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Effects of prenatal alcohol exposure (PAE): insights into FASD using mouse models of PAE[1]

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Abstract

The potential impact of prenatal alcohol exposure (PAE) varies considerably among exposed individuals, with some displaying serious alcohol-related effects and many others showing few or no overt signs of fetal alcohol spectrum disorder (FASD). In animal models, variables such as nutrition, genetic background, health, other drugs, and stress, as well as dosage, duration, and gestational timing of exposure to alcohol can all be controlled in a way that is not possible in a clinical situation. In this review we examine mouse models of PAE and focus on those with demonstrated craniofacial malformations, abnormal brain development, or behavioral phenotypes that may be considered FASD-like outcomes. Analysis of these data should provide a valuable tool for researchers wishing to choose the PAE model best suited to their research questions or to investigate established PAE models for FASD comorbidities. It should also allow recognition of patterns linking gestational timing, dosage, and duration of PAE, such as recognizing that binge alcohol exposure(s) during early gestation can lead to severe FASD outcomes. Identified patterns could be particularly insightful and lead to a better understanding of the molecular mechanisms underlying FASD.

[1]This Invited Review is one of a selection of papers covering various aspects of fetal alcohol spectrum disorder (FASD).

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Keywords

fetal alcohol spectrum disorder (FASD); prenatal alcohol exposure (PAE); mouse models; craniofacial and brain malformations; behavioral outcomes

Introduction

Clinical and experimental studies have clearly demonstrated that alcohol (ethanol) consumption during pregnancy can produce a range of pervasive and long-lasting developmental, neurobehavioral, neurobiological, and physiological impairments in the offspring (Sokol et al. 2003; Chudley et al. 2005). The first paper describing the adverse effects of prenatal alcohol exposure was published in 1968 by a French pediatrician, Paul Lemoine, who noted facial abnormalities as well as a range of cognitive and behavioral alterations in children of alcoholic parents (Lemoine et al. 1968). However, this work was largely unrecognized, and it was not until the publications of Jones, Smith, and colleagues in 1973 that the effects of prenatal alcohol exposure gained widespread attention (Jones and Smith 1973; Jones et al. 1973). These investigators reported growth deficiencies, cognitive deficits, and facial dysmorphology similar to what Lemoine had described and coined the term fetal alcohol syndrome (FAS) to describe this cluster of abnormalities. Since then, thousands of clinical studies have confirmed and significantly extended these early findings, describing numerous deficits in multiple domains, including neurocognitive, self-regulatory, and adaptive function impairments (Streissguth et al. 1985; Spadoni et al. 2007; Sowell et al. 2008; Norman et al. 2009; Mattson et al. 2011; Bower et al. 2013). Studies utilizing animal models of prenatal alcohol exposure began shortly after FAS was first identified and have been critical in confirming that alcohol itself is a teratogen, mirroring and extending the clinical work on behavioral, functional, and neurobiological effects of alcohol, and elucidating the mechanisms underlying the adverse effects of alcohol (Sulik and Johnston 1983; Sulik et al. 1988; Parnell et al. 2009; Godin et al. 2010; Lipinski et al. 2012).

A variety of diagnostic guidelines have been developed over the years to assist clinicians in recognizing and diagnosing children affected by exposure to alcohol in utero (Benz et al. 2009). Following the identification of FAS, Clarren and Smith (1978) introduced the term suspected fetal alcohol effects (FAE) to describe partial expression of FAS, in recognition of the fact that alcohol exposure results in a range of deficits and that facial features may not be prominent. In 1980, Rosett (1980) proposed criteria specific for diagnosis of FAS based on a review of 245 cases by Clarren and Smith (1978) and discussions of the Fetal Alcohol Study Group of the Research Society on Alcoholism. These included pre- and post-natal growth retardation, central nervous system (CNS) involvement, including neurological abnormalities, developmental delay, and intellectual impairment, and a characteristic facial dysmorphology. Like Clarren and Smith (1978), Rosett also noted that exposure to alcohol results in a wide range of effects, with FAS at the far end of the spectrum. Sokol and Clarren's paper in 1989 (Sokol and Clarren 1989) built on this work with the goal of providing "guidelines for use by investigators, care providers, and others that will enhance comparability of results of clinical observations, scientific studies, and public health reporting." They supported continued use of the 1980 Fetal Alcohol Study Group guidelines

with minor modifications (Rosett 1980). FAS was still defined by deficits in the 3 areas listed above. ARBD was defined as “a term that connotes attribution of an observed anatomic or functional outcome to the impact of alcohol.” In addition, they noted that the term fetal alcohol effects (FAE) was not specific enough and was being misapplied by clinicians, and recommended that it no longer be used. A major advance occurred in 1994, when a committee convened by the Institute of Medicine was charged with conducting a study of FAS and related birth defects to improve the understanding of available research knowledge and experience in relation to tools and approaches for diagnosing FAS and related disorders, as well as prevalence of FAS, surveillance systems, and prevention and treatment programs. This committee built on previous diagnostic criteria, with minor changes to try to resolve some of the issues causing confusion in the clinical and research communities. They identified 4 alcohol-related clinical diagnostic categories (Stratton et al. 1996): fetal alcohol syndrome (FAS:evidence of characteristic craniofacial dysmorphology, prenatal and postnatal growth restriction, and central nervous system (CNS) neurodevelopmental deficits); partial fetal alcohol syndrome (pFAS: some but not all of the characteristics features of FAS and confirmed maternal alcohol exposure); alcohol-related neurodevelopmental disorder (ARND: evidence of CNS neurodevelopmental abnormalities and (or) of a complex pattern of behavioral or cognitive abnormalities, and confirmed maternal alcohol exposure); and alcohol-related birth defects (ARBD: one or more congenital anomalies and confirmed maternal alcohol exposure), with the latter two categories not mutually exclusive. However, this system did not specify clinical criteria by which diagnoses could be assigned. To address this issue, Astley and Clarren developed the FASD 4-digit diagnostic code (Astley and Clarren 2000), with the goal of developing objective and quantitative scales to “operationalize” the IOM criteria. The code utilizes a 4-point Likert scale (1 = absence of the feature, 4 = strong presentation of the feature) that reflects the magnitude of expression of the 4 key diagnostic features of FAS: growth deficiency, the FAS facial phenotype, central nervous system dysfunction, and gestational exposure to alcohol. This system has been updated several times to enhance clarity and specificity as well as to account for racial differences in facial features, and is now widely used. The term fetal alcohol spectrum disorder (FASD) was first introduced around 2000 to recognize more specifically the broad spectrum of effects that result from prenatal alcohol exposure and was formalized in April 2004, when the National Organization on Fetal Alcohol Syndrome brought the National Institutes of Health, the CDC, and the Substance Abuse and Mental Health Services Administration, as well as additional experts in the field, to develop a consensus definition of FASD: “FASD is an umbrella term describing the range of effects that can occur in an individual whose mother drank alcohol during pregnancy,” noting that, “These effects include physical, mental, behavioral, and (or) learning disabilities with possible lifelong implications. The term FASD encompasses all other diagnostic terms, such as FAS, and is not intended for use as a clinical diagnosis.” Three other diagnostic guidelines are important to highlight. Hoyme and colleagues published updated clinical guidelines for the diagnosis of FASD based on the evaluation of >10 000 children in clinical settings and epidemiologic studies, together with studies funded by the National Institute on Alcohol Abuse and Alcoholism, as well as the Collaborative Initiative on Fetal Alcohol Spectrum Disorders, and the Collaboration on FASD Prevalence (Hoyme et al. 2016). These authors kept the 4 original IOM diagnostic categories (FAS, partial FAS, ARND, ARBD) but

provided more specific and rigorous criteria for each. A set of Canadian diagnostic guidelines for FASD was proposed in 2005 (Chudley et al. 2005) and was recently updated (Cook et al. 2016). Similar to the US guidelines, a large group of individuals, selected by the Canada Fetal Alcohol Spectrum Disorder Research Network, and with broad expertise came together to develop these guidelines. Input was also obtained from diagnostic centers across Canada and multiple focus groups. Interestingly, the 2016 revision made FASD a diagnostic term and indicated that making a diagnosis of FASD requires a multidisciplinary team and involves a complex physical and neurodevelopmental assessment. As well, the diagnostic categories were collapsed to two: (*i*) FASD with sentinel facial features and evidence of impairment in 3 or more identified neurodevelopmental domains, with pre-natal alcohol exposure either confirmed or unknown and (*ii*) FASD without sentinel facial features, with evidence of impairment in 3 or more identified neurodevelopmental domains, and confirmed prenatal alcohol exposure. They also specify a category called “At risk for neurodevelopmental disorder and FASD, associated with prenatal alcohol exposure,” which may help to identify individuals who are at risk. Finally, the Diagnostic and Statistical Manual, 5th edition, from the American Psychiatric Association, now includes “neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE)” (American Psychiatric Association 2013). ND-PAE was listed in the “Conditions for Further Study” and also given as an example under “Other Specified Neurodevelopmental Disorder (315.8)”. ND-PAE is proposed as a type of umbrella term and goes beyond physically based diagnostic criteria to allow clinical assessment that encompasses the neurodevelopmental and mental health symptoms associated with PAE (Kable et al. 2016). It can be diagnosed in either the presence or absence of physical effects of PAE, requires confirmed gestational exposure to alcohol, and includes symptoms in 3 domains, neurocognitive functioning, self-regulation, and adaptive functioning, which adversely impact quality of life. Clearly, the field is moving forward in terms of both recognizing the multiple adverse outcomes associated with pre-natal alcohol exposure and developing diagnostic guidelines that can be utilized effectively by physicians.

In addition to the formal diagnosis of an FASD, psychopathologies (e.g., anxiety, depression, other mood disorders, and substance use disorders), often referred to as “secondary disabilities,” are expressed at disproportionally higher rates in individuals prenatally exposed to alcohol (Roebuck et al. 1999; O’Connor et al. 2006; Kodituwakku 2007, 2009; Mattson et al. 2011; Riley et al. 2011; Kable et al. 2016), which increases the health risks of these individuals as well as the emotional and financial cost to the individual, the family, and the health care system (Stade et al. 2009). Interestingly, a wealth of animal data (reviewed in Patten et al. 2014) suggest that these “secondary disabilities” may actually be primary effects of alcohol itself, or at least have a primary component in terms of alcohol-induced neurobiological alterations that increase vulnerability in these individuals. Overall, the estimated incidence of FASD is thought to be approximately 1 in 100 live births, although recent epidemiological studies indicate prevalence rates of combined FAS and pFAS of 1.1%–2.5%, and of the full FASD spectrum as high as 2%–5% in the US (May et al. 2009, 2013, 2014, 2015).

In this review we examine mouse models of PAE and focus on those with demonstrated craniofacial malformations, abnormal brain development, or behavioral phenotypes that may

be considered FASD-like outcomes. First, we provide a brief overview of FASD and the clinical features of the disorder, which include the sentinel features as well as the secondary disabilities associated with FASD. Second, we provide an in-depth assessment of factors that may cause FASD, including dosage, duration, and timing of alcohol exposure, maternal genetics, and maternal nutrition and metabolism, while also exploring the molecular mechanisms of alcohol teratogenicity as found in seminal animal model PAE research. Finally, the main focus of the review will be devoted to mouse models of PAE, specifically focusing on the paradigms (dosage, duration, and timing of alcohol exposure) used, the PAE-induced craniofacial and brain malformations, and behavioral outcomes observed, and how these PAE mouse model phenotypes (malformations and outcomes) relate to the clinical manifestations of FASD. This approach will allow recognition of patterns linking gestational timing, dosage, and duration of PAE with FASD-like outcomes. The latter could be particularly insightful and lead to a better understanding of the molecular mechanisms underlying FASD as well as gaps in the literature related to our current understanding of FASD.

Risk factors for alcohol teratogenicity and FASD

The potential impact of prenatal alcohol exposure varies considerably among exposed individuals, with some infants displaying serious alcohol-related effects and others showing few or even no overt signs of an FASD (Khoale et al. 2004). Therefore, it is important to understand the variables that may increase or decrease the probability that PAE will produce deleterious effects on fetal development. These factors include the gestational timing, duration, and dose of alcohol (Astley et al. 1999), amount consumed per drinking session (Khoale et al. 2004), genetic and epigenetic factors (Kaminen-Ahola et al. 2010*a*; Kleiber et al. 2014; Lussier et al. 2015), maternal and fetal stress (Glavas et al. 2007; Uban et al. 2013; Raineki et al. 2014), nutritional status (Weinberg 1984, 1985; Keen et al. 2010), and the mother's ability to metabolize alcohol, which are all known to be critically involved in alcohol teratogenicity (Ramchandani et al. 2001; Riley et al. 2011).

Dose, duration, and timing of alcohol exposure

Early studies by Chernoff (1977), and Randall and Taylor (1979), utilizing animal models, verified a positive correlation between maternal blood alcohol concentration (BAC) and developmental malformations or deficits; a finding further validated by the seminal research of West and colleagues (Pierce and West 1986*a*, 1986*b*, 1987; Pierce et al. 1989). The use of varying alcohol treatment regimens in animal models has been critical for elucidating the teratogenic effects of alcohol. Regimens may involve acute maternal administration (i.e., 1 or 2 doses) during critical periods of fetal development (Sulik et al. 1981; Godin et al. 2010; Idrus and Napper 2012), trimester-equivalent fetal and (or) neonatal alcohol exposure (Bake et al. 2012; Hamilton et al. 2012; Wagner et al. 2013; Jablonski and Stanton 2014), intermittent binge-like exposure (Ramadoss et al. 2008; Sawant et al. 2013), or chronic exposure throughout gestation through gavage, inhalation, or liquid diets (Lee et al. 2000; Green et al. 2005; Iqbal et al. 2005, 2006; Hellemans et al. 2010; Probyn et al. 2013; Uban et al. 2013).

Timing of alcohol exposure may be equally as important as BAC. In the early and mid-1980s, Webster et al. (1980), as well as Sulik and colleagues (Sulik et al. 1981; Sulik and Johnston 1983; Sulik and Schoenwolf 1985) demonstrated that the timing of alcohol exposure was crucial to the development of craniofacial anomalies, a signature feature of FAS in humans. In addition, the timing of exposure has major implications for brain development. The teratogenic effects of alcohol may be particularly detrimental to CNS development during critical periods of vulnerability, which include the first half or two-thirds of the first trimester when brain and organ development are extremely rapid, and the brain growth spurt that occurs in the third trimester in the human and in the early postnatal period in rodents (Dobbing and Sands 1979; Bockhorst et al. 2008). Indeed, using a rat model of PAE, early postnatal exposure to alcohol (third trimester equivalent) has highlighted the enduring effects of PAE on neurogenesis, a process that continues throughout life in select areas of the brain, most notably the dentate gyrus within the hippocampus (Klintsova et al. 2007; Sliwowska et al. 2010). Nevertheless, PAE can impair brain development throughout all stages of gestation, including effects on neurogenesis, differentiation, and synaptogenesis (Dobbing and Sands 1979; West et al. 1994; Guerri 1998; Cudd 2005).

