Perinatal exposure to alcohol: implications for lung development and disease

Danielle Giliberti, MD, PhD, Sowmya S. Mohan, MD, Lou Ann S. Brown, PhD, and Theresa W. Gauthier, MD
Department of Paediatrics Division of Neonatal-Perinatal Medicine Emory University Emory Children's Centre for Developmental Lung Biology 2015 Uppergate Dr. NE Atlanta, GA USA 30322

Summary

In utero alcohol exposure dramatically increases the risk of premature delivery. However, the majority of premature and term newborns exposed to alcohol remain undetected by medical caregivers. There is a desperate need for reliable and accurate biomarkers of alcohol exposure for the term and premature newborn population. The inability to identify the exposed newborn severely limits our understanding of alcohol's pathophysiological effects on developing organs such as the lung. This chapter will review potential advancements in future biomarkers of alcohol exposure for the newborn population. We will discuss alcohol's effects on redox homeostasis and cellular development of the neonatal lung. Finally, we will present the evidence describing in utero alcohol's derangement of innate and adaptive immunity and risk for infectious complications in the lung. Continued investigations into the identification and understanding of the mechanisms of alcohol-induced alterations in the premature lung will advance the care of this vulnerable patient population.

Keywords

Foetal alcohol; pregnancy; reactive oxygen species; immunity; lung; infection

Introduction

Clinical and animal-based investigations continue to address the gap of knowledge regarding the effects of prenatal exposure to alcohol on pathophysiological effects to the vulnerable developing lung. In this chapter, we will identify the need for more accurate biomarkers to assist in the identification of the exposed premature newborn. We will review the growing body of evidence implicating prenatal alcohol's detrimental effects on lung development. Finally, we will discuss the effects of in utero alcohol on the developing immune system resulting in impaired immune defences in the exposed neonatal lung.

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Corresponding Author Phone: 404-727-3360 Fax: 404-727-3236 tgauthi@emory.edu
danielle.giliberti@emory.edu/mohan@emory.edu/lbrow03@emory.edu.

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Conflict of interest statement: The authors acknowledge that they have no financial disclosures to declare.
Alcohol Use during Pregnancy

According to the Centres for Disease Control and Prevention (CDC), prenatal alcohol exposure is the leading preventable cause of birth defects, developmental disabilities, and mental retardation in the USA. In 2002, the prevalence of drinking during pregnancy ranged from 0.5-2 cases/1,000 live births. While women may stop drinking once they know they are pregnant, nearly 50% of pregnancies are unplanned making binge drinking early in gestation a significant problem to the exposed newborns. Alcohol is the most prominent behavioural teratogen in the world and the consequences of this teratogen can be devastating to the developing foetus.

Exposure during pregnancy results in Foetal Alcohol Spectrum Disorders (FASD) that can negatively impact growth, development, cognition, behaviour, and reproductive physiology over the child's entire lifespan. Approximately 1 in 33 pregnant women consume alcohol at levels shown to increase the risk of having a baby with FASD. The majority of affected infants do not have the recognized phenotypic changes at birth. Although the evidence that in utero alcohol exposure is detrimental to the developing foetus is clear, identifying and determining the clinical effects on the newborn relies on maternal self-reporting of alcohol use. In one study, a diagnosis of FASD was missed in 100% of term newborns subsequently diagnosed later in childhood. This failure to recognize the alcohol-exposed newborn means that the detrimental effects of in utero alcohol exposure on the newborn remain poorly understood.

Alarmingly, alcohol use increases the risk of extreme premature delivery over 35 fold, demonstrating that a significant proportion of premature newborns are also alcohol-exposed. Therefore, given the various morbidities associated with prematurity, it is crucial to develop accurate and reliable methods for identifying alcohol-exposed infants. The search for biomarkers of alcohol exposure has become crucial to the early and accurate identification of exposed infants. Multiple standardized questionnaires have attempted to accurately define exposure during pregnancy; however, the uncertainty of maternal admittance to use remains problematic. One of the most reliable direct biological markers of prenatal exposure to alcohol is fatty acid ethyl esters (FAEEs), formed via esterification of alcohol with endogenous free fatty acids. Multiple researchers have evaluated FAEEs in meconium of term newborns as a potential biomarker and studies in premature meconium are ongoing. Other metabolites of alcohol remain under investigation as potential biomarkers including ethyl glucuronide (EtG) and ethyl sulphate (EtS). Correlations between FAEE, EtG, and EtS in the meconium, placenta, and hair of patients with FASD have been demonstrated. Phosphatidylethanol (PEt) holds a similar potential but it has not been validated in the alcohol-exposed newborn. Continued research is necessary to develop reliable and accurate biomarkers to assist in the identification of both full term and premature babies exposed to alcohol in utero.

Effects of Foetal Alcohol Exposure on Lung Development

Foetal alcohol exposure promotes toxicity to developing organs, including the lung. At moderate levels, alcohol is primarily metabolized through alcohol dehydrogenase with class I and class IV alcohol dehydrogenase the primary isoforms in the developing lung. Since each polymorphism is associated with a different enzymatic activity, there will also be variances in alcohol metabolism within the developing lung. Alcohol is also metabolized by mixed function oxidases such cytochrome P450 generating acetaldehyde and other reactive oxygen species. Although its activity is minimal at baseline, chronic alcohol exposure increases cytochrome P450 2E1 (CYP2E1) activity which also generates acetaldehyde but is particularly active in generating reactive oxygen species. CYP2E1 polymorphisms can contribute to variances in acetaldehyde generation and subsequent...
injury. Significant levels of CYP2E1 have been detected between gestational days 45 and 53 as organogenesis begins, increasing the probability that alcohol is metabolized by CYP2E1 in the developing lung. Whether alcohol is metabolized by alcohol dehydrogenase or P450s, the generation of the oxidant acetaldehyde or other reactive oxygen species promotes oxidative stress and antioxidant depletion. With an imbalance between reactive oxygen species and antioxidants, an increased burden of reactive oxygen species can lead to protein oxidation, lipid peroxidation and mutations because of DNA oxidation. With foetal alcohol exposure, there are increased lipid peroxidation products, proteins containing lipid peroxidation adducts, and increased DNA oxidation, demonstrating that reactive oxygen species are generated and that macromolecule damage occurs.

Since developing tissues such as the lung have poorly developed antioxidant systems, they are particularly vulnerable to alcohol-induced oxidant stress and antioxidant depletion. For the lung, oxidant stress can lead to arrested alveolarization and vascular development. Multiple animal models of foetal alcohol exposure have demonstrated alterations in neonatal lung development (Table 1). In rats, foetal alcohol impairs lung development resulting in inhibition of cellular growth and hypoplastic lungs. In mice, foetal alcohol during the second trimester at the pseudo glandular stage of lung development resulted in decreased lung mass and delayed lung maturation. These effects of in utero alcohol were also associated with increased expression of the homeobox-containing gene Hoxb5 which is critical for bronchiolar patterning and airway branching morphogenesis during the saccular phase of development. However, dramatic decreases in Hoxb5 expression are needed for bronchiolo elongation and further lung development. Thus, persistent Hoxb5 expression further supports the concept that foetal alcohol exposure impairs lung development.

In preterm lambs with foetal exposure during the last trimester of pregnancy, there was decreased expression of vascular endothelial growth factor (VEGF) which is critical for angiogenesis, endothelial cell maturation and alveolar formation. There was also decreased gene and protein expression of VEGF receptors (VEGFR-1 and VEGFR-2) as well as hypoxiainducible factors 1α and 2α, two transcription factors that up regulate VEGF gene transcription. In another study of term lambs with alcohol exposure during the last trimester, impaired lung development was observed as evidenced by decreased composition and amount of surfactant. In contrast to rodent models, this study demonstrated that alcohol exposure during the last trimester of pregnancy did not alter prenatal or postnatal lung growth, lung architecture or alveolarization during early postnatal life. The differences in the effects of alcohol on lung development between the rodent and sheep models are unclear. However, these lamb studies with exposure during the last trimester did not demonstrate the characteristic alcohol-induced decreases in foetal body weight. This suggested that these differences between rodent and lamb studies may be related to the developmental stage of alcohol exposure or the amount of alcohol consumed. Since abusive drinking patterns often occur before women know they are pregnant, it is important to determine if impaired lung development and maturation occurs when there is foetal alcohol exposure throughout pregnancy or during the first trimester.

One mechanism for alcohol-induced impairment of lung development may be inhibition of glucose uptake or hormonal factors that regulate growth. In the lung, foetal alcohol exposure decreases the release of insulin-like growth factor II (IGF-II) and further decreases IGF-II bioavailability through increased release of IGF-binding proteins. Another growth factor essential for lung development is vitamin A (retinoic acid), its analogues (retinoids) and corresponding receptors. Foetal alcohol exposure affects the expression and activation of retinoic acid receptors in neuronal cells but whether foetal alcohol exposure has these effects in the developing lung remains to be determined.
In order to understand the impaired lung development observed with foetal alcohol exposure, additional studies on the effects of alcohol on growth factors, corresponding receptors, and transcription factors in the developing lung as well as different cell types that contribute to angiogenesis, endothelial cell maturation and alveolar formation are needed. Whether these events are strictly related to oxidant stress and antioxidant depletion should also be addressed. Finally, foetal alcohol exposure impacts lung development but additional studies are needed to understand the role of the gestational period of exposure and the foetal alcohol blood levels in delayed lung maturation.

**Alcohol and Impaired Pulmonary Immunity**

Alcohol use alters the immune response of the body, increasing the risk and severity of infection in the vulnerable adult population\(^{32}\). Newborns have an increased baseline risk for pneumonia as many normal adult lung defences are compromised in the foetus and newborn including the ciliary escalator, airway macrophages and dendritic cells, secretory antibodies, and antimicrobial proteins and antigens\(^{33}\). It has been estimated that pneumonia contributes to 1 million neonatal deaths annually, accounting for 10% of childhood mortality\(^{34}\).

Infection is a leading cause of death in the early neonatal period with the greatest risk in the smallest infants\(^{35}\). In the US, from 2005-2008 there were an estimated 390 deaths annually due to early onset sepsis, with half in premature infants\(^{36}\). Foetal alcohol exposure has been shown to increase the risk of neonatal sepsis in both the term and premature neonatal populations. Late preterm and term small for gestational age infants whose mothers reported any alcohol use had a 2.5 times increased risk of infection, while excessive alcohol use increased the risk 3–4-fold\(^{37}\). In very low birth weight infants, this effect was more pronounced. Early onset sepsis was 15-fold higher in the alcohol exposed very low birth weight infants\(^{38}\). Pneumonia occurs frequently in neonates with sepsis although it often goes underreported or undetected. Increased incidence of neonatal pneumonia is multifactorial; however, animal models show an increased risk for pneumonia in foetal alcohol exposure\(^{20}\).

Data from a variety of animal models of alcohol exposure show impairment of the innate and adaptive immune responses in the lung (**Table 2**). Surfactant is best known for its role in reducing surface tension and its deficiency underlies the pathophysiology of respiratory distress syndrome in premature newborns; however, this lipoprotein complex also plays an important role in immune function. Of the four major surfactant proteins (SP-A, SP-B, SP-C, and SP-D), the hydrophilic proteins, SP-A and SP-D, play a major role in innate immunity. SP-A and SP-D have been shown to aggregate pathogens preventing their spread, stimulate phagocytosis and killing, and modulate inflammation through pro- and anti-inflammatory actions\(^{39}\). They have direct bactericidal activity\(^{40}\) and link to the adaptive immune system by inhibiting T-cell proliferation via IL-2 independent and dependent mechanisms\(^{41}\).

When exposed to alcohol *in utero*, surfactant protein expression is decreased. Lambs exposed to alcohol during the third trimester demonstrated alterations in the lung microenvironment including decreased SP-A mRNA expression when born prematurely and decreased SP-A expression and reduced ciliary beat frequency when born at term\(^{42}\). Other research has confirmed decreased surfactant protein mRNA levels and suppression of pro-inflammatory cytokines in lambs exposed to alcohol prenatally\(^{27}\).

Prenatal alcohol exposure increases oxidant stress in the alveolar macrophage and decreases the ability of the cells to phagocytize foreign particles *in vitro* and *in vivo*, suggesting that *in utero* ethanol exposure impairs the macrophage via decreased glutathione, an essential antioxidant in the lung\(^{43,44}\). In an adult guinea pig model, alcohol inhibited the terminal
differentiation of the interstitial and alveolar macrophage\(^45\). Further animal models confirmed that foetal alcohol exposure caused exaggerated oxidant stress that impaired terminal differentiation and phagocytic function of the neonatal alveolar macrophage\(^46\).

Foetal alcohol exposure may also affect the adaptive immune system by inhibition of B and T cell function. In an animal model of chronic ethanol abuse, proliferation and antigen-specific CD8\(^+\) T cell function were reduced \textit{in vitro} and \textit{in vivo}\(^47\). Alcohol-exposed dendritic cells also have a decreased number of receptors for inflammatory mediators and therefore lack the input needed to stimulate T cells\(^48\). Whole animal models of influenza virus infection show that chronic alcohol ingestion increases disease morbidity, mortality, enhanced pulmonary lesion severity, and pulmonary virus titres\(^49\). These changes correlate with decreased numbers of flu-antigen specific CD8\(^+\) T cells\(^49\). Similar models have interrogated \textit{in utero} ethanol exposure and its long term effects on response to influenza virus infection. McGill, \textit{et al.} exposed mice to maternal ethanol prenatally and during nursing. After infection with influenza virus, these mice exhibited increased disease severity, and increased and sustained pulmonary viral titres. The lungs demonstrated decreased numbers of pulmonary flu-antigen specific CD8\(^+\) T cells and also a decreased number and size of pulmonary B cell foci resulting in a decreased amount of specific antiviral antibody\(^50\).

Therefore, clinical studies suggest that \textit{in utero} alcohol increases the risk of infection in term and premature newborns, but more investigations are warranted. Animal models demonstrate that \textit{in utero} alcohol exposure deranges both the innate and adaptive arms of immune defences in the developing lung.

**Conclusions**

Although alcohol exposure dramatically increases the risk of extreme premature delivery, accurate identification of the exposed premature newborn remains clinically challenging. Establishing an accurate biomarker panel will allow for the necessary advancement in understanding the mechanisms of \textit{in utero} alcohol’s effects on the developing lung. Although in utero alcohol induces significant alterations in oxidant stress, much research is needed to fully understand alcohol’s effect on development of the lung. In utero alcohol exposure alters multiple arms of the developing immune system and decreases pulmonary defences against both bacterial and viral infection. Continued research is required to fully identify and understand the effects of in utero alcohol on infection risk and infectious-mediated pulmonary morbidities, particularly in the at-risk premature newborn.

**Acknowledgments**

Supported in part by NIAAA 5T32AA013528-10 (DG), 1 F32 AA019880-01 (SSM), 1P50 AA013757 (LAB, TWG), 1R01AA016348 (LAB, TWG), and the Emory Children’s Lung Developmental Biology Centre (LAB, TWG).

**References**


Table 1
EFFECTS OF FOETAL ALCOHOL EXPOSURE ON LUNG DEVELOPMENT

This table identifies the timing and effects of foetal alcohol exposure on neonatal lung development in a variety of animal models.

<table>
<thead>
<tr>
<th>Timing of Ethanol Exposure</th>
<th>Animal Model</th>
<th>Developmental Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Trimesters</td>
<td>Rat</td>
<td>Inhibition of cellular growth Hypoplastic lungs&lt;sup&gt;24&lt;/sup&gt;</td>
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<tr>
<td></td>
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<tr>
<td>Second Trimester</td>
<td>Mouse</td>
<td>Decreased lung mass Delayed lung maturation Persistence of Hoxb5 expression&lt;sup&gt;25&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td>Third Trimester</td>
<td>Preterm Lamb</td>
<td>Decreased VEGF protein &amp; gene expression&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Term Lamb</td>
<td>Decreased surfactant production Altered surfactant content&lt;sup&gt;27&lt;/sup&gt;</td>
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</tbody>
</table>
**TABLE 2**

**ALCOHOL ASSOCIATED IMMUNE DYSFUNCTION**

This table summarizes studies showing alcohol related derangements of the innate and adaptive immune system.

<table>
<thead>
<tr>
<th>Alcohol Exposure</th>
<th>Model</th>
<th>Functional Deficit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foetal (Third Trimester)</td>
<td>Preterm Neonatal Lamb</td>
<td>Decreased SP-A mRNA&lt;sup&gt;27, 42&lt;/sup&gt;</td>
</tr>
<tr>
<td>Foetal (Third Trimester)</td>
<td>Term Neonatal Lamb</td>
<td>Decreased SP-A and ciliary beat frequency&lt;sup&gt;42&lt;/sup&gt;</td>
</tr>
<tr>
<td>Foetal</td>
<td>Preterm &amp; Term Neonatal Guinea Pig</td>
<td>Decreased AM phagocytosis&lt;sup&gt;in vitro and in vivo&lt;/sup&gt;&lt;sup&gt;43, 44&lt;/sup&gt;</td>
</tr>
<tr>
<td>Foetal</td>
<td>Term Neonatal Guinea Pig</td>
<td>Increased risk of GBS pneumonia and sepsis Decreased AM phagocytosis&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic</td>
<td>Adult Guinea Pig</td>
<td>Impaired AM differentiation&lt;sup&gt;45&lt;/sup&gt;</td>
</tr>
<tr>
<td>Foetal</td>
<td>Term Neonatal Mouse</td>
<td>Impaired AM differentiation&lt;sup&gt;46&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic</td>
<td>Adult Mouse</td>
<td>Decreased number of CD8+ T cells and specific Ag presentation&lt;sup&gt;47&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic</td>
<td>Adult Mouse</td>
<td>Decreased receptors for inflammatory mediators on airway dendritic cells&lt;sup&gt;48&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chronic</td>
<td>Adult Mouse</td>
<td>Increased flu severity and viral titres. Decreased number of Ag-specific CD8+ T cells&lt;sup&gt;49&lt;/sup&gt;</td>
</tr>
<tr>
<td>Foetal</td>
<td>Adolescent/ Adult Mouse</td>
<td>Increased flu severity. Increased &amp; sustained viral titres. Decreased number of Ag-specific CD8+ T cells. Decrease number &amp; size of B cell foci. Decreased amount of specific antibody&lt;sup&gt;50&lt;/sup&gt;</td>
</tr>
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SGA: Small for gestational age; SP-A: Surfactant protein A; AM: Alveolar macrophage; GBS: Group B Streptococcus; Ag: Antigen.