molecules, epigenetics and miRNA expression [4–6]. The most current research has advanced the field tremendously by uncovering fetal biological markers via DNA arrays and real-time RT-PCR [7,8]. The reports described by Ismail *et al.* [4], disclosing cutting-edge investigations into the effects of ethanol on miRNAs expression and those describing epigenetics [5,6] are some of the most intriguing. In essence, they reveal exciting new avenues of exploration into the underlying biological mechanisms of FASD.

One focus of my laboratory is miRNAs, a précis of which would describe them as 19–21 bases of noncoding RNA sequences that complimentarily bind to mRNA molecules, regulating their translation into protein [9,10]. Remarkably, ethanol has been shown to alter miRNA expression especially in the developing nervous system [11] with at least 15 miRNAs known to be up- or down-regulated in response to physiological concentrations of ethanol. Although miRNA research is still in its infancy (less than 10 years) a basic foundation of how they regulate translation has already been laid. Less understood is the regulation of miRNAs themselves. A few transcription factors, such as c-Myc, have been found to upregulate the transcription of specific miRNAs early in development [12]; however, perhaps equally as interesting is a recently described intersection between miRNAs and epigenetics. miRNA gene promoters have been found to be methylated ten times more than protein-coding genes, leaving them more vulnerable to disturbances in the methylation pathway [13,14]. In essence, combining fields of miRNAs and epigenetics unlocks a Pandora's box of variations/combinations of events that ethanol can usurp to significantly contribute to the various forms of FASD. When these new promoter regulation data are combined with current research demonstrating that miRNAs have multiple mRNA targets, a fascinating and entirely new research frontier for FASD researchers opens up. That said, the amount of work needed to properly decipher what is actually occurring with respect to miRNA expression, epigenetics and FASD may take years to understand; however, clues do abound if one looks outside the FASD research box and focuses on the role of miRNAs during development and epigenetics.

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With respect to FASD research, it is these types of studies that have led us and others to generate the hypothesis that ethanol causes FASD by interfering with epigenetic

programming and thus gene and/or miRNA expression, during development. One problem with this hypothesis is that it will not be easy to fully test; however, variations of the experiments that are necessary are prevalent in the literature. For example, *in vitro* neural developmental analyses, specifically looking at the effects of ethanol on miRNAs during development, could be modeled using the neural differentiation potential of embryonic, induced pluripotent, neural or germ line pluripotent stem cells [15,16]. An initial experiment could encompass identifying which miRNAs are targeted in relevant cell types treated with ethanol, followed by identifying which proteins are or are not translated. If an miRNA is upregulated as a result of ethanol exposure (leading to the downregulation of a target mRNA and its protein), the antisense inhibitor of that miRNA could be transfected into these cells to see if protein levels, and phenotype(s), are rescued. Conversely, if an miRNA is downregulated as a result of ethanol exposure (leading to upregulation of a target mRNA and its protein) the miRNA mimic could be transfected into cells to see if proper protein levels and the normal phenotype is restored. While all the possible permutations may seem daunting, in the end, it is becoming evident that a complete understanding of miRNA expression and epigenetics is vitally important to the future of FASD research.

Presently, there is no cure for FASD, but children who are diagnosed early and receive appropriate physical and educational interventions, especially those in a stable and nurturing home, are more likely to have better outcomes than those who are not [4]. There have been studies attempting to treat FASD *in utero* but these attempts are still at the basic research stage and will need extensive clinical data before they are deemed safe and efficacious for clinical use.

That said, FASD is, for all intents and purposes, completely preventable if the mother is well-versed on drinking and pregnancy and has access to the social and economic resources necessary for education and comprehensive prenatal care. A caveat to this is the fact that many women do not know they are pregnant at precisely the time the fetus is most susceptible to ethanol exposure: the first few weeks of pregnancy. In light of this, research is still critical to not only determine the treatability of FASD but also explicate the mechanistic biology and etiology of ethanol during early development.

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