Genetics, maternal nutrition, alcohol metabolism

Various maternal factors act to increase the risk of alcohol's deleterious effects in the developing fetus. First, the presence of genetic polymorphisms of alcohol-metabolizing enzymes may significantly increase or decrease alcohol's deleterious effects in the fetus. For example, maternal polymorphisms manifesting as increased alcohol dehydrogenase activity and enhanced alcohol metabolism have been associated with a decreased incidence of alcohol teratogenicity (Eriksson 2001; Jacobson et al. 2006). Second, inadequate maternal nutrition may increase the risk of alcohol teratogenicity throughout fetal development. Deficiencies in folic acid, iron, choline, zinc, and omega-3 fatty acid are associated with alcohol-induced fetal growth restriction and neurobehavioral teratogenicity in postnatal offspring (Thomas et al. 2010; Rufer et al. 2012; Idrus et al. 2013; Patten et al. 2013). Third, the capacity of the maternal–fetal unit to metabolize alcohol may greatly affect the risk of alcohol teratogenicity. The capacity for alcohol metabolism among pregnant women varies up to 8-fold (from 0.0025 to 0.0200 $g \cdot dL^{-1} \cdot h^{-1}$), which may help to explain the variation in phenotypic presentation of FASD following maternal consumption of similar doses of alcohol (Burd et al. 2012). The capacity for alcohol metabolism also varies with age, ethnicity, hormonal status, body composition and lean body weight, liver size, and food intake (Ramchandani et al. 2001). Ultimately, the peak BAC achieved from maternal alcohol consumption is critical to the risk of alcohol's deleterious effects and depends on the rate of drinking, gastric emptying, and phase I biotransformation, involving alcohol dehydrogenase and cytochrome P450 2E1, as well as the nutritional status of the mother and whether alcohol is consumed alone or with a meal (Ramchandani et al. 2001). In general, not surprisingly, mothers of children with FASD consumed more alcohol and achieved a higher BAC than mothers who consumed alcohol but did not give birth to a child with FASD (Khoale et al. 2004).

Mechanism of alcohol's teratogenic effects

The toxic effects of alcohol on the embryo are well known. Alcohol readily crosses the placental and blood–brain barriers and can thus affect developing fetal cells. Alcohol is known to act on or modulate many different target molecules, and multiple mechanisms, activated at different stages of development or at different dose thresholds of exposure, probably contribute to the diverse phenotypes seen in FASD (Goodlett et al. 2005). In this review, we will briefly highlight some of the major mechanisms shown to be involved in mediating, at least in part, alcohol's adverse effects.

Studies on non-mammalian species, where the organism develops outside the mother and there are no effects of the placenta or the maternal environment, have provided unique insights into the teratogenic effects of alcohol. While this work is largely outside the scope of this review, it is noteworthy that studies utilizing *C. elegans* (Davis et al. 2008), and chicken models (Pennington et al. 1983; Cartwright and Smith 1995) have revealed important adverse effects of alcohol on craniofacial development, growth, the developmental trajectory, reproductive maturity, and overall longevity, and have elucidated how the dose and timing of alcohol exposure may contribute to outcomes. In support of the significance of these findings in non-mammalian models is work on the effects of alcohol on mammalian embryos. Studies have shown dose-dependent embryonic retardation of growth and differentiation, with specific reduction in both DNA and protein content (Brown et al. 1979). Consistent with these findings, data from in vivo studies have shown that alcohol exposure in utero or in the early postnatal period (third trimester equivalent model) can reduce brain, heart, and kidney weight in newborns, and can inhibit protein synthesis and decrease RNA and (or) DNA content in the fetal and neonatal brain and other organs (Rawat 1975; Gallo and Weinberg 1986). Alcohol-induced disruptions in the proliferation of stem cell populations are another mechanism of alcohol's actions on the fetus, leading to a reduction in the generation of both new neurons and new glial cells (Miller 1992; Guerri et al. 1993, 2009; Guerri 1998). Neuronal cell damage and (or) cell death can also occur through both programmed cell death (Ikonomidou et al. 2000) and inhibition or disruption of enzymes that play a role in metabolism in neural tissue (Goodlett et al. 2005). Of particular interest to both researchers and clinicians, there appear to be fairly large differences in the susceptibility of different brain regions to alcohol, including the hippocampus, amygdala, and cerebellum, depending on dose and timing of exposure, which could underlie at least some of the behavioral alterations seen in FASD (Michaelis 1990; Guerri 1998; Spadoni et al. 2007; Sowell et al. 2008). As well, alcohol exposure may result in a disorganized cortical architecture, which could ultimately influence the pattern of communication in and across regions involved in higher cognitive function (Clarren 1986; Spadoni et al. 2007; Sowell et al. 2008).

Other mechanisms mediating alcohol's adverse effects on neurobiological and neurobehavioral outcomes include: nutritional deprivation or deficiencies (e.g., calories, protein, zinc, folate, vitamin A); abnormalities in calcium signaling; altered prostaglandin synthesis/degradation; placental dysmorphology/dysfunction; alcohol-induced circulatory changes in placenta and (or) fetus; disrupted cell–cell interactions (cell adhesion); interference with growth factors or other cell signaling mechanisms that mediate cell

proliferation, growth, differentiation, migration, and maturation; oxidative stress and damage by free radicals; disruption of neuronal development in specific cell populations (e.g., serotonergic neurons); alteration/disruption of endocrine balance and neuroendocrine function (Michaelis 1990; Randall et al. 1990; West et al. 1994; Guerri 1998; Shibley et al. 1999; Goodlett et al. 2005; Bake et al. 2012; Uban et al. 2013; Wieczorek et al. 2015), and increased neuroinflammation (Weinberg and Gallo 1982; Randall et al. 1987, 1989; Weinberg and Bezio 1987; Taylor et al. 1988; Weinberg 1989, 1992, 1993; Lee et al. 1990, 2000; LeBel and Bondy 1991; Halasz et al. 1993; Henderson et al. 1995; Kotch et al. 1995; Lee and Rivier 1996; Ramanathan et al. 1996; Gabriel et al. 1998; Bearer 2001*a*, 2001*b*; Spong et al. 2001; Wilkemeyer et al. 2002, 2003; Zhang et al. 2005, 2012; Glavas et al. 2007; Gubitosi-Klug et al. 2007; Weinberg et al. 2008; Sari 2009; Dong et al. 2010; Bodnar and Weinberg 2013; Dou and Charness 2014; Drew et al. 2015; Bodnar et al. 2016).

Of particular relevance to our research, it is known that the endocrine, immune, and nervous systems exist within a complex interactive regulatory network (Besedovsky and del Rey 1996, 2007; Blalock and Smith 2007). Reciprocal expression of receptors for hormones, neurotransmitters, and neuropeptides, and shared ligands underlie the bidirectional communication that allows immune, endocrine, and neural cells to “speak a common biochemical language” (Blalock and Smith 2007), and influence each other to maintain homeostasis. PAE can exert teratogenic effects at all levels of this neuro–endo–immune circuit. Indeed, alcohol-induced disruptions of HPA (hypothalamic–pituitary–adrenal) activity and regulation, and thus the normal neuroendocrine–immune interactions, may provide a route through which early life experiences, including prenatal alcohol exposure, can have long term effects on both endocrine and immune function and thus contribute to some of the long-term adverse effects of PAE, including metabolic, cognitive, and immune dysfunction (McEwen and Stellar 1993). Together, this strong and extensive body of research on mechanisms of alcohol’s teratogenic effects highlights the adverse and pervasive outcomes of prenatal alcohol exposure and suggests possible directions for future PAE research.

Mouse models of prenatal alcohol exposure

In animal models, variables such as nutrition, genetic background, health, other drugs, and stress, as well as dosage, duration, and gestational timing of alcohol exposure can all be controlled in a way not possible in the clinical situation (Becker et al. 1996). In this section we examine mouse models of PAE and focus on those with demonstrated craniofacial abnormalities, abnormal brain development, or behavioral phenotypes that may be considered FASD-like outcomes. Animal models have significantly advanced our insight into the multiple variables that may influence how alcohol affects the developing embryo and fetus, including possible mechanisms that mediate alcohol’s adverse effects, and some of the risk and resilience factors that play a role in the etiology of FASD.

Mouse models of PAE provide researchers with several advantages over other genetic animal models. First, mouse models are biologically and genetically the closest animal model available to study human genetics. Second, mouse models have well-developed genetic engineering tools for genetic modeling. These advantages allow researchers to study PAE

using genetically modified mice to assess specific contributions of genes, genetic variants, or signalling pathways to the outcomes observed. Moreover, epigenetic signatures can be extensively studied in mouse models of PAE compared with other animal models because there is a wealth of non-PAE genetic data sets now available. Third, mice, specifically the C57BL/6 strain, readily adapt to alcohol consumption ad libitum, allowing more reliable alcohol dosing in chronic models and higher blood alcohol concentrations in the dams. Last, craniofacial and brain development including morphology has been extensively characterized in the mouse, providing researchers with a wealth of data to compare the craniofacial and brain (developmental) malformations. For the purpose of this review, PAE paradigms that include behavioral studies were sought out to highlight phenotypes observed in the various mouse models of PAE.

The criteria for literature inclusion in this review were publications that used validated (multiple publications using the same paradigm) and robust (reproducible and relevant FASD-like phenotypes) models. Occasionally, PAE paradigms showing phenotypes reminiscent of clinical features (or co-morbidities) of FASD are also highlighted. We first examine mouse models of PAE based on gestational alcohol exposure (human trimester equivalent): first trimester (gestational day [GD]0–10), second trimester (GD11–20), and third trimester (PN1–10), in the context of alcohol dose and duration of exposure. Second, in the context of gestation stage, we examine robust FASD-like phenotypes, including craniofacial, physiological/brain, and behavioral phenotypes that reflect clinical features of FASD observed in children. These analyses should provide a valuable tool for researchers wishing to choose the PAE model best suited to their research or to investigate established PAE models for FASD comorbidities. It should also allow recognition of patterns linking gestational timing, dosage, and duration of PAE with FASD-like outcomes. The latter could be particularly insightful and lead to a better understanding of the molecular mechanisms underlying FASD.

It was also important to review the mouse models in the context of alcohol exposure, which will allow alcohol-related phenotypes to be categorized by dosage, duration, and timing of alcohol exposure and to correlate those findings to clinical FASD cases according to the drinking patterns seen in pregnant (and unknowingly pregnant —if early first trimester) women. A recent CDCP report has indicated that 10.2% of pregnant women consume alcohol, and 3.1% of women binge drink throughout pregnancy (Tan et al. 2015). In an Australian population based cohort study, 54% of pregnant women indicated drinking during the first trimester (27%) and throughout pregnancy (27%). Moreover, 18.5% of women binge drank prior to pregnancy recognition (usually in the first trimester of pregnancy) (Muggli et al. 2016). In a systematic review and meta-analysis, the global prevalence of alcohol use during pregnancy is 9.8%, and it is estimated that 1 in 67 women who consumed alcohol during pregnancy would deliver a child with FAS (Popova et al. 2017). Based on maternal drinking patterns, gestational exposure, and overall consumption of alcohol during pregnancies, this review of mouse models of PAE is unique because it categorizes these paradigms based on maternal alcohol consuming practices (dose, duration, and timing of alcohol exposure).

First trimester PAE

The first trimester equivalent in the mouse is considered to be from gestational day 0–10. It starts at the moment of conception and includes the pre-implantation period, implantation period, gastrulation, and the beginning of neurulation. First trimester mouse models of PAE tend to use either an acute delivery of alcohol during the gastrulation–neurulation developmental stages or chronic exposure to alcohol during the conception–gastrulation stages. Maternal alcohol exposure during the first trimester is most often by oral gavage, intraperitoneal (i.p.) injection, liquid diet, or voluntary ad libitum drinking. Both oral gavage and i.p. injection can result in high BACs (>200 mg/dL) and are typically utilized for acute exposure studies but have the disadvantage of being more stressful to the dam. Liquid diet and ad libitum drinking generally result in low to moderate BACs (80–150 mg/dL), but involve less handling and stress and are usually utilized for chronic exposure studies (Patten et al. 2014). The majority of mouse strains (CD1, 129/SV, BALB/c, DBA, etc.) will not consume alcohol voluntarily, at least not at a level that is pharmacologically relevant. Interestingly, C57BL/6 strains prefer alcohol over water and are commonly used in ad libitum drinking paradigms (Wahlsten et al. 2006). This section will focus on chronic exposures spanning the first trimester and acute exposures during gastrulation and early neurulation. Chronic and acute exposures in the pre-implantation and implantation periods are extensively reviewed elsewhere in this special issue.

Recent studies have utilized chronic first trimester exposure to alcohol spanning GD0–10. Kaminen-Ahola et al. utilized a 10% *v*/*v* alcohol ad libitum drinking paradigm, with exposure from GD0.5 to 8.5 (pre-implantation period to late gastrulation/early neurulation), as a chronic low–moderate BAC model (Kaminen-Ahola et al. 2010*a*, 2010*b*). This paradigm results in midfacial dysmorphologies, specifically a wider interorbital distance (the distance between the eye sockets) and cranium width, with a shorter midface compared with wild-type (WT) controls (Kaminen-Ahola et al. 2010*a*). Moreover, at PN30, these mice demonstrate premaxillary deviation of the midface (curved snout) and loss of the interfrontal bone that is normally found between the maxillary and parietal bone regions of the cranium in WT controls (Kaminen-Ahola et al. 2010*a*). Furthermore, these PAE offspring consistently exhibit a lower body weight and smaller skull size, suggesting intra-uterine growth restriction and microcephaly compared with controls (Kaminen-Ahola et al. 2010*a*, 2010*b*). Interestingly, mid-facial and premaxillary malformations of the skull are seen in individuals with FAS, where midfacial hypoplasia and premaxillary rotational effects are common (Suttie et al. 2013). Intrauterine growth restriction, including small skull size (<3rd percentile), are also common features of children with FAS (Astley et al. 2016).

Acute first trimester prenatal alcohol exposure studies are typically done at the gastrulation and early neurulation developmental stages (GD7–9). Seminal research by Sulik and colleagues has shown that a single acute dose of alcohol on each of these 3 days can produce distinct and profound craniofacial malformations that are reminiscent of sentinel clinical features of FAS (Parnell et al. 2009, 2013; Godin et al. 2010; Lipinski et al. 2012). Sulik and colleagues used 2 doses of alcohol = 2.9 g/kg (4 h apart, i.p.) at gestational day 7.0 to model an acute high BAC (Sulik and Johnston 1983; Godin et al. 2010). This single day binge exposure model results in frontonasal prominence malformations: deficient medial nasal

processes (closely set nostrils), abnormally longer maxillary processes (long upper lip), abnormal/deficient pre-maxillary bone, eye malformations including anophthalamia, microphthalmia, colobomas, and incidence of forebrain exencephaly (Sulik and Johnston 1983; Godin et al. 2010). Varying the timing of PAE exposures by plus or minus 4 h from GD7.0 results in a strikingly different craniofacial phenotypes. Incidence of frontonasal prominence malformations in embryos assessed at GD14 was 59.6%, 44.4%, or 27% compared with non-exposed controls, depending on whether the PAE occurred at GD7 – 4 h, GD7.0 or GD7 + 4 h, respectively. Conversely, embryo resorption rates were 9.5%, 18.2%, or 45.9% in the same PAE groups (Sulik and Johnston 1983). This study demonstrates that early gastrulation window is very sensitive to the effects of PAE on craniofacial development and viability.

Impact of acute GD7 PAE on frontonasal prominence abnormalities can be further characterized at GD17. The frontonasal prominence is an early embryonic structure that develops into the future forebrain and face, as directed first by retinoic acid and later on by sonic hedgehog (SHH) during early neurulation (Carlson 2014). Abnormal frontonasal prominence structure during embryogenesis is indicative of future forebrain and facial abnormalities (DeMyer et al. 1964). GD17 embryos exhibited many craniofacial malformations, including closely set nostrils, a long upper lip, and eye malformations, as well as midfacial hypoplasia and clefting, cleft palate, and micrognathia (Godin et al. 2010; Hong and Krauss 2012; Lipinski et al. 2012). At GD17, GD7 PAE embryos can exhibit forebrain dysmorphologies reminiscent of holoprosencephaly (HPE): pituitary agenesis, third ventricle dilation, reduction/loss of olfactory bulbs, reduction in cerebral cortex size, and malformed lateral ventricles (DeMyer et al. 1964; Sulik and Johnston 1983; Godin et al. 2010). Magnetic resonance imaging (MRI) studies of adolescent mice (at PN45) found that the anterior commissure was significantly reduced in PAE mice exposed at GD7 compared with control mice. Moreover, the myelin content of the 3 largest white matter tract bundles (the anterior commissure, corpus callosum, and hippocampal commissure) were significantly reduced (Cao et al. 2014). These studies demonstrate that acute PAE at early gastrulation is sufficient to induce developmental changes in brain and face structure as well as grey matter and white matter tracts that continue well into adolescence. Interestingly, Wieczorek et al. found, in an acute model of GD7 PAE, that PAE affects anxiety-like behavior in a sexually dimorphic manner: PAE males showed increases in anxiety-like behavior whereas females exhibited decreases in anxiety-like behavior (Wieczorek et al. 2015).

In addition to the ability to target alcohol exposure to a specific gestational period, another advantage of the mouse for studies of FASD is the ability to take advantage of robust transgenic mouse models to elucidate underlying developmental mechanisms of FASD at the genetic level. Moreover, alcohol-sensitized transgenic mouse models allow reproducible (and more severe) phenotypes elucidating pathways affected by targeted alcohol exposure. *Shh* is an important gene required for proper craniofacial formation and growth. Heterozygous/knock-out transgenic mouse models of *Shh* and downstream *Shh* pathway genes (*Cdon*, *Ptch1*, *Gas1*, and *Gli2*) have shown increased incidence of HPE (Roessler and Muenke 2010). Interestingly, an acute dose of alcohol administered during late gastrulation–early neurulation to a $Shh^{+/-}$ pregnant female or a pregnant female heterozygous for one of

the downstream SHH pathway genes increases the incidence and severity of HPE (Roessler and Muenke 2010). PAE *Cdon*$^{-/-}$ offspring on a 129S6 background show a significant increase in Alobar and Lobar HPE incidence compared with *Cdon*$^{+/-}$ and WT PAE controls (Hong and Krauss 2012) (Table 1). Moreover, there is a significant incidence of FASD-related pathologies in PAE *Cdon*$^{-/-}$ offspring, including external midline defects, deficient philtrum, shortened and fused pre-maxillary bone, and under-developed maxillary region (Hong and Krauss 2012). In situ hybridization experiments on PAE *Cdon*$^{-/-}$ GD10.25 embryos show reduced mRNA expression of *Gli1* and *Ptch1*, both downstream SHH pathway genes (Hong and Krauss 2012). PAE *Shh*$^{+/-}$ and *Gli2*$^{+/-}$ (haplo-insufficient) offspring show an increased incidence of FASD-related facial dysmorphology: medial facial deficiency, reduced/loss of internasal distance, and loss of lip notch/longer philtrum compared with haplo-insufficient and WT PAE controls (Kietzman et al. 2014) (Table 1). *Shh*$^{+/-}$ and *Gli2*$^{+/-}$ PAE embryos had a 3.2- and 6.6-fold increase, respectively, in the facial dysmorphology score compared with WT littermates. Interestingly, for *Shh*$^{+/-}$ and *Gli2*$^{+/-}$ PAE embryos, the severity of HPE correlated with the severity of facial dysmorphology (Kietzman et al. 2014). Similar observations were found in *Rdh10*$^{-/-}$ and *Raldh2*$^{-/-}$ mice, linking aberrant retinoic acid signalling to the occurrence of midfacial defects (Niederreither et al. 2002; Rhinn et al. 2011). Taken together, the use of transgenic mice in PAE models allows for the identification of certain developmental genes and their downstream pathways that may play a role in the mechanism of FASD. Moreover it allows researchers to study the role of maternal/offspring genetics, as well as epigenetic signatures in alcohol's effects and thus the discovery of biomarkers and potential risk factors of FASD.

Data from studies of acute GD8 (late gastrulation) PAE show less severe frontonasal prominence abnormalities than those seen in GD7 PAE. GD8–8.5 PAE craniofacial malformations include: eye malformations (microphthalmia and colobomas), midfacial hypoplasia, reduced snout width, shorter upper lip length, and micrognathia/mandibular hypoplasia (Parnell et al. 2009; Lipinski et al. 2012). Interestingly, a craniofacial feature characteristic of FASD that was seen in GD7 PAE embryos, a longer philtrum/upper lip length, was no longer seen in GD8 or 8.5 PAE embryos. Instead, a shorter upper lip length was seen, a feature that is more reminiscent of Di George Syndrome; a rare genetic disorder that at one time some considered was associated with FASD (Sulik et al. 1986). GD8–8.5 PAE embryos also do not exhibit clefting or cleft palate malformations at these later ages, as opposed to what was observed in GD7 PAE embryos.

Using MRI, Parnell et al. demonstrated that GD8 PAE embryos have an 8.2% reduction in crown–rump lengths and a 25% reduction in total body volume (including a 19.5% smaller brain volume) compared with controls (Parnell et al. 2009). By contrast, GD8 PAE embryos studied at GD17 show a significant reduction in brain volume, with forebrain dysmorphologies not in the form of HPE, but instead a reduction in olfactory bulbs, and in hippocampus and cerebellum volumes, compared with the controls (Parnell et al. 2009). The pituitary, septal region, and ventricles all were significantly larger in GD8 PAE embryos compared with the controls, even though total brain volumes were reduced. However, GD8.5 PAE embryos studied at GD17 show a significant increase in volume of the olfactory bulb, hippocampus, third ventricle, and pituitary regions compared with the controls (Lipinski et al. 2012). These data suggest that late gastrulation PAE (GD8–8.5) produces a different

craniofacial phenotype compared with early gastrulation PAE (GD7) when using the same dose and acute duration.

Acute GD9 and GD10 PAE studies show negligible frontonasal prominence abnormalities compared with GD7 and GD8 PAE embryos. Mouse models of early neurulation GD9–10 PAE show brain volume abnormalities compared with the controls but do not result in facial dysmorphologies (O'Leary-Moore et al. 2010; Parnell et al. 2013). In their MRI studies, Parnell and colleagues demonstrated that in contrast to GD7–8 PAE embryos, GD9–10 PAE embryos have no statistically significant reduction in crown–rump length or total body volume compared with their stage-matched controls (O'Leary-Moore et al. 2010; Parnell et al. 2013). GD9–10 PAE embryos also have a significant reduction in total brain volume of ~13.6% compared with the stage-matched controls (O'Leary-Moore et al. 2010; Parnell et al. 2013). These results provide support for the hypothesis that without facial dysmorphologies, there are no severe brain abnormalities, although a reduction in brain volume could still have an adverse impact (DeMyer et al. 1964; Sulik and Johnston 1983). Moreover, the reduction in total brain volume in GD9–10 embryos was driven by changes in specific brain regions only. GD9 PAE embryos studied at GD17 showed a significant reduction in cerebellar volume of ~14%, whereas lateral, third, and fourth ventricles showed a significant increase in volume and altered morphology compared with the controls (Parnell et al. 2013). GD10 PAE embryos examined at GD17 exhibit a reduction in cerebral cortex volume, a significant increase in third ventricle volume, and further alterations in morphology in the lateral, third, and fourth ventricles compared with the controls (O'Leary-Moore et al. 2010).

These data suggest that early neurulation PAE (GD9–10) predominantly results in changes to brain volume but not in morphology. Brain regions affected in GD9 PAE embryos are similar to those affected in GD8 PAE embryos; importantly, cerebellar reduction and ventricular enlargement are both features seen in individuals with FASD (Spadoni et al. 2007). Furthermore, the ventricular enlargement seen in GD9–10 PAE embryos likely indicates increased cerebrospinal fluid and a loss of neurons and glia at the cellular level, as overall brain volumes remain the same. In addition, in GD9 PAE embryos the cerebral cortex volume is reduced by ~5%; although not statistically significant, this suggests that enlargement of the lateral ventricle affected development of the cortex (Parnell et al. 2013). In addition, in GD10 PAE embryos the enlarged third ventricle likely affects the development of the thalamus and hypothalamus, which are directly adjacent to the third ventricle. It is commonly known that alcohol exposure in mice during early gestation causes exencephaly, microcephaly, dilated ventricles, and various brain structure defects (Becker et al. 1996). Similar findings are reported in individuals with FASD, whereby MRI studies have shown reduced overall brain volume and dilated ventricles (Sowell et al. 2008). Moreover, individuals with FASD who have sentinel feature(s) may have brain volumes 2 standard deviations smaller than the mean, which could possibly underlie the observed impairments in cognition, learning and memory, anxiety, and attention deficits, culminating in a lower IQ (Spadoni et al. 2007). These changes in brain volume and morphology most likely reflect changes at the cellular level. Indeed, PAE during early gestation modulates neural progenitor cells, by inhibiting their proliferation and differentiation, and causing cell

death in brain regions such as the hippocampus (Nixon and Crews 2002; He et al. 2005; Tateno et al. 2005).

Recent studies have investigated the role of epigenetic modifications in mediating the adverse effects of PAE (Haycock 2009; Ramsay 2010; Lussier et al. 2017). Mouse models of PAE are being used to study global methylation patterns and identify specific genes implicated in the brain abnormalities reported in FASD (Kaminen-Ahola et al. 2010*a*; Kleiber et al. 2012, 2013, 2014; Hill et al. 2014). Many of these studies have used robust acute and chronic dosage paradigms discussed in this review. Using an acute GD9–11 early neurulation (3.0 g/kg, gavage) model, Garro et al. (1991) demonstrated decreased *Dnmt1* activity causing reduced global DNA methylation (using the whole embryo), indicating an increase in active gene transcription compared with what typically occurs at GD11. Furthermore, acute PAE at GD9 (5.8 g/kg, intragastric intubation) caused a decrease in *Igf2* DNA methylation in the whole embryo, causing an increase in *Igf2* expression at GD17 (Downing et al. 2011). Studies of chronic ad libitum alcohol consumption during the first trimester (GD0.5–8.5, 10% *v*/*v* alcohol) have shown increased DNA methylation of *Vmn2r64* and *Olfr110* in male PN28 mice (Kaminen-Ahola et al. 2010*a*), and decreased DNA methylation of *Vglut2* in male PN87 mice (Zhang et al. 2015). Taken together, measurements of DNA methylation provides PAE researchers with new tools to help elucidate the mechanisms of FASD.

Second trimester PAE (with or without first trimester exposure)

The second trimester in mice is from gestational day 11–20. It starts during neurulation, includes organogenesis, and ends at birth. Second trimester PAE mouse models often use a chronic alcohol exposure model or acute exposure to alcohol at specific second trimester gestational time points. Maternal alcohol exposure during the second trimester is done using a variety of methods, including oral gavage, i.p. injection, liquid diet, or ad libitum drinking of alcohol.

Second trimester studies on PAE have assessed craniofacial malformations, brain abnormalities, behavioral consequences, organ development, cerebral blood flow, and gene expression in developing embryos (Anthony et al. 2010; Cui et al. 2010; Akers et al. 2011; El Shawa et al. 2013; Shen et al. 2013). Anthony and colleagues utilized a 4.8% *v*/*v* alcohol liquid diet, administered from GD0 to 19 as a 2-trimester chronic low–moderate model of BAC. They observed marked midfacial dysmorphologies: a reduction in upper- and mid-facial depth measurements in both C57BL/6J and C57BL/6N substrains. C57BL/6N mice also showed a reduction in snout height and a narrowed bigonial line (width of mouth/lips) (Anthony et al. 2010). In a follow-up study, researchers showed that a similar PAE paradigm (4.2% *v*/*v* alcohol, liquid diet model), but with a shorter PAE duration (GD7 to 16, again overlapping part of the first trimester), produced a smaller skull volume and circumference compared with controls at PN7 and PN21 (Shen et al. 2013). Interestingly, it is most likely the exposure to alcohol during the first trimester (first to second trimester exposure paradigm) that caused the craniofacial malformations present in these mice (Table 1). Mice at PN7 exhibited a significant reduction in the parietal region of the skull, whereas assessment at PN21 showed a significant reduction in frontal, parietal, occipital, and

mandibular regions. Shen et al. observed that the cranium (frontal, parietal, occipital regions of the skull) was more sensitive to PAE than the face (nasal and mandibular regions of the skull). The study by Anthony et al. (2010), however, found significant reductions in length, height, and width of the facial region using an earlier/longer PAE duration and slightly higher alcohol concentrations. This suggests that earlier (extending back into the first trimester) and (or) longer chronic PAE may produce FASD-like midfacial malformations, and that cranium and brain abnormalities may be seen if the facial malformations are present (DeMyer et al. 1964). Increasing the dosage of this chronic PAE paradigm results in increased severity of the phenotype (Akers et al. 2011). A liquid diet of 10% *v*/*v* alcohol resulted in a reduction in olfactory bulb, hippocampus (specifically the granule cell layer of the dentate gyrus), and fourth ventricle volumes at P60 in offspring exposed from trimesters 1 through 3 (Akers et al. 2011); these regions are also affected in individuals with FASD (Sowell et al. 2008). Moreover, these PAE mice had impaired odor discrimination, with impaired ability to discriminate enantiomers R- and S-carvone compared with the controls (Akers et al. 2011). Interestingly, neural precursor cells in the subependymal zone of the olfactory bulbs appear to be the most affected (Akers et al. 2011). This study also found that the basal forebrain, posterior anterior commissure, thalamus, and amygdala were larger in PAE mice (Akers et al. 2011). Reduction in the olfactory bulbs and hippocampal volume are consistent findings, using acute first trimester alcohol exposure and in clinical cases of FASD. By contrast, increases in basal fore-brain, thalamus, and amygdala are not commonly seen in mouse models of PAE or in clinical cases of FASD (Coulter et al. 1993; Parnell et al. 2009; Godin et al. 2010; Lipinski et al. 2012).

Cui et al. (2010), using 2 and 4 g/kg gavages on GD5 to 19 as a chronic high BAC model extending into part of the first trimester, reported abnormal dendritic spines of pyramidal neurons in the visual cortex. Not only was there a decrease in overall number of dendritic spines in PN30 mice, but the remaining dendritic spines had an increased mean length and a further reduction of synaptic vesicles compared with the controls (Cui et al. 2010). Moreover, this dendritic spine phenotype was more severe in the 4 g/kg cohort compared with the 2 g/kg cohort, suggesting that persistent high levels of alcohol exposure during the first and second trimesters produces visual cortex abnormalities, in addition to the eye malformations that are common in first trimester mouse models of PAE and found in individuals with FAS (Coulter et al. 1993; Abdelrahman and Conn 2009; Godin et al. 2010). Although this latter study did not investigate craniofacial malformations, brain abnormalities, or behavioral deficits, it is possible that these may have occurred, at least to some extent, given the high level of alcohol exposure and the extended exposure period. The finding that acute alcohol exposure by gavage during the first trimester (as discussed above), using comparable PAE doses but on only one day (during gastrulation), found multiple FASD-like dysmorphologies highlights the importance of timing of exposure in the alcohol effects that are observed.

Behavioral deficits associated with brain abnormalities are also seen in mouse models of PAE, and are commonly found with chronic exposure. El Shawa et al. used 25% *v*/*v* alcohol in an ad libitum drinking model, with exposure from GD0 to 19, as a chronic moderate BAC model. Using CD1 mice, the study showed abnormal somatosensory and visual cortex neuron development, specifically, significantly longer frontal cortex neurons projecting to an

ectopically caudal position in visual cortex regions, as well as malformed cortical layers 1–4, causing overlapping intraneocortical connections that appear disorganized (El Shawa et al. 2013). A similar PAE paradigm, with the only variation being 18% (*v*/*v*) alcohol, showed that cerebellar Purkinje cells presented decreased voltage-gated calcium currents and increased firing rates (Servais et al. 2007). Moreover, PAE mice had an increased latency to fall in rotarod testing, supporting the data indicating that ataxia and motor learning impairments are primarily caused by increased Punkinje cell firing rates (Servais et al. 2007). An inhalation based, chronic GD12–19 and PN2–9 exposure paradigm, modeling high (4.5 g/dL) BAC, demonstrated reductions in cerebellar Purkinje cells in lobules II, IV, V, and IX, with reductions in GABAergic cells only in lobule II (Nirgudkar et al. 2016). Furthermore, PAE mice exhibited a reduction in cerebellar volume, specifically in lobules II, IV, V, VI, VII, IX, and X, suggesting that cerebellar Punkinje cells are more sensitive to alcohol than GABAergic cells (Nirgudkar et al. 2016). Conversely, Cuzon et al. used 2% (*w*/*v*) alcohol in an ad libitum drinking model, with exposure from GD0 to 14.5, as a chronic low BAC model. Using *LHX6-eGFP* and *GAD67-GFP* C57BL/6 mice to express GFP in GABAergic cells (where GABA serves as a transmitter), the study showed that PAE increases the density of GABAergic cells in the marginal, intermediate, and subventricular zones of the cerebral cortex in GD14.5 embryos (Cuzon et al. 2008). Moreover, PAE caused premature tangential migration of primordial GABAergic interneurons from the medial ganglionic eminence to the marginal zone–cortical plate, intermediate, and subventricular–ventricular zones (Cuzon et al. 2008). The data suggest that PAE drives medial ganglionic eminence-derived GABAergic cells toward differentiation and causes increased numbers of GABAergic neurons. It was later found that mice receiving the same paradigm exhibited altered patterning of spontaneous GABA-mediated synaptic barrages and increased GABA-mediated synaptic activity, while showing an increase in Cajal-Reizius cell numbers in cortical layer I (Skorput and Yeh 2015). Similar PAE-induced frontal cortex neuron abnormalities have been previously demonstrated in other mouse models implementing a chronic liquid diet for PAE, including the loss of GABAergic neurons in an acute 3rd trimester PAE paradigm (Skorput and Yeh 2015; Smiley et al. 2015; Abbott et al. 2016), in parallel with the altered neuronal circuitry seen in individuals with FASD (Rema and Ebner 1999; Sowell et al. 2008). Importantly, at PN20 PAE, offspring show increased anxiety and impaired gross and fine motor coordination (El Shawa et al. 2013). In a similar drinking model of PAE using 10% (*w*/*v*) ad libitum, with exposure from GD0 to 19 as a low-moderate BAC paradigm study, PAE mice did not respond to an enriched environment with enhanced neuronal progenitor survival or differentiation in the subgranular zone of the dentate gyrus (Choi et al. 2005). In a follow-up study using the same PAE paradigm but limiting alcohol exposure to 4 h per day, it was shown that post-mitotic neurons from PAE mice housed in an enriched environment are not capable of survival in the dentate gyrus (Kajimoto et al. 2013). Moreover, the neurogenesis deficit was associated with impaired spatial pattern recognition and caused by dentate gyrus-mediated neuronal (learning) impairments in these PAE mice (Kajimoto et al. 2013). Thus, while dosage of alcohol in the PAE mouse paradigms discussed might vary, it appears that there are changes in brain morphology and cellular differentiation at any dosage of alcohol exposure, although some cell types are more resilient than others. Furthermore, abnormalities in forebrain/frontal cortex structure as seen in these studies and in acute first trimester PAE studies can result in

anxiety, learning deficits, and motor coordination impairments reminiscent of the altered or impaired function seen in individuals with FASD (Rasmussen et al. 2008; Sowell et al. 2008; Norman et al. 2009).

While impaired organ development, such as heart and skeletal alterations, may be seen in individuals with FAS (at the most severe end of the FASD spectrum) (Hofer and Burd 2009; Giliberti et al. 2013; Assadi 2014), mouse studies have typically not focused on this. However, some first-to-second trimester models of PAE do show altered organ development, including underdeveloped lungs and kidneys, cardiac anomalies, and gastrointestinal abnormalities. A PAE study using acute exposure to 3.75 g/kg alcohol (resulting in high BACs) from GD11.5 to 13.5 observed that GD18 embryos have reduced body weight and lung weight as well as significantly reduced lung/body weight ratio compared with the control embryos. Histological analysis of the lungs of these PAE embryos found that they were developmentally immature (Wang et al. 2007). Interestingly, there were no craniofacial malformations in these embryos, in contrast to those seen in some first trimester exposure models. Another study using acute alcohol exposure, 3.0 g/kg, once daily by i.p. injection at GD12.5–14.5, observed, using ultrasound, that PAE embryos had significantly reduced arterial blood acceleration and velocity from the umbilical cord to the cerebral arteries. Interestingly this effect was not due to a change in heart rate. Rather, each binge exposure suppressed blood flow velocity for a minimum of 24 h (Bake et al. 2012). The reduction in arterial blood flow to the brain and to other organs like the lungs, heart, and kidneys compromises their development during organogenesis, which is a critical developmental stage. Not only is a reduction in oxygen (causing hypoxia or hypoxic conditions) a worry, but reduction in blood flow velocity carries other concerns like nutritional deficiencies leading to further cellular stress and production of reactive oxygen species (ROS) (Wellen and Thompson 2010).

Caldwell et al. used a liquid diet model with 5.0% (*v*/*v*) alcohol, ad libitum, with exposure from GD0 to GD19, as a chronic low–moderate BAC first-to-second trimester paradigm (alcohol exposure via nursing during PN0–6). This exposure produced a depressive-like phenotype in PN60–90 female mice (Caldwell et al. 2008), including increased immobility in the Porsolt forced swim test and learned helplessness in the Coulbourn Habitest shuttle box, consistent with findings in other recent studies (Hales et al. 2014; Landgraf et al. 2015). The fact that Caldwell et al. did not find anxiety-like behavior may be due to the use of only female mice for their study. As previously described, Wieczorek et al. found that PAE affects anxiety-like behavior in a sexually dimorphic manner; PAE males showed increases in anxiety-like behavior, whereas females exhibited a decrease in anxiety like behavior (Wieczorek et al. 2015). Furthermore, mRNA transcript levels of BDNF (brain-derived neurotrophic factor) were reduced in the medial frontal cortex and hippocampus, and BDNF protein levels were reduced in the medial frontal cortex, suggesting a role for BDNF in depressive-like behavior. Similar reductions in BDNF were seen post-mortem in the hippocampi of clinically depressed and suicidal individuals (Martinowich et al. 2007; Caldwell et al. 2008; Lee and Kim 2010; Autry and Monteggia 2012).

Depressive-like behavior in PAE mice and depression in clinical cases of FASD may be linked to impaired glucocorticoid signalling, specifically, reductions in glucocorticoid

receptors. In follow-up studies, again using a liquid alcohol diet (this time 10.0% *v*/*v* alcohol) ad libitum, from GD0 to GD19 (alcohol exposure via nursing during PN0–6), Caldwell and associates observed learning deficits in hippocampal- and frontal-cortex-dependent tasks. Male PN40–50 PAE mice showed a decrease in delay and trace fear conditioning; a hippocampal-dependent learning task (Brady et al. 2012). Similarly, male PN90–120 PAE mice demonstrated slower reversal learning; a predominantly frontal cortex-dependent task (Allan et al. 2014). Furthermore, these same mice had reduced glucocorticoid receptor nuclear localization in the medial prefrontal cortex (Allan et al. 2014). Glucocorticoid-mediated gene transcription is facilitated through glucocorticoid receptor signalling and is specifically required for adaptive and flexible decision-making strategies during stressful situations, as well as for learning and memory (Diorio et al. 1993; Matsubara et al. 2006). Glucocorticoid signalling also regulates the neuronal connections between the medial prefrontal cortex, hippocampus and amygdala, and ultimately regulates HPA-axis activation in response to stress by negative-feedback (Diorio et al. 1993; Matsubara et al. 2006). Thus, downregulation of glucocorticoid receptors results in exaggerated HPA-axis activity, resulting in an increase in glucocorticoid (corticosterone in most rodents) hormone levels (Diorio et al. 1993). Reduced glucocorticoid receptor mRNA has been reported, post-mortem, in the cortex and hippocampus of brains from individuals with major depressive disorder (Webster et al. 2002; Knable et al. 2004). Moreover, glucocorticoid receptor mRNA is reduced in the lymphocytes of individuals with major depressive disorder, both while they are depressed and during remission (Matsubara et al. 2006). A high incidence of mental health problems, including depression (~45%), is found in children and adults with FASD (Rasmussen et al. 2008). It is noteworthy that postmortem studies of brains from individuals with major depressive disorder and brains of PAE mice show alterations in glucocorticoid receptor signalling in the cortex and hippocampus, suggesting the possibility that alterations in glucocorticoid receptor function and thus in ivity and regulation may underlie at least some of the mental health problems in individuals with FASD.

These studies demonstrate that chronic first-to-second trimester PAE mouse models can produce similar, but not as severe, craniofacial malformations or brain abnormalities as acute exposure (GD7, 8, 8.5, 9) PAE models and compared to the first trimester exposure studies of Sulik's group (Parnell et al. 2009, 2013; Godin et al. 2010; Lipinski et al. 2012), nor do those malformations resemble the FASD sentinel facial features. Furthermore, the brain alterations seen tend to reflect changes in specific brain regions such as the olfactory bulbs, hippocampus, and cerebral cortex (Akers et al. 2011; El Shawa et al. 2013; Abbott et al. 2016). Thus, the alterations produced by chronic first-to-second trimester PAE exposure may resemble the types of alterations observed in FASD, rather than the more severe craniofacial malformations normally observed in children with FASD with sentinel features (Chudley et al. 2005; Cook et al. 2016). An advantage of using first-to-second trimester models of PAE is that it allows researchers to study behavioral outcomes and possibly link them to the structural deficits observed (Cui et al. 2010; El Shawa et al. 2013; Abbott et al. 2016). Furthermore, acute second trimester PAE models allow researchers to study the effects of alcohol exposure during organogenesis, and the effect of alcohol on organ development in particular (Wang et al. 2007; Bake et al. 2012).

Third trimester PAE (with or without first and (or) second trimester exposure)

The third trimester in mice is considered to be the 10 day period from postnatal day 1–10 (equivalent to the human third trimester comprising months 7–9) and includes developmental events that occur during the third trimester of human pregnancy. Third trimester PAE models use either chronic alcohol exposure or acute delivery of alcohol. Alcohol exposure can occur through maternal alcohol exposure (suckling of the pups), by oral gavage, i.p. injection, subcutaneous (s.c.) injection, or liquid diet. As well, alcohol may be delivered directly to the pups, most often by oral gavage or i.p. injection.

Unlike the studies on first and second trimester PAE, there is a plethora of recent chronic and acute third and first-to-third trimester studies of PAE in mice to assess brain abnormalities, behavioral consequences, and changes in gene expression (Kleiber et al. 2011, 2013). Using a first-to-third trimester PAE paradigm, Kleiber et al. observed significantly delayed developmental milestones (negative geotaxis, auditory startle, cliff aversion, and air righting) in PAE offspring during the pre-weaning period when compared to controls (Kleiber et al. 2011). Of relevance, delays in motor coordination, balance, and muscle tone are common in infants with FASD (Staisey and Fried 1983; Kalberg et al. 2006). In addition, PN25–70 PAE offspring demonstrated anxiety-like behaviors in the open field and learning and memory deficits in the Barnes maze when compared with the controls (Kleiber et al. 2011). Whole brain tissue qPCR studies revealed a down-regulation of *Glra1* and *Grin2c*, involved in the glycine receptor complex and glutamate NMDA receptor complexes, respectively (Kleiber et al. 2011). Interestingly, *GRIN* and *GLRA* gene family subunits are down-regulated in individuals with schizophrenia and autism, respectively (Marín 2012; Pilorge et al. 2016). These findings provide further evidence for a possible link between PAE and neurodevelopmental/psychiatric disorders.

Data from PAE models also show pro-inflammatory signalling and abnormal neuronal development in the hypothalamus, hippocampus, and cerebellum —brain regions known to be affected in individuals with FASD (Volgin 2008; Coleman et al. 2012; Drew et al. 2015; Smiley et al. 2015). Drew et al. administered 4.0 $g \cdot kg^{-1} \cdot day^{-1}$ alcohol by oral gavage, in pups from postnatal days 4–9, as a chronic high BAC third trimester alcohol exposure model. They found upregulation of mRNA levels for the pro-inflammatory cytokines IL1β and TNFα in hippocampus, cerebral cortex, and cerebellum that reflects an alcohol-induced activation of microglia in this PAE paradigm. This is significant, as both animal model and clinical studies have shown that alcohol has a significant adverse impact on the hippocampus, cerebral cortex, and cerebellum (Sowell et al. 2008; Parnell et al. 2009, 2013). Moreover, recent studies suggest that microglia play a critical role in normal brain development, including the neuronal synaptic function involved in refinement of brain wiring and synaptic circuitry through synaptic pruning (Paolicelli et al. 2011). Microglia are directly activated by alcohol through TLR2 and TLR4 signalling, triggering a cascade of cytokine, ROS, and NO production (Fernandez-Lizarbe et al. 2013). Moreover, alcohol-activated pro-inflammatory microglia have been shown to facilitate clearance of developing hypothalamic and cerebellar (Purkinje) neurons following the apoptotic insult caused by PAE (Kane et al. 2011; Boyadjieva and Sarkar 2013). Alcohol has also been shown to cause microglial cell death as well as a decrease in intracellular levels of BDNF in hypothalamic

neurons in vitro (Kane et al. 2011; Boyadjieva and Sarkar 2013). Taken together, PAE in the third trimester is detrimental to the developing fetal brain, resulting in inflammation and microglial activation that alter the normal course of brain development and produce behavioral outcomes consistent with those seen in individuals with FASD.

Nitric oxide synthase (NOS) produces nNOS, one of 3 isoforms found almost exclusively expressed in the central and peripheral nervous system (Förstermann and Sessa 2012). It is a key signalling molecule for normal synaptic plasticity in the brain. Karacay et al. used alcohol doses of 2.2 or 4.4 g/kg, i.p, on PN4–9, in either *nNos*$^{-/-}$ knockout transgenic offspring (on a C57BL/6 × 129S6 background) or WT controls. PAE *nNos*$^{-/-}$ offspring showed a significant decrease in adult (PN110–120) brain weight and increased incidence of microcephaly (Karacay et al. 2015). Moreover, *nNos*$^{-/-}$ mice demonstrated severe PAE-induced cerebral cortex pyramidal neuronal loss compared with the WT controls, suggesting abnormalities in brain wiring/connection (Karacay et al. 2015). Furthermore, there was a significant increase in anxiety-like behavior, an impaired startle response, and impaired learning and memory (Morris Water Maze) in PN85–90 mice; behavior outcomes that have previously been observed in other PAE mouse models (Brady et al. 2012; Allan et al. 2014). Increased anxiety and deficits in learning and memory have also been observed in children with FASD (Clarke and Gibbard 2003). In comparison, only the 4.4 g/kg cohort of WT mice experienced a decrease in body weight at PN9–10 and had learning and memory deficits (Karacay et al. 2015). Taken together, normal nNos activity appears to have a protective role, while *nNos* mutations worsen brain abnormalities and behavioral outcomes in the presence of PAE when compared with the PAE WT controls. Interestingly, gene mutations (loss of gene activity) in *Bax* and *tPA* (tissue plasminogen activator) protect the brain from alcohol exposure in mouse models of PAE (Young et al. 2003; Noel et al. 2011).

Using a dose of 3 g/kg alcohol by oral gavage during the third trimester from days PN3–10 or PN3–20, Dursun et al. showed that PAE mice exhibit altered retinal ganglion cell morphology and a decrease in the number of neurons in the ganglion cell layer and in the dorsolateral geniculate nucleus, indicating that alcohol exposure during the third trimester impairs development of the visual system (Dursun et al. 2011, 2013). Coleman et al. observed a reduction in total brain volume (~5%), including a reduction in frontal cortex neurons: parvalbumin GABAergic neurons (18%) and pyramidal neurons (15%) in PN82 mice, using an acute PAE model with an alcohol dose of 2.5 g/kg, s.c. on PN7, modeling high BAC from exposure to alcohol during the third trimester. The hypothalamus, hippocampus, cerebral cortex, corpus callosum, and amygdala were some of the 14 brain regions significantly reduced in volume compared with those in the controls (Coleman et al. 2012). Using a similar dose of alcohol (2.5 g/kg; administered twice over a 2 h interval) PAE paradigm, Smiley et al. observed a reduction in total brain volume (~10%), including in frontal cortex neurons (parvalbumin and calretinin GABAergic neurons; ~30%) in PN72–89 mice (Smiley et al. 2015). In a follow-up study using the same PAE paradigm, adult PAE mice exhibited hyper-activity in their home cages, reduced and fragmented slow-wave sleep, and reduced slow-wave sleep duration (Wilson et al. 2016). Furthermore, PAE mice exhibited impaired contextual fear conditioning memory that was significantly correlated with slow-wave sleep fragmentation (Wilson et al. 2016). Interestingly, mice with impairments in $GABA_A$ receptors (or their subunits) have learning and memory deficits,

hyperactivity, and dysregulated sleep patterns, impairments reminiscent of mouse models of acute third trimester PAE (DeLorey et al. 1998; Volgin 2008). While sleep disturbance patterns have been a relatively well-known co-morbidity of FASD, sleeping problems in children with FASD often goes undiagnosed, and can be associated with deficits in emotional, behavioral, and cognitive function. Moreover, sleep problems are found to occur in 50%–80% and 70% of children with Autism Spectrum Disorder and ADHD, respectively (Hanlon-Dearman et al. 2018). These studies show that acute exposure to alcohol during the third trimester produces significantly reduced brain volumes, including a substantial loss of GABAergic and pyramidal neurons in the developing cerebral cortex, reduced slow-wave sleep, and hyperactivity. These observations are consistent with findings from the first and second trimester of acute and chronic PAE models, suggesting that PAE exposure at any time during gestation causes brain abnormalities that may be associated with abnormal brain function (Sowell et al. 2008).

Mouse models: dosage, duration, and gestational timing of PAE and FASD-like models

To provide an overview of the data from the numerous studies discussed in this review, we color-coded FASD-like outcomes for the 3 general domains of craniofacial malformations, brain abnormalities, and behavioral alterations, and then clustered them by the experimental PAE model parameters of gestational timing, dose and duration of exposure (Table 1). We hypothesized that this approach would reveal areas where the PAE models show particular strength, and hopefully, generate new insights into the neurodevelopmental/neurobiological processes underlying the alterations observed in FASD (Table 1).

Areas of PAE model strength become immediately obvious when evaluating the heat map, which clusters the multiple robust PAE models, exposure timing, dosage, and duration of alcohol insult described in this review. Severe craniofacial malformations, including facial and eye dysmorphologies, are most robustly expressed using a first trimester acute PAE paradigm. Impaired behavioral outcomes, including learning deficits and anxiety- and (or) depression-like behavior are best studied using chronic third trimester PAE models. These evaluations showing that acute PAE early in gestation causes the FAS facial phenotype and that chronic PAE late in gestation causes the behavioral impairments that are consistent with data from multiple PAE mouse model and FASD clinical studies, validating the efficacy of the PAE mouse model heat map. In this regard, the analysis can be a tool for many researchers to quickly assess and understand how any PAE model may best suit their own research question. It also quickly reveals the gaps in our understanding of FASD-like outcomes, such as the impairments seen in neurodevelopment compared with behavior. From the heat map, it can be extrapolated that acute first-trimester PAE results in reduced brain volume (microcephaly) and brain region abnormalities, yet very few studies using this model performed behavioral assessments. Much of our research in mouse models of PAE focuses on the craniofacial or brain malformations that underlie problematic behavior and learning, yet few mouse studies focus on the spectrum of FASD co-morbidities, such as seen in individuals with FASD. These obvious strengths and weaknesses identify immediate avenues of opportunity for research initiatives that will have significant impact.

Additional interesting patterns begin to emerge. Both acute and chronic first trimester models of PAE clearly indicate that one of the most sensitive windows for craniofacial malformations, the sentinel diagnostic and most recognizable features for FAS, is at early gastrulation. This appears to recapitulate in mammalian models what has already been well-demonstrated more broadly in other vertebrate models (Kot-Leibovich and Fainsod 2009; Lovely et al. 2016). These PAE studies suggest that binge alcohol exposure(s) during early gestation can lead to severe FASD outcomes. In humans, this would be the fourth-to-fifth week of pregnancy —a point where most women may only begin to suspect they are pregnant. Add to this that the majority of pregnancies are not planned and that binge drinking is increasingly prevalent among youth, one must recognize that the major prevention strategy —don't drink if you are pregnant — will fail most Canadians. Education and prevention messaging and strategies urgently need to be reassessed to incorporate this reality about the timing of PAE and FASD outcomes.

A second and related pattern to emerge is that acute first-trimester PAE is associated with severe craniofacial outcomes and correlates with developmental brain abnormalities. The 4-digit code shows that craniofacial abnormalities correlate with brain malformations in individuals with FASD, specifically individuals with FAS (Astley and Clarren 2001). In an MRI study, individuals with FASD who had the most severe brain malformations (smaller frontal lobes) also had increased risks for underlying structural abnormalities, and also showed severe brain impairment (Astley et al. 2009). It is possible that seemingly disparate neural crest and neuroectodermal cell lineage-derived outcomes may have common developmental or signalling pathways. It should be mentioned that the sentinel features for FASD are midline anomalies derived from the anterior frontal neural crest progenitor cells of the frontonasal prominence region (early face and forebrain) (Johnston 1975). Therefore, it is possible that the common developmental and (or) signalling pathways forming the frontonasal prominence during early gestation are affected by binge alcohol exposure.

Another interesting observation from the heat map is that acute first-trimester PAE models do not have any significant behavioral impairment. This most likely reflects the fact that behavioral studies have not been performed in these models, as either the craniofacial malformations were too severe and offspring may have been too impaired for testing, or perhaps researchers assessing brain abnormalities were focused primarily on those and did not do behavioral testing. Using the heat map as a tool, one would assume that if the craniofacial and brain malformations are present in a mouse model, one would expect that behavioral impairments would also be present (as per first-second and first-third trimester phenotypic outcomes; Table 1). Moreover, one would expect the most significant adverse outcomes in mouse models of PAE that phenocopy the craniofacial, brain, and behavioral outcomes seen in children with FASD. To further develop robust behavioral PAE mouse models, acute first-trimester models need to be tested for behavioral outcomes where possible.

FAS phenotypes are also observed in chronic low BAC first trimester models of PAE spanning conception to gastrulation stages, suggesting that the incidence of FASD may be further influenced (synergistic with alcohol exposure) by genetic modulation, including epigenetic methylation signatures in the offspring as well as maternal/offspring genetics and

metabolism (Kaminen-Ahola et al. 2010*a*). Having strong robust models of PAE can facilitate the use of genetic models to interrogate candidate signalling pathways affected by PAE. The models of PAE using transgenic mice outlined in this review serve as a reproducible tool with which to demonstrate that transgenic *Shh*, *Glia2*, *Cdon*, and *nNos* mouse models can increase or prevent FASD-like outcomes: craniofacial malformations, brain abnormalities, and behavioral deficits. This also demonstrates that the incidence and severity of FASD may be further influenced by genetic modulation. The PAE transgenic mouse models used in these studies also tend to examine the cellular changes that play a role in the brain abnormalities and behavioral deficits that underlie FASD-like pathophysiology. The link between brain abnormalities and behavioral deficits may lie in changes at the neural cell level (Caldwell et al. 2008; Allan et al. 2014; Smiley et al. 2015). Changes in developing neural cells can lead to changes in brain wiring and connections that are associated with behavioral outcomes (Kleiber et al. 2011; El Shawa et al. 2013; Allan et al. 2014; Karacay et al. 2015).

Finally, examination of these patterns suggests that there are tools that could strengthen the use and analysis of PAE models. For example, directly measuring the actual BAC in the various models of PAE could allow much better assessment of outcomes and allow better comparisons among PAE models. In addition, further research is needed to understand the physiological relevance of BAC in the PAE models to increase our understanding of PAE in pregnant women (Table 2). All of these tools will further allow researchers to choose a specific PAE model with the respective dosage, duration, and gestational timing of alcohol exposure relevant to their research questions. These PAE models will robustly, consistently, and reliably demonstrate the craniofacial malformations, brain abnormalities, and behavioral deficits associated with FASD-like outcomes. This will allow future research using mouse models of PAE to further elucidate the etiology of FASD and discover more targeted treatments and interventions for this disorder.

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References

Abbott CW, Kozanian OO, Kanaan J, Wendel KM, Huffman KJ. The impact of prenatal ethanol exposure on neuroanatomical and behavioral development in mice. Alcohol Clin Exp Res. 2016; 40(1):122–133. DOI: 10.1111/acer.12936 [PubMed: 26727530]

Abdelrahman A, Conn R. Eye abnormalities in fetal alcohol syndrome. Ulster Med J. 2009; 78(3):164–165. [PubMed: 19907681]

Akers KG, Kushner SA, Leslie AT, Clarke L, van der Kooy D, Lerch JP, Frankland PW. Fetal alcohol exposure leads to abnormal olfactory bulb development and impaired odor discrimination in adult mice. Mol Brain. 2011; 4:29.doi: 10.1186/1756-6606-4-29 [PubMed: 21736737]

American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-5. 5. APA; Arlington, Va: 2013.

Allan AM, Goggin SL, Caldwell KK. Prenatal alcohol exposure modifies glucocorticoid receptor subcellular distribution in the medial pre-frontal cortex and impairs frontal cortex-dependent learning. PLoS One. 2014; 9(4):e96200.doi: 10.1371/journal.pone.0096200 [PubMed: 24755652]

Anthony B, Vinci-Booher S, Wetherill L, Ward R, Goodlett C, Zhou FC. Alcohol-induced facial dysmorphology in C57BL/6 mouse models of fetal alcohol spectrum disorder. Alcohol. 2010; 44(7–8):659–671. DOI: 10.1016/j.alcohol.2010.04.002 [PubMed: 20570474]

Assadi F. Renal dysfunction in fetal alcohol syndrome: a potential contributor on developmental disabilities of offspring. J Renal Inj Prev. 2014; 3(4):83–86. DOI: 10.12861/jrip.2014.24 [PubMed: 25610884]

Astley SJ, Clarren SK. Diagnosing the full spectrum of fetal alcohol-exposed individuals: introducing the 4-digit diagnostic code. Alcohol Alcohol. 2000; 35(4):400–410. DOI: 10.1093/alcalc/35.4.400 [PubMed: 10906009]

Astley SJ, Clarren SK. Measuring the facial phenotype of individuals with prenatal alcohol exposure: correlations with brain dysfunction. Alcohol Alcohol. 2001; 36(2):147–159. DOI: 10.1093/alcalc/36.2.147 [PubMed: 11259212]

Astley SJ, Magnuson SI, Omnell LM, Clarren SK. Fetal alcohol syndrome: changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. Teratology. 1999; 59(3):163–172. DOI: 10.1002/(SICI)1096-9926(199903)59:3<163::AID-TERA8>3.0.CO;2-8 [PubMed: 10194807]

Astley SJ, Aylward EH, Olsen HC, Kerns K, Brooks A, Coggins TE, et al. Magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2009; 33(10):1671–1689. DOI: 10.1111/j.1530-0277.2009.01004.x [PubMed: 19572986]

Astley SJ, Bledsoe JM, Davies JK. The essential role of growth deficiency in the diagnosis of fetal alcohol spectrum disorder. Adv Pediatr Res. 2016; 3:9.doi: 10.12715/apr.2016.3.9

Autry AE, Monteggia LM. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev. 2012; 64(2):238–258. DOI: 10.1124/pr.111.005108 [PubMed: 22407616]

Bake S, Tingling JD, Miranda RC. Ethanol exposure during pregnancy persistently attenuates cranially directed blood flow in the developing fetus: evidence from ultrasound imaging in a murine second trimester equivalent model. Alcohol Clin Exp Res. 2012; 36(5):748–758. DOI: 10.1111/j.1530-0277.2011.01676.x [PubMed: 22141380]

Bearer CF. L1 cell adhesion molecule signal cascades: targets for ethanol developmental neurotoxicity. Neurotoxicology. 2001a; 22(5):625–633. DOI: 10.1016/S0161-813X(01)00034-1 [PubMed: 11770884]

Bearer CF. Mechanisms of brain injury: L1 cell adhesion molecule as a target for ethanol-induced prenatal brain injury. Semin Pediatr Neurol. 2001b; 8(2):100–107. DOI: 10.1053/spen.2001.25227 [PubMed: 11464956]

Becker HC, Diaz-Granados JL, Randall CL. Teratogenic actions of ethanol in the mouse: a minireview. Pharmacol Biochem Behav. 1996; 55(4):501–513. DOI: 10.1016/S0091-3057(96)00255-9 [PubMed: 8981580]

Benz J, Rasmussen C, Andrew G. Diagnosing fetal alcohol spectrum disorder: history, challenges and future directions. Paediatr Child Health. 2009; 14(4):231–237. DOI: 10.1093/pch/14.4.231 [PubMed: 20357921]

Besedovsky HO, del Rey A. Immune-neuroendocrine interactions: facts and hypotheses. Endocr Rev. 1996; 17:64–102. DOI: 10.1210/edrv-17-1-64 [PubMed: 8641224]

Besedovsky HO, del Rey A. Physiology of psychoneuroimmunology: a personal view. Brain, Behav, Immun. 2007; 21:34–44. DOI: 10.1016/j.bbi.2006.09.008 [PubMed: 17157762]

Blalock JE, Smith EM. Conceptual development of the immune system as a sixth sense. Brain, Behav, Immun. 2007; 21:23–33. DOI: 10.1016/j.bbi.2006.09.004 [PubMed: 17088044]

Bockhorst KH, Narayana PA, Liu R, Ahobila-Vijjula P, Ramu J, Kamel M, et al. Early postnatal development of rat brain: in vivo diffusion tensor imaging. J Neurosci Res. 2008; 86(7):1520–1528. DOI: 10.1002/jnr.21607 [PubMed: 18189320]

Bodnar, T., Weinberg, J. Prenatal alcohol exposure: impact on neuroendocrine-neuroimmune networks. In: Cui, C.Grandison, L., Noronha, A., editors. Neural-immune interactions in brain function and alcohol related disorders. Springer; Boston, Mass: 2013. p. 307-357.

Bodnar TS, Hill LA, Weinberg J. Evidence for an immune signature of prenatal alcohol exposure in female rats. Brain, Behav, Immun. 2016; 58:130–141. DOI: 10.1016/j.bbi.2016.05.022 [PubMed: 27263429]

Bower E, Szajer J, Mattson SN, Riley EP, Murphy C. Impaired odor identification in children with histories of heavy prenatal alcohol exposure. Alcohol. 2013; 47(4):275–278. DOI: 10.1016/j.alcohol.2013.03.002 [PubMed: 23683527]

Boyadjieva NI, Sarkar DK. Cyclic adenosine monophosphate and brain-derived neurotrophic factor decreased oxidative stress and apoptosis in developing hypothalamic neuronal cells: role of microglia. Alcohol Clin Exp Res. 2013; 37(8):1370–1379. DOI: 10.1111/acer.12104 [PubMed: 23550806]

Brady ML, Allan AM, Caldwell KK. A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. Alcohol Clin Exp Res. 2012; 36(3):457–466. DOI: 10.1111/j.1530-0277.2011.01644.x [PubMed: 21933200]

Brown NA, Goulding EH, Fabro S. Ethanol embryotoxicity: direct effects on mammalian embryos in vitro. Science. 1979; 206(4418):573–575. DOI: 10.1126/science.573922 [PubMed: 573922]

Burd L, Blair J, Dropps K. Prenatal alcohol exposure, blood alcohol concentrations and alcohol elimination rates for the mother, fetus and newborn. J Perinatol. 2012; 32(9):652–659. DOI: 10.1038/jp.2012.57 [PubMed: 22595965]

Caldwell KK, Sheema S, Paz RD, Samudio-Ruiz SL, Laughlin MH, Spence NE, et al. Fetal alcohol spectrum disorder-associated depression: evidence for reductions in the levels of brain-derived neurotrophic factor in a mouse model. Pharmacol Biochem Behav. 2008; 90(4):614–624. DOI: 10.1016/j.pbb.2008.05.004 [PubMed: 18558427]

Cao W, Li W, Han H, O'Leary-Moore SK, Sulik KK, Allan Johnson G, Liu C. Prenatal alcohol exposure reduces magnetic susceptibility contrast and anisotropy in the white matter of mouse brains. NeuroImage. 2014; 102:748–755. DOI: 10.1016/j.neuroimage.2014.08.035 [PubMed: 25175539]

Carlson, BM. Human embryology and developmental biology. 5. Elsevier; Philadelphia, Pa: 2014. Head and neck; p. 294-334.

Cartwright MM, Smith SM. Increased cell death and reduced neural crest cell numbers in ethanol-exposed embryos: partial basis for the fetal alcohol syndrome phenotype. Alcohol Clin Exp Res. 1995; 19(2):378–386. DOI: 10.1111/j.1530-0277.1995.tb01519.x [PubMed: 7625573]

Chernoff GF. The fetal alcohol syndrome in mice: An animal model. Teratology. 1977; 15(3):223–229. [PubMed: 560725]

Choi IY, Allan AM, Cunningham LA. Moderate fetal alcohol exposure impairs the neurogenic response to an enriched environment in adult mice. Alcohol Clin Exp Res. 2005; 29(11):2053–2062. DOI: 10.1097/01.alc.0000187037.02670.59 [PubMed: 16340464]

Chudley AE, Conry J, Cook JL, Loock C, Rosales T, LeBlanc N. Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. Can Med Assoc J. 2005; 172:S1–S21. DOI: 10.1503/cmaj.1040302 [PubMed: 15738468]

Clarke ME, Gibbard WB. Overview of FASD for mental health professionals. Can Child Adolesc Psychiatr Rev. 2003; 12(3):57–63. [PubMed: 19030526]

Clarren, SK. Neuropathology in fetal alcohol syndrome. In: West, JR., editor. Alcohol and brain development. Oxford University Press; New York: 1986. p. 158-166.

Clarren SK, Smith DW. The fetal alcohol syndrome. N Engl J Med. 1978; 298(19):1063–1067. DOI: 10.1056/NEJM197805112981906 [PubMed: 347295]

Coleman LG Jr, Oguz I, Lee J, Styner M, Crews FT. Postnatal day 7 ethanol treatment causes persistent reductions in adult mouse brain volume and cortical neurons with sex specific effects on neurogenesis. Alcohol. 2012; 46(6):603–612. DOI: 10.1016/j.alcohol.2012.01.003 [PubMed: 22572057]

Cook JL, Green CR, Lilley CM, Anderson SM, Baldwin ME, Chudley AE, et al. Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. Can Med Assoc J. 2016; 188(3): 191–197. DOI: 10.1503/cmaj.141593 [PubMed: 26668194]

Coulter CL, Leech RW, Schaefer GB, Scheithauer BW, Brumback RA. Midline cerebral dysgenesis, dysfunction of the hypothalamic-pituitary axis, and fetal alcohol effects. Arch Neurol. 1993; 50(7): 771–775. DOI: 10.1001/archneur.1993.00540070083022 [PubMed: 8323485]

Cudd TA. Animal model systems for the study of alcohol teratology. Exp Biol Med. 2005; 230(6):389–393. DOI: 10.1177/15353702-0323006-06

Cui ZJ, Zhao KB, Zhao HJ, Yu DM, Niu YL, Zhang JS, Deng JB. Prenatal alcohol exposure induces long-term changes in dendritic spines and synapses in the mouse visual cortex. Alcohol Alcohol. 2010; 45(4):312–319. DOI: 10.1093/alcalc/agq036 [PubMed: 20543181]

Cuzon VC, Yeh PWL, Yanagawa Y, Obata K, Yeh HH. Ethanol consumption during early pregnancy alters the disposition of tangentially migrating GABAergic interneurons in the fetal cortex. J Neurosci. 2008; 28(8):1854–1864. DOI: 10.1523/JNEUROSCI.5110-07.2008 [PubMed: 18287502]

Davis JR, Li Y, Rankin CH. Effects of developmental exposure to ethanol on *Caenorhabditis elegans*. Alcohol Clin Exp Res. 2008; 32(5):853–867. DOI: 10.1111/j.1530-0277.2008.00639.x [PubMed: 18336629]

DeLorey TM, Handforth A, Anagnostaras SG, Homanics GE, Minassian BA, Asatourian A, et al. Mice lacking the Beta-3 subunit of the GABA A receptor have the epilepsy phenotype and many of the behavioral characteristics of Angelman syndrome. J Neurosci. 1998; 18(20):8505–8514. [PubMed: 9763493]

DeMyer W, Zeman W, Palmer CG. The face predicts the brain: diagnostic significance of median facial anomalies for holoprosencephaly (arhinencephaly). Pediatrics. 1964; 34:256–263. [PubMed: 14211086]

Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic–pituitary–adrenal responses to stress. J Neurosci. 1993; 13(9):3839–3847. [PubMed: 8396170]

Dobbing J, Sands J. Comparative aspects of the brain growth spurt. Early Hum Dev. 1979; 3(1):79–83. DOI: 10.1016/0378-3782(79)90022-7 [PubMed: 118862]

Dong J, Sulik KK, Chen S-y. The role of NOX enzymes in ethanol-induced oxidative stress and apoptosis in mouse embryos. Toxicol Lett. 2010; 193:94–100. DOI: 10.1016/j.toxlet.2009.12.012 [PubMed: 20026259]

Dou X, Charness ME. Effect of lipid raft disruption on ethanol inhibition of L1 adhesion. Alcohol Clin Exp Res. 2014; 38(11):2707–2711. DOI: 10.1111/acer.12556 [PubMed: 25421507]

Downing C, Johnson TE, Larson C, Leakey TI, Siegfried RN, Rafferty TM, Cooney CA. Subtle decreases in DNA methylation and gene expression at the mouse Igf2 locus following prenatal alcohol exposure: effects of a methyl-supplemented diet. Alcohol (Fayetteville, NY). 2011; 45:65–71. DOI: 10.1016/j.alcohol.2010.07.006

Drew PD, Johnson JW, Douglas JC, Phelan KD, Kane CJM. Pioglitazone blocks ethanol induction of microglial activation and immune responses in the hippocampus, cerebellum, and cerebral cortex in a mouse model of fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2015; 39(3):445–454. DOI: 10.1111/acer.12639 [PubMed: 25703036]

Dursun I, Jakubowska-Dog ru E, van der List D, Leits LC, Coombs JL, Berman RF. Effects of early postnatal exposure to ethanol on retinal ganglion cell morphology and numbers of neurons in the dorsolateral geniculate in mice. Alcohol Clin Exp Res. 2011; 35(11):2063–2074. DOI: 10.1111/j.1530-0277.2011.01557.x [PubMed: 21651582]

Dursun I, Jakubowska-Dog ru E, Elibol-Can B, van der List D, Chapman B, Qi L, Berman RF. Effects of early postnatal alcohol exposure on the developing retinogeniculate projections in C57BL/6 mice. Alcohol. 2013; 47(3):173–179. DOI: 10.1016/j.alcohol.2012.12.013 [PubMed: 23402901]

El Shawa H, Abbott CW, Huffman KJ. Prenatal ethanol exposure disrupts intraneocortical circuitry, cortical gene expression, and behavior in a mouse model of FASD. J Neurosci. 2013; 33(48): 18893–18905. DOI: 10.1523/JNEUROSCI.3721-13.2013 [PubMed: 24285895]

Eriksson CJP. The role of acetaldehyde in the actions of alcohol. Alcohol Clin Exp Res. 2001; 25(s1): 15S–32S. DOI: 10.1111/j.1530-0277.2001.tb02369.x [PubMed: 11391045]

Fernandez-Lizarbe S, Montesinos J, Guerri C. Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells. J Neurochem. 2013; 126(2):261–273. DOI: 10.1111/jnc.12276 [PubMed: 23600947]

Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012; 33(7): 829–837. DOI: 10.1093/eurheartj/ehr304 [PubMed: 21890489]

Gabriel K, Hofmann C, Glavas M, Weinberg J. The hormonal effects of alcohol use on the mother and the fetus. Alcohol Health Res World. 1998; 22:170–177. [PubMed: 15706792]

Gallo PV, Weinberg J. Organ growth and cellular development in ethanol-exposed rats. Alcohol. 1986; 3(4):261–267. DOI: 10.1016/0741-8329(86)90035-2.PMID:3755955 [PubMed: 3755955]

Garro AJ, McBeth DL, Lima V, Lieber CS. Ethanol consumption inhibits fetal DNA methylation in mice: implications for the fetal alcohol syndrome. Alcohol Clin Exp Res. 1991; 15:395–398. [PubMed: 1877725]

Giliberti D, Mohan SS, Brown LAS, Gauthier TW. Perinatal exposure to alcohol: implications for lung development and disease. Paediatr Respir Rev. 2013; 14(1):17–21. DOI: 10.1016/j.prrv.2012.05.005 [PubMed: 23347657]

Glavas MM, Ellis L, Yu WK, Weinberg J. Effects of prenatal ethanol exposure on basal limbic-hypothalamic-pituitary-adrenal regulation: role of corticosterone. Alcohol Clin Exp Res. 2007; 31:1598–1610. DOI: 10.1111/j.1530-0277.2007.00460.x [PubMed: 17760789]

Godin EA, O'Leary-Moore SK, Khan AA, Parnell SE, Ament JJ, Dehart DB, et al. Magnetic resonance microscopy defines ethanol-induced brain abnormalities in prenatal mice: effects of acute insult on gestational day 7. Alcohol Clin Exp Res. 2010; 34(1):98–111. DOI: 10.1111/j.1530-0277.2009.01071.x [PubMed: 19860813]

Goodlett CR, Horn KH, Zhou FC. Alcohol teratogenesis: mechanisms of damage and strategies for intervention. Exp Biol Med. 2005; 230(6):394–406. DOI: 10.1177/15353702-0323006-07

Green CR, Kobus SM, Ji Y, Bennett BM, Reynolds JN, Brien JF. Chronic prenatal ethanol exposure increases apoptosis in the hippocampus of the term fetal guinea pig. Neurotoxicol Teratol. 2005; 27(6):871–881. DOI: 10.1016/j.ntt.2005.07.006 [PubMed: 16112842]

Gubitosi-Klug R, Larimer CG, Bearer CF. L1 cell adhesion molecule is neuroprotective of alcohol induced cell death. Neurotoxicology. 2007; 28(3):457–462. DOI: 10.1016/j.neuro.2006.11.008 [PubMed: 17267039]

Guerri C. Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. Alcohol Clin Exp Res. 1998; 22(2):304–312. DOI: 10.1111/j.1530-0277.1998.tb03653.x [PubMed: 9581633]

Guerri C, Saez R, Portoles M, Renau-Piqueras J. Derangement of astrogliogenesis as a possible mechanism involved in alcohol-induced alterations of central nervous system development. Alcohol Alcohol Suppl. 1993; 2:203–208. [PubMed: 7748301]

Guerri C, Bazinet A, Riley EP. Foetal alcohol spectrum disorders and alterations in brain and behaviour. Alcohol Alcohol. 2009; 44(2):108–114. DOI: 10.1093/alcalc/agn105 [PubMed: 19147799]

Halasz I, Aird F, Li L, Prystowsky MB, Redei E. Sexually dimorphic effects of alcohol exposure in utero on neuroendocrine and immune functions in chronic alcohol-exposed adult rats. Mol Cell Neurosci. 1993; 4:343–353. DOI: 10.1006/mcne.1993.1044 [PubMed: 19912941]

Hales CA, Stuart SA, Anderson MH, Robinson ESJ. Modelling cognitive affective biases in major depressive disorder using rodents. Br J Pharmacol. 2014; 171(20):4524–4538. DOI: 10.1111/bph.12603 [PubMed: 24467454]

Hamilton GF, Boschen KE, Goodlett CR, Greenough WT, Klintsova AY. Housing in environmental complexity following wheel running augments survival of newly generated hippocampal neurons in a rat model of binge alcohol exposure during the third trimester equivalent. Alcohol Clin Exp Res. 2012; 36(7):1196–1204. DOI: 10.1111/j.1530-0277.2011.01726.x [PubMed: 22324755]

Hanlon-Dearman A, Chen ML, Carmichael Olsen H. Understanding and managing sleep disruption in children with FASD. Biochem Cell Biol. 2018; 96 This issue. doi: 10.1139/bcb-2017-0064

Haycock PC. Fetal alcohol spectrum disorders: The epigenetic perspective. Biol Reprod. 2009; 81(4): 607–617. [PubMed: 19458312]

He J, Nixon K, Shetty AK, Crews FT. Chronic alcohol exposure reduces hippocampal neurogenesis and dendritic growth of newborn neurons. European J Neurosci. 2005; 21:2711–2720. DOI: 10.1111/j.1460-9568.2005.04120.x [PubMed: 15926919]

Hellemans KGC, Verma P, Yoon E, Yu WK, Young AH, Weinberg J. Prenatal alcohol exposure and chronic mild stress differentially alter depressive- and anxiety-like behaviors in male and female offspring. Alcohol Clin Exp Res. 2010; 34(4):633–645. DOI: 10.1111/j.1530-0277.2009.01132.x [PubMed: 20102562]

Henderson GI, Devi BG, Perez A, Schenker S. In utero ethanol exposure elicits oxidative stress in the rat fetus. Alcohol Clin Exp Res. 1995; 19(3):714–720. DOI: 10.1111/j.1530-0277.1995.tb01572.x [PubMed: 7573798]

Hill AJ, Drever N, Yin H, Tamayo E, Saade G, Bytautiene E. The role of NADPH oxidase in a mouse model of fetal alcohol syndrome. Am J Obstet Gynecol. 2014; 210(5):466e1–466.e5. DOI: 10.1016/j.ajog.2013.12.019 [PubMed: 24334207]

Hofer R, Burd L. Review of published studies of kidney, liver, and gastrointestinal birth defects in fetal alcohol spectrum disorders. Birth Defects Res, Part A. 2009; 85(3):179–183. DOI: 10.1002/bdra.20562

Hong M, Krauss RS. *Cdon* mutation and fetal ethanol exposure synergize to produce midline signaling defects and holoprosencephaly spectrum disorders in mice. PLoS Genet. 2012; 8(10):e1002999.doi: 10.1371/journal.pgen.1002999 [PubMed: 23071453]

Hoyme HE, Kalberg WO, Elliott AJ, Blankenship J, Buckley D, Marais AS, et al. Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders. Pediatrics. 2016; 138(2):e20154256.doi: 10.1542/peds.2015-4256 [PubMed: 27464676]

Idrus NM, Napper RMA. Acute and long-term Purkinje cell loss following a single ethanol binge during the early third trimester equivalent in the rat. Alcohol Clin Exp Res. 2012; 36(8):1365–1373. DOI: 10.1111/j.1530-0277.2012.01743.x [PubMed: 22404759]

Idrus NM, Happer JP, Thomas JD. Cholecalciferol attenuates perseverative behavior associated with developmental alcohol exposure in rats in a dose-dependent manner. J Steroid Biochem Mol Biol. 2013; 136:146–149. DOI: 10.1016/j.jsbmb.2012.10.012 [PubMed: 23104117]

Ikonomidou C, Bittigau P, Ishimaru MJ, Wozniak DF, Koch C, Genz K, et al. Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. Science. 2000; 287(5455):1056–1060. DOI: 10.1126/science.287.5455.1056 [PubMed: 10669420]

Iqbal U, Brien JF, Banjanin S, Andrews MH, Matthews SG, Reynolds JN. Chronic prenatal ethanol exposure alters glucocorticoid signalling in the hippocampus of the postnatal guinea pig. J Neuroendocrinol. 2005; 17(9):600–608. DOI: 10.1111/j.1365-2826.2005.01349.x [PubMed: 16101899]

Iqbal U, Brien JF, Kapoor A, Matthews SG, Reynolds JN. Chronic prenatal ethanol exposure increases glucocorticoid-induced glutamate release in the hippocampus of the near-term foetal guinea pig. J Neuroendocrinol. 2006; 18(11):826–834. DOI: 10.1111/j.1365-2826.2006.01479.x [PubMed: 17026532]

Jablonski SA, Stanton ME. Neonatal alcohol impairs the context preexposure facilitation effect in juvenile rats: dose-response and post-training consolidation effects. Alcohol. 2014; 48(1):35–42. DOI: 10.1016/j.alcohol.2013.11.002 [PubMed: 24387902]

Jacobson SW, Carr LG, Croxford J, Sokol RJ, Li TK, Jacobson JL. Protective effects of the alcohol dehydrogenase-ADH1B allele in children exposed to alcohol during pregnancy. J Pediatr. 2006; 148(1):30–37. DOI: 10.1016/j.jpeds.2005.08.023 [PubMed: 16423594]

Johnston M. The neural crest in abnormalities of the face and brain. Birth Defects, Orig Artic Ser. 1975; 11(7):1–18.

Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. Lancet. 1973; 302:999–1001. DOI: 10.1016/S0140-6736(73)91092-1.PMID:4127281 [PubMed: 4127281]

Jones KL, Smith DW, Ulleland CN, Streissguth AP. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet. 1973; 301:1267–1271. DOI: 10.1016/S0140-6736(73)91291-9

Kable JA, O'Connor MJ, Olson HC, Paley B, Mattson SN, Anderson SM, Riley EP. Neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE): proposed DSM-5 diagnosis. Child Psychiatry Hum Dev. 2016; 47(2):335–346. DOI: 10.1007/s10578-015-0566-7 [PubMed: 26202432]

Kajimoto K, Allan A, Cunningham LA. Fate analysis of adult hippocampal progenitors in a murine model of fetal alcohol spectrum disorder (FASD). PLoS One. 2013; 8(9):e73788.doi: 10.1371/journal.pone.0073788 [PubMed: 24040071]

Kalberg WO, Provost B, Tollison SJ, Tabachnick BG, Robinson LK, Eugene Hoyme H, et al. Comparison of motor delays in young children with fetal alcohol syndrome to those with prenatal alcohol exposure and with no prenatal alcohol exposure. Alcohol Clin Exp Res. 2006; 30(12): 2037–2045. DOI: 10.1111/j.1530-0277.2006.00250.x [PubMed: 17117969]

Kaminen-Ahola N, Ahola A, Maga M, Mallitt KA, Fahey P, Cox TC, et al. Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model. PLoS Genet. 2010a; 6(1):e1000811.doi: 10.1371/journal.pgen.1000811 [PubMed: 20084100]

Kaminen-Ahola N, Ahola A, Flatscher-Bader T, Wilkins SJ, Anderson GJ, Whitelaw E, Chong S. Postnatal growth restriction and gene expression changes in a mouse model of fetal alcohol syndrome. Birth Defects Res, Part A. 2010b; 88(10):818–826. DOI: 10.1002/bdra.20729

Kane CJM, Phelan KD, Han L, Smith RR, Xie J, Douglas JC, Drew PD. Protection of neurons and microglia against ethanol in a mouse model of fetal alcohol spectrum disorders by peroxisome proliferator-activated receptor-gamma agonists. Brain, Behav, Immun. 2011; 25:S137–S145. DOI: 10.1016/j.bbi.2011.02.016 [PubMed: 21376806]

Karacay B, Mahoney J, Plume J, Bonthius DJ. Genetic absence of nNOS worsens fetal alcohol effects in mice. II: Microencephaly and neuronal losses. Alcohol Clin Exp Res. 2015; 39(2):221–231. DOI: 10.1111/acer.12615 [PubMed: 25664654]

Keen CL, Uriu-Adams JY, Skalny A, Grabeklis A, Grabeklis S, Green K, et al. The plausibility of maternal nutritional status being a contributing factor to the risk for fetal alcohol spectrum disorders: the potential influence of zinc status as an example. BioFactors. 2010; 36(2):125–135. [PubMed: 20333752]

Khoale NCO, Ramchandani VA, Viljoen DL, Li TK. A pilot study of alcohol exposure and pharmacokinetics in women with or without children with fetal alcohol syndrome. Alcohol Alcohol. 2004; 39(6):503–508. DOI: 10.1093/alcalc/agh089 [PubMed: 15351745]

Kietzman HW, Everson JL, Sulik KK, Lipinski RJ. The teratogenic effects of prenatal ethanol exposure are exacerbated by sonic Hedgehog or Gli2 haploinsufficiency in the mouse. PLoS One. 2014; 9(2):e89448.doi: 10.1371/journal.pone.0089448 [PubMed: 24586787]

Kleiber ML, Wright E, Singh SM. Maternal voluntary drinking in C57BL/6J mice: advancing a model for fetal alcohol spectrum disorders. Behav Brain Res. 2011; 223(2):376–387. DOI: 10.1016/j.bbr.2011.05.005 [PubMed: 21601595]

Kleiber ML, Laufer BI, Wright E, Diehl EJ, Singh SM. Long-term alterations to the brain transcriptome in a maternal voluntary consumption model of fetal alcohol spectrum disorders. Brain Res. 2012; 1458:18–33. DOI: 10.1016/j.brainres.2012.04.016 [PubMed: 22560501]

Kleiber ML, Mantha K, Stringer RL, Singh SM. Neurodevelopmental alcohol exposure elicits long-term changes to gene expression that alter distinct molecular pathways dependent on timing of exposure. J Neurodev Disord. 2013; 5(1):6.doi: 10.1186/1866-1955-5-6 [PubMed: 23497526]

Kleiber ML, Diehl EJ, Laufer BI, Mantha K, Chokroborty-Hoque A, Alberry B, Singh SM. Long-term genomic and epigenomic dys-regulation as a consequence of prenatal alcohol exposure: a model for fetal alcohol spectrum disorders. Front Genet. 2014; 5:161. [PubMed: 24917881]

Klintsova AY, Helfer JL, Calizo LH, Dong WK, Goodlett CR, Greenough WT. Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. Alcohol Clin Exp Res. 2007; 31:2073–2082. DOI: 10.1111/j.1530-0277.2007.00528.x [PubMed: 17949464]

Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF. Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium. Mol Psychiatry. 2004; 9(6):609–620. DOI: 10.1038/sj.mp.4001471 [PubMed: 14708030]

Kodituwakku PW. Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: a review. Neurosci Biobehav Rev. 2007; 31(2):192–201. DOI: 10.1016/j.neubiorev.2006.06.020 [PubMed: 16930704]

Kodituwakku PW. Neurocognitive profile in children with fetal alcohol spectrum disorders. Dev Disabil Res Rev. 2009; 15(3):218–224. DOI: 10.1002/ddrr.73 [PubMed: 19731385]

Kotch LE, Chen S, Sulik KK. Ethanol-induced teratogenesis: free radical damage as a possible mechanism. Teratology. 1995; 52(3):128–136. DOI: 10.1002/tera.1420520304 [PubMed: 8638252]

Kot-Leibovich H, Fainsod A. Ethanol induces embryonic malformations by competing for retinaldehyde dehydrogenase activity during vertebrate gastrulation. Dis Models & Mech. 2009; 2(5–6):295–305. DOI: 10.1242/dmm.001420

Landgraf D, Long J, Der-Avakian A, Streets M, Welsh DK. Dissociation of learned helplessness and fear conditioning in mice: a mouse model of depression. PLoS One. 2015; 10(4):e0125892.doi: 10.1371/journal.pone.0125892 [PubMed: 25928892]

LeBel CP, Bondy SC. Oxygen radicals: common mediators of neurotoxicity. Neurotoxicol Teratol. 1991; 13(3):341–346. DOI: 10.1016/0892-0362(91)90081-7 [PubMed: 1886545]

Lee BH, Kim YK. The roles of BDNF in the pathophysiology of major depression and in antidepressant treatment. Psychiatry Invest. 2010; 7(4):231–235. DOI: 10.4306/pi.2010.7.4.231

Lee S, Rivier C. Gender differences in the effect of prenatal alcohol exposure on the hypothalamic–pituitary–adrenal axis response to immune signals. Psychoneuroendocrinology. 1996; 21:145–155. DOI: 10.1016/0306-4530(95)00038-0 [PubMed: 8774059]

Lee S, Imaki T, Vale W, Rivier C. Effect of prenatal exposure to ethanol on the activity of the hypothalamic–pituitary–adrenal axis of the offspring: importance of the time of exposure to ethanol and possible modulating mechanisms. Mol Cell Neurosci. 1990; 1:168–177. DOI: 10.1016/1044-7431(90)90022-V [PubMed: 19912768]

Lee S, Schmidt D, Tilders F, Rivier C. Increased activity of the hypothalamic-pituitary-adrenal axis of rats exposed to alcohol in utero: role of altered pituitary and hypothalamic function. Mol Cell Neurosci. 2000; 16(4):515–528. DOI: 10.1006/mcne.2000.0890 [PubMed: 11085885]

Lemoine P, Harousseau H, Borteyru J, Menuet J. Les enfants des parents alcooliques: anomalies observees apropos de 127 cas. Ouest Med. 1968; 21:476–482.

Lipinski RJ, Hammond P, O'Leary-Moore SK, Ament JJ, Pecevich SJ, Jiang Y, et al. Ethanol-induced face-brain dysmorphology patterns are correlative and exposure-stage dependent. PLoS One. 2012; 7(8):e43067.doi: 10.1371/journal.pone.0043067 [PubMed: 22937012]

Lovely CB, Fernandes Y, Eberhart JK. Fishing for fetal alcohol spectrum disorders: zebrafish as a model for ethanol teratogenesis. Zebrafish. 2016; 13(5):391–398. DOI: 10.1089/zeb.2016.1270 [PubMed: 27186793]

Lussier AA, Weinberg J, Kobor MS. Epigenetics studies of fetal alcohol spectrum disorder: where are we now? Epigenomics. 2017; 9:291–311. DOI: 10.2217/epi-2016-0163 [PubMed: 28234026]

Lussier AA, Stepien KA, Neuman SM, Pavlidis P, Kobor MS, Weinberg J. Prenatal alcohol exposure alters steady-state and activated gene expression in the adult rat brain. Alcohol Clin Exp Res. 2015; 39(2):251–261. DOI: 10.1111/acer.12622 [PubMed: 25684047]

Marín O. Interneuron dysfunction in psychiatric disorders. Nat Rev Neurosci. 2012; 13(2):107–120. DOI: 10.1038/nrn3155 [PubMed: 22251963]

Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nat Neurosci. 2007; 10(9):1089–1093. DOI: 10.1038/nn1971 [PubMed: 17726474]

Matsubara T, Funato H, Kobayashi A, Nobumoto M, Watanabe Y. Reduced glucocorticoid receptor alpha expression in mood disorder patients and first-degree relatives. Biol Psychiatry. 2006; 59(8):689–695. DOI: 10.1016/j.biopsych.2005.09.026 [PubMed: 16458268]

Mattson SN, Crocker N, Nguyen TT. Fetal alcohol spectrum disorders: neuropsychological and behavioral features. Neuropsychol Rev. 2011; 21(2):81–101. DOI: 10.1007/s11065-011-9167-9 [PubMed: 21503685]

May PA, Gossage JP, Kalberg WO, Robinson LK, Buckley D, Manning M, Hoyme HE. Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on

recent in-school studies. Dev Disabil Res Rev. 2009; 15(3):176–192. DOI: 10.1002/ddrr.68 [PubMed: 19731384]

May PA, Blankenship J, Marais AS, Gossage JP, Kalberg WO, Barnard R, et al. Approaching the prevalence of the full spectrum of fetal alcohol spectrum disorders in a South African population-based study. Alcohol Clin Exp Res. 2013; 37(5):818–830. DOI: 10.1111/acer.12033 [PubMed: 23241076]

May PA, Baete A, Russo J, Elliott AJ, Blankenship J, Kalberg WO, et al. Prevalence and characteristics of fetal alcohol spectrum disorders. Pediatrics. 2014; 134(5):855–866. DOI: 10.1542/peds.2013-3319 [PubMed: 25349310]

May PA, Keaster C, Bozeman R, Goodover J, Blankenship J, Kalberg WO, et al. Prevalence and characteristics of fetal alcohol syndrome and partial fetal alcohol syndrome in a Rocky Mountain Region City. Drug Alcohol Depend. 2015; 155:118–127. DOI: 10.1016/j.drugalcdep.2015.08.006 [PubMed: 26321671]

McEwen BS, Stellar E. Stress and the individual. Mechanisms leading to disease. Arch Intern Med. 1993; 153:2093–2101. [PubMed: 8379800]

Michaelis EK. Fetal alcohol exposure: cellular toxicity and molecular events involved in toxicity. Alcohol Clin Exp Res. 1990; 14(6):819–826. DOI: 10.1111/j.1530-0277.1990.tb01821.x [PubMed: 1982397]

Miller, M., editor. Development of the central nervous system: effects of alcohol and opiates. Wiley-Liss; New York: 1992. The effects of prenatal exposure to ethanol on cell proliferation and neuronal migration; p. 47-69.

Muggli E, O'Leary C, Donath S, Orsini F, Forster D, Anderson PJ, et al. "Did you ever drink more?". A detailed description of pregnant women's drinking patterns. BMC Public Health. 2016; 16:683.doi: 10.1186/s12889-016-3354-9 [PubMed: 27485120]

Niederreither K, Vermot J, Schuhbaur B, Chambon P, Dollé P. Embryonic retinoic acid synthesis is required for forelimb growth and anteroposterior patterning in the mouse. Development. 2002; 129:3563–3574. [PubMed: 12117807]

Nirgudkar P, Taylor DH, Yanagawa Y, Valenzuela CF. Ethanol exposure during development reduces GABAergic/glycinergic neuron numbers and lobule volumes in the mouse cerebellar vermis. Neurosci Lett. 2016; 632:86–91. DOI: 10.1016/j.neulet.2016.08.039 [PubMed: 27565053]

Nixon K, Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem. 2002; 83(5):1087–1093. [PubMed: 12437579]

Noel M, Norris EH, Strickland S. Tissue plasminogen activator is required for the development of fetal alcohol syndrome in mice. Proc Natl Acad Sci USA. 2011; 108(12):5069–5074. DOI: 10.1073/pnas.1017608108 [PubMed: 21383198]

Norman AL, Crocker N, Mattson SN, Riley EP. Neuroimaging and fetal alcohol spectrum disorders. Dev Disabil Res Rev. 2009; 15(3):209–217. DOI: 10.1002/ddrr.72 [PubMed: 19731391]

O'Connor MJ, Frankel F, Paley B, Schonfeld AM, Carpenter E, Laugeson EA, Marquardt R. A controlled social skills training for children with fetal alcohol spectrum disorders. J Consult Clin Psychol. 2006; 74(4):639–648. DOI: 10.1037/0022-006X.74.4.639 [PubMed: 16881771]

O'Leary-Moore SK, Parnell SE, Godin EA, Dehart DB, Ament JJ, Khan AA, et al. Magnetic resonance microscopy-based analyses of the brains of normal and ethanol-exposed fetal mice. Birth Defects Res, Part A. 2010; 88:953–964. DOI: 10.1002/bdra.20719

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, et al. Synaptic pruning by microglia is necessary for normal brain development. Science. 2011; 333(6048):1456–1458. DOI: 10.1126/science.1202529 [PubMed: 21778362]

Parnell SE, O'Leary-Moore SK, Godin EA, Dehart DB, Johnson BW, Allan Johnson G, et al. Magnetic resonance microscopy defines ethanol-induced brain abnormalities in prenatal mice: effects of acute insult on gestational day 8. Alcohol Clin Exp Res. 2009; 33(6):1001–1011. DOI: 10.1111/j.1530-0277.2009.00921.x [PubMed: 19302087]

Parnell SE, Holloway HT, O'Leary-Moore SK, Dehart DB, Paniaqua B, Oguz I, et al. Magnetic resonance microscopy-based analyses of the neuroanatomical effects of gestational day 9 ethanol exposure in mice. Neurotoxicol Teratol. 2013; 39:77–83. DOI: 10.1016/j.ntt.2013.07.009 [PubMed: 23911654]

Patten AR, Gil-Mohapel J, Wortman RC, Noonan A, Brocardo PS, Christie BR. Effects of ethanol exposure during distinct periods of brain development on hippocampal synaptic plasticity. Brain Sci. 2013; 3(3):1076–1094. DOI: 10.3390/brainsci3031076 [PubMed: 24961522]

Patten AR, Fontaine CJ, Christie BR. A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. Front Pediatr. 2014; 2:93.doi: 10.3389/fped.2014.00093 [PubMed: 25232537]

Pennington SN, Boyd JW, Kalmus GW, Wilson RW. The molecular mechanism of fetal alcohol syndrome (FAS). I Ethanol-induced growth suppression. Neurobehav Toxicol Teratol. 1983; 5(2): 259–262. [PubMed: 6306496]

Pierce DR, West JR. Blood alcohol concentration: a critical factor for producing fetal alcohol effects. Alcohol. 1986a; 3(4):269–272. DOI: 10.1016/0741-8329(86)90036-4 [PubMed: 3638973]

Pierce DR, West JR. Alcohol-induced microencephaly during the third trimester equivalent: relationship to dose and blood alcohol concentration. Alcohol. 1986b; 3(3):185–191. DOI: 10.1016/0741-8329(86)90043-1 [PubMed: 3741615]

Pierce DR, West JR. Differential deficits in regional brain growth induced by postnatal alcohol. Neurotoxicol Teratol. 1987; 9(2):129–141. DOI: 10.1016/0892-0362(87)90089-4 [PubMed: 3657748]

Pierce DR, Goodlett CR, West JR. Differential neuronal loss following early postnatal alcohol exposure. Teratology. 1989; 40(2):113–126. DOI: 10.1002/tera.1420400205 [PubMed: 2772847]

Pilorge M, Fassier C, Le Corronc H, Potey A, Bai J, De Gois S, et al. Genetic and functional analyses demonstrate a role for abnormal glycinergic signaling in autism. Mol Psychiatry. 2016; 21:936–945. DOI: 10.1038/mp.2015.139 [PubMed: 26370147]

Popova S, Lange S, Probst C, Gmel G, Rehm J. Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. Lancet Global Health. 2017; 5(3):e290–e299. DOI: 10.1016/S2214-109X(17)30021-9 [PubMed: 28089487]

Probyn ME, Parsonson KR, Gårdebjer EM, Ward LC, Wlodek ME, Anderson ST, Moritz KM. Impact of low dose prenatal ethanol exposure on glucose homeostasis in Sprague–Dawley rats aged up to eight months. PLoS One. 2013; 8(3):e59718.doi: 10.1371/journal.pone.0059718 [PubMed: 23533642]

Raineki C, Hellemans KGC, Bodnar T, Lavigne KM, Ellis L, Woodward TS, Weinberg J. Neurocircuitry underlying stress and emotional regulation in animals prenatally exposed to alcohol and subjected to chronic mild stress in adulthood. Front Endocrinol. 2014; 5:5.doi: 10.3389/fendo.2014.00005

Ramadoss J, Wu G, Cudd TA. Chronic binge ethanol-mediated acidemia reduces availability of glutamine and related amino acids in maternal plasma of pregnant sheep. Alcohol. 2008; 42(8): 657–666. DOI: 10.1016/j.alcohol.2008.08.008 [PubMed: 19038697]

Ramanathan R, Wilkemeyer MF, Mittal B, Perides G, Charness ME. Alcohol inhibits cell-cell adhesion mediated by human L1. J Cell Biol. 1996; 133(2):381–390. DOI: 10.1083/jcb.133.2.381 [PubMed: 8609170]

Ramchandani VA, Bosron WF, Li TK. Research advances in ethanol metabolism. Pathol Biol. 2001; 49(9):676–682. DOI: 10.1016/S0369-8114(01)00232-2 [PubMed: 11762128]

Ramsay M. Genetic and epigenetic insights into fetal alcohol spectrum disorders. Genome Med. 2010; 2:27–34. [PubMed: 20423530]

Randall CL, Taylor WJ. Prenatal ethanol exposure in mice: Teratogenic effects. Teratology. 1979; 19(3):305–311. [PubMed: 473082]

Randall CL, Anton RF, Becker HC. Alcohol, pregnancy, and prostaglandins. Alcohol Clin Exp Res. 1987; 11(1):32–36. DOI: 10.1111/j.1530-0277.1987.tb01256.x [PubMed: 3551661]

Randall CL, Anton RF, Becker HC, White NM. Role of prostaglandins in alcohol teratogenesis. Ann N Y Acad Sci. 1989; 562:178–182. DOI: 10.1111/j.1749-6632.1989.tb21016.x [PubMed: 2742275]

Randall CL, Ekblad U, Anton RF. Perspectives on the pathophysiology of fetal alcohol syndrome. Alcohol Clin Exp Res. 1990; 14(6):807–812. DOI: 10.1111/j.1530-0277.1990.tb01818.x [PubMed: 2088115]

Rasmussen C, Andrew G, Zwaigenbaum L, Tough S. Neurobehavioural outcomes of children with fetal alcohol spectrum disorders: a Canadian perspective. Paediatr Child Health. 2008; 13(3): 185–191. [PubMed: 19252695]

Rawat A. Ribosomal proteins synthesis in the fetal and neonatal rat brain as influenced by maternal ethanol consumption. Res Commun Chem Pathol Pharmacol. 1975; 12(4):723–732. [PubMed: 1215660]

Rema V, Ebner FF. Effect of enriched environment rearing on impairments in cortical excitability and plasticity after prenatal alcohol exposure. J Neurosci. 1999; 19(24):10993–11006. [PubMed: 10594080]

Rhinn M, Schuhbaur B, Niederreither K, Dollé P. Involvement of retinol dehydrogenase 10 in embryonic patterning and rescue of its loss of function by maternal retinaldehyde treatment. Proc Natl Acad Sci. 2011; 108(40):16687–16692. DOI: 10.1073/pnas.1103877108 [PubMed: 21930923]

Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: an overview. Neuropsychol Rev. 2011; 21(2):73–80. DOI: 10.1007/s11065-011-9166-x [PubMed: 21499711]

Roebuck TM, Mattson SN, Riley EP. Behavioral and psychosocial profiles of alcohol-exposed children. Alcohol Clin Exp Res. 1999; 23(6):1070–1076. DOI: 10.1111/j.1530-0277.1999.tb04227.x [PubMed: 10397293]

Roessler E, Muenke M. The molecular genetics of holoprosencephaly. Am J Med Genet, Part C. 2010; 154C(1):52–61. DOI: 10.1002/ajmg.c.30236 [PubMed: 20104595]

Rosett HL. A clinical perspective of the fetal alcohol syndrome. Alcohol Clin Exp Res. 1980; 4(2): 119–122. DOI: 10.1111/j.1530-0277.1980.tb05626.x [PubMed: 6990814]

Rufer ES, Tran TD, Attridge MM, Andrzejewski ME, Flentke GR, Smith SM. Adequacy of maternal iron status protects against behavioral, neuroanatomical, and growth deficits in fetal alcohol spectrum disorders. PLoS One. 2012; 7(10):e47499.doi: 10.1371/journal.pone.0047499 [PubMed: 23094056]

Sari Y. Activity-dependent neuroprotective protein-derived peptide, NAP, preventing alcohol-induced apoptosis in fetal brain of C57BL/6 mouse. Neuroscience. 2009; 158(4):1426–1435. DOI: 10.1016/j.neuroscience.2008.11.021 [PubMed: 19073235]

Sawant OB, Lunde ER, Washburn SE, Chen WA, Goodlett CR, Cudd TