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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.edusmohan@emory.edulbrow03@emory.edu.

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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 ¹. While women may stop drinking once they know they are pregnant, nearly 50% of pregnancies are unplanned ² making binge drinking early in gestation a significant problem to the exposed newborns ³. Alcohol is the most prominent behavioural teratogen in the world⁴ 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 ⁵. 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 ⁶. This failure to recognize the alcohol-exposed newborn means that the detrimental effects of *in utero* alcohol exposure on the newborn remain poorly understood.

Alarming, alcohol use increases the risk of extreme premature delivery over 35 fold, ⁷ 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^{8,9}, EtG ¹⁰, and EtS ¹¹ in the meconium, placenta, and hair of patients with FASD have been demonstrated ¹¹⁻¹³. Phosphatidylethanol (PEt) holds a similar potential ¹⁴ 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 ¹⁵. Since each polymorphism is associated with a different enzymatic activity, there will also be variances in alcohol metabolism within the developing lung ¹⁶. Alcohol is also metabolized by mixed function oxidases such cytochrome P450 generating acetaldehyde and other reactive oxygen species ¹⁶. 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

injury¹⁶. Significant levels of CYP2E1 have been detected between gestational days 45 and 53 as organogenesis begins¹⁷ 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 depletion¹⁸. 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 occurs¹⁹.

Since developing tissues such as the lung have poorly developed antioxidant systems, they are particularly vulnerable to alcohol-induced oxidant stress and antioxidant depletion^{20,21}. For the lung, oxidant stress can lead to arrested alveolarization and vascular development^{22,23}. 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 lungs²⁴. In mice, foetal alcohol during the second trimester at the pseudo glandular stage of lung development resulted in decreased lung mass and delayed lung maturation²⁵. 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 bronchiole 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 formation²⁶. There was also decreased gene and protein expression of VEGF receptors (VEGFR-1 and VEGFR-2) as well as hypoxia-inducible factors 1 α , and 2 α , 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 surfactant²⁷. 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)²⁸ and further decreases IGF-II bioavailability through increased release of IGF-binding proteins²⁹. Another growth factor essential for lung development is vitamin A (retinoic acid), its analogues (retinoids) and corresponding receptors³⁰. Foetal alcohol exposure affects the expression and activation of retinoic acid receptors in neuronal cells³¹ 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 population³². 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 antigens³³. It has been estimated that pneumonia contributes to 1 million neonatal deaths annually, accounting for 10% of childhood mortality³⁴.

Infection is a leading cause of death in the early neonatal period with the greatest risk in the smallest infants³⁵. In the US, from 2005-2008 there were an estimated 390 deaths annually due to early onset sepsis, with half in premature infants³⁶. 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-fold³⁷. 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 infants³⁸. 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 exposure²⁰.

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 actions³⁹. They have direct bactericidal activity⁴⁰ and link to the adaptive immune system by inhibiting T-cell proliferation via IL-2 independent and dependent mechanisms⁴¹.

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 term⁴². Other research has confirmed decreased surfactant protein mRNA levels and suppression of pro-inflammatory cytokines in lambs exposed to alcohol prenatally²⁷.

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 macrophage⁴⁵. Further animal models confirmed that foetal alcohol exposure caused exaggerated oxidant stress that impaired terminal differentiation and phagocytic function of the neonatal alveolar macrophage⁴⁶.

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 *in vitro* and *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 cells⁴⁸. Whole animal models of influenza virus infection show that chronic alcohol ingestion increases disease morbidity, mortality, enhanced pulmonary lesion severity, and pulmonary virus titres⁴⁹. These changes correlate with decreased numbers of flu-antigen specific CD8+ T cells⁴⁹. Similar models have interrogated *in utero* ethanol exposure and its long term effects on response to influenza virus infection. McGill, *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 antibody⁵⁰.

Therefore, clinical studies suggest that *in utero* alcohol increases the risk of infection in term and premature newborns, but more investigations are warranted. Animal models demonstrate that *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 *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.

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References

1. Center for Disease Control and Prevention C. Alcohol use among childbearing-age women - United States 1991-1999. MMWR Morbidity and Mortality Weekly Report. 2002; 51:273-6. [PubMed: 11952279]
2. Finer LB, Henshaw SK. Disparities in rates of unintended pregnancy in the United States, 1994 and 2001. Perspect Sex Reprod Health. 2006; 38:90-6. [PubMed: 16772190]
3. Grant TM, Huggins JE, Sampson PD, Ernst CC, Barr HM, Streissguth AP. Alcohol use before and during pregnancy in western Washington, 1989-2004: implications for the prevention of fetal alcohol spectrum disorders. Am J Obstet Gynecol. 2009; 200:278, e1-8. [PubMed: 19027093]

4. Warren KR, Hewitt BG. Fetal alcohol spectrum disorders: when science, medicine, public policy, and laws collide. *Dev Disabil Res Rev.* 2009; 15:170–5. [PubMed: 19731390]
5. Center for Disease Control and Prevention C. Alcohol consumption among women who are pregnant or who might become pregnant - United States, 2002. *MMWR Morbidity and Mortality Weekly Report.* 2004; 53:1178–81. [PubMed: 15614234]
6. Little BB, Snell LM, Rosenfeld CR, Gilstrap LC 3rd, Gant NF. Failure to recognize fetal alcohol syndrome in newborn infants. *Am J Dis Child.* 1990; 144:1142–6. [PubMed: 2403097]
7. Sokol RJJJ, Louis JM, Bailey BN, Ager J, Jacobson SW, Jacobson JL. Extreme Prematurity: An Alcohol-Related Birth Effect. *Alcoholism: Clinical and Experimental Research.* 2007; 31:1031–7.
8. Bearer CF, Santiago LM, O'Riordan MA, Buck K, Lee SC, Singer LT. Fatty Acid ethyl esters: quantitative biomarkers for maternal alcohol consumption. *J Pediatr.* 2005; 146:824–30. [PubMed: 15973326]
9. Gareri J, Brien J, Reynolds J, Koren G. Potential role of the placenta in fetal alcohol spectrum disorder. *Paediatr Drugs.* 2009; 11:26–9. [PubMed: 19127948]
10. Chang G, Goetz MA, Wilkins-Haug L, Berman S. Identifying prenatal alcohol use: screening instruments versus clinical predictors. *Am J Addict.* 1999; 8:87–93. [PubMed: 10365188]
11. Morini L, Marchei E, Vagnarelli F, et al. Ethyl glucuronide and ethyl sulfate in meconium and hair-potential biomarkers of intrauterine exposure to ethanol. *Forensic Sci Int.* 2010; 196:74–7. [PubMed: 20060246]
12. Kulaga V, Gareri J, Fulga N, Koren G. Agreement between the fatty acid ethyl ester hair test for alcohol and social workers' reports. *Ther Drug Monit.* 2010; 32:294–9. [PubMed: 20431508]
13. Tarcomnicu I, van Nuijs AL, Aerts K, De Doncker M, Covaci A, Neels H. Ethyl glucuronide determination in meconium and hair by hydrophilic interaction liquid chromatography-tandem mass spectrometry. *Forensic Sci Int.* 2010; 196:121–7. [PubMed: 20061101]
14. Stewart SH, Law TL, Randall PK, Newman R. Phosphatidylethanol and alcohol consumption in reproductive age women. *Alcohol Clin Exp Res.* 2010; 34:488–92. [PubMed: 20028353]
15. Ang HL, Deltour L, Zgombic-Knight M, Wagner MA, Duester G. Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. *Alcohol Clin Exp Res.* 1996; 20:1050–64. [PubMed: 8892527]
16. Gemma S, Vichi S, Testai E. Metabolic and genetic factors contributing to alcohol induced effects and fetal alcohol syndrome. *Neurosci Biobehav Rev.* 2007; 31:221–9. [PubMed: 16908065]
17. Brzezinski MR, Boutelet-Bochan H, Person RE, Fantel AG, Juchau MR. Catalytic activity and quantitation of cytochrome P-450 2E1 in prenatal human brain. *J Pharmacol Exp Ther.* 1999; 289:1648–53. [PubMed: 10336564]
18. Olivares IP, Bucio L, Souza V, Carabez A, Gutierrez-Ruiz MC. Comparative study of the damage produced by acute ethanol and acetaldehyde treatment in a human fetal hepatic cell line. *Toxicology.* 1997; 120:133–44. [PubMed: 9184200]
19. Maffi SK, Rathinam ML, Cherian PP, et al. Glutathione content as a potential mediator of the vulnerability of cultured fetal cortical neurons to ethanol-induced apoptosis. *J Neurosci Res.* 2008; 86:1064–76. [PubMed: 18058941]
20. Gauthier TW, Young PA, Gabelaia L, et al. In utero ethanol exposure impairs defenses against experimental group B streptococcus in the term Guinea pig lung. *Alcohol Clin Exp Res.* 2009; 33:300–6. [PubMed: 19032578]
21. Sozo F, O'Day L, Maritz G, et al. Repeated ethanol exposure during late gestation alters the maturation and innate immune status of the ovine fetal lung. *Am J Physiol Lung Cell Mol Physiol.* 2009; 296:L510–8. [PubMed: 19112099]
22. Davis JM. Role of oxidant injury in the pathogenesis of neonatal lung disease. *Acta Paediatr Suppl.* 2002; 91:23–5. [PubMed: 12200893]
23. Asikainen TM, White CW. Pulmonary antioxidant defenses in the preterm newborn with respiratory distress and bronchopulmonary dysplasia in evolution: implications for antioxidant therapy. *Antioxid Redox Signal.* 2004; 6:155–67. [PubMed: 14713347]
24. Inselman LS, Fisher SE, Spencer H, Atkinson M. Effect of intrauterine ethanol exposure on fetal lung growth. *Pediatr Res.* 1985; 19:12–4. [PubMed: 2578634]

25. Wang X, Gomutputra P, Wolgemuth DJ, Baxi L. Effects of acute alcohol intoxication in the second trimester of pregnancy on development of the murine fetal lung. *Am J Obstet Gynecol.* 2007; 197:269, e1–4. [PubMed: 17826415]
26. Lazic T, Sow FB, Van Geelen A, Meyerholz DK, Gallup JM, Ackermann MR. Exposure to ethanol during the last trimester of pregnancy alters the maturation and immunity of the fetal lung. *Alcohol.* 2011; 45:673–80. [PubMed: 21163613]
27. Sozo F, Vela M, Stokes V, et al. Effects of prenatal ethanol exposure on the lungs of postnatal lambs. *Am J Physiol Lung Cell Mol Physiol.* 2011; 300:L139–47. [PubMed: 21036920]
28. Mauceri HJ, Lee WH, Conway S. Effect of ethanol on insulin-like growth factor-II release from fetal organs. *Alcohol Clin Exp Res.* 1994; 18:35–41. [PubMed: 8198224]
29. Fatayerji N, Engelmann GL, Myers T, Handa RJ. In utero exposure to ethanol alters mRNA for insulin-like growth factors and insulin-like growth factor-binding proteins in placenta and lung of fetal rats. *Alcohol Clin Exp Res.* 1996; 20:94–100. [PubMed: 8651471]
30. Masuyama H, Hiramatsu Y, Kudo T. Effect of retinoids on fetal lung development in the rat. *Biol Neonate.* 1995; 67:264–73. [PubMed: 7544161]
31. Kumar A, Singh CK, DiPette DD, Singh US. Ethanol impairs activation of retinoic acid receptors in cerebellar granule cells in a rodent model of fetal alcohol spectrum disorders. *Alcohol Clin Exp Res.* 2010; 34:928–37. [PubMed: 20201933]
32. de Roux A, Cavalcanti M, Marcos MA, et al. Impact of alcohol abuse in the etiology and severity of community-acquired pneumonia. *Chest.* 2006; 129:1219–25. [PubMed: 16685012]
33. Nissen MD. Congenital and neonatal pneumonia. *Paediatric Respiratory Reviews.* 2007; 8:195–203. [PubMed: 17868917]
34. The Child Health Research Project. Reducing perinatal and neonatal mortality: report of a meeting. Vol. 3. Baltimore, Maryland: 1999. p. 6-12.
35. Murphy SL, Xu J, Kochanek KD. Deaths: Preliminary Data for 2010. *National Vital Statistics Reports.* 2012; 60:1–68.
36. Weston EJ, Pondo T, Lewis MM, et al. The Burden of Invasive Early-onset Neonatal Sepsis in the United States, 2005–2008. *The Pediatric Infectious Disease Journal.* 2011; 30:937–41. [PubMed: 21654548]
37. Gauthier TW, Drews-Botsch C, Falek A, Coles C, Brown LA. Maternal alcohol abuse and neonatal infection. *Alcohol Clin Exp Res.* 2005; 29:1035–43. [PubMed: 15976530]
38. Gauthier TW, Manar MH, Brown LA. Is maternal alcohol use a risk factor for early-onset sepsis in premature newborns? *Alcohol.* 2004; 33:139–45. [PubMed: 15528011]
39. Haagsman HP, Hogenkamp A, van Eijk M, Veldhuizen EJA. Surfactant Collectins and Innate Immunity. *Neonatology.* 2008; 93:288–94. [PubMed: 18525212]
40. Wu H, Kuzmenko A, Wan S, et al. Surfactant proteins A and D inhibit the growth of Gram-negative bacteria by increasing membrane permeability. *J Clin Invest.* 2003; 111:1589–602. [PubMed: 12750409]
41. Borron PJ, Mostaghel EA, Doyle C, Walsh ES, McHeyzer-Williams MG, Wright JR. Pulmonary surfactant proteins A and D directly suppress CD3+/CD4+ cell function: evidence for two shared mechanisms. *J Immunol.* 2002; 169:5844–50. [PubMed: 12421966]
42. Lazic T, Wyatt TA, Matic M, et al. Maternal alcohol ingestion reduces surfactant protein A expression by preterm fetal lung epithelia. *Alcohol.* 2007; 41:347–55. [PubMed: 17889311]
43. Gauthier TW, Ping X-D, Harris FL, Wong M, Elbahesh H, Brown LAS. Fetal Alcohol Exposure Impairs Alveolar Macrophage Function via Decreased Glutathione Availability. *Pediatric Research.* 2005; 57:76–81. [PubMed: 15531743]
44. Ping XD, Harris FL, Brown LA, Gauthier TW. In vivo dysfunction of the term alveolar macrophage after in utero ethanol exposure. *Alcohol Clin Exp Res.* 2007; 31:308–16. [PubMed: 17250624]
45. Brown SD, Gauthier TW, Brown LA. Impaired terminal differentiation of pulmonary macrophages in a Guinea pig model of chronic ethanol ingestion. *Alcohol Clin Exp Res.* 2009; 33:1782–93. [PubMed: 19645732]

46. Gauthier TW, Ping XD, Gabelaia L, Brown LA. Delayed neonatal lung macrophage differentiation in a mouse model of in utero ethanol exposure. *Am J Physiol Lung Cell Mol Physiol*. 2010; 299:L8–16. [PubMed: 20382747]
47. Gurung P, Young BM, Coleman RA, et al. Chronic ethanol induces inhibition of antigen-specific CD8+ but not CD4+ immunodominant T cell responses following *Listeria monocytogenes* inoculation. *Journal of Leukocyte Biology*. 2008; 85:34–43. [PubMed: 18820175]
48. Fan J, Edsen-Moore MR, Turner LE, et al. Mechanisms by Which Chronic Ethanol Feeding Limits the Ability of Dendritic Cells to Stimulate T-Cell Proliferation. *Alcoholism: Clinical and Experimental Research*. 2011; 35:47–59.
49. Meyerholz DK, Edsen-Moore M, McGill J, Coleman RA, Cook RT, Legge KL. Chronic alcohol consumption increases the severity of murine influenza virus infections. *J Immunol*. 2008; 181:641–8. [PubMed: 18566431]
50. McGill J, Meyerholz DK, Edsen-Moore M, et al. Fetal Exposure to Ethanol Has Long-Term Effects on the Severity of Influenza Virus Infections. *The Journal of Immunology*. 2009; 182:7803–8. [PubMed: 19494304]

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.

Timing of Ethanol Exposure	Animal Model	Developmental Abnormality
All Trimesters	Rat	Inhibition of cellular growth Hypoplastic lungs ²⁴
Second Trimester	Mouse	Decreased lung mass Delayed lung maturation Persistence of Hoxb5 expression ²⁵
Third Trimester	Preterm Lamb	Decreased VEGF protein & gene expression ²⁶
	Term Lamb	Decreased surfactant production Altered surfactant content ²⁷

TABLE 2
ALCOHOL ASSOCIATED IMMUNE DYSFUNCTION

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

Alcohol Exposure	Model	Functional Deficit
Foetal (Third Trimester)	Preterm Neonatal Lamb	Decreased SP-A mRNA ^{27, 42}
Foetal (Third Trimester)	Term Neonatal Lamb	Decreased SP-A and ciliary beat frequency ⁴²
Foetal	Preterm & Term Neonatal Guinea Pig	Decreased AM phagocytosis <i>in vitro</i> and <i>in vivo</i> ^{43, 44}
Foetal	Term Neonatal Guinea Pig	Increased risk of GBS pneumonia and sepsis Decreased AM phagocytosis ²⁰
Chronic	Adult Guinea Pig	Impaired AM differentiation ⁴⁵
Foetal	Term Neonatal Mouse	Impaired AM differentiation ⁴⁶
Chronic	Adult Mouse	Decreased number of CD8+ T cells and specific Ag presentation ⁴⁷
Chronic	Adult Mouse	Decreased receptors for inflammatory mediators on airway dendritic cells ⁴⁸
Chronic	Adult Mouse	Increased flu severity and viral titres. Decreased number of Ag-specific CD8+ T cells ⁴⁹
Foetal	Adolescent/ Adult Mouse	Increased flu severity. Increased & sustained viral titres. Decreased number of Ag-specific CD8+ T cells. Decrease number & size of B cell foci. Decreased amount of specific antibody ⁵⁰

SGA: Small for gestational age; SP-A: Surfactant protein A; AM: Alveolar macrophage; GBS: Group B Streptococcus; Ag: Antigen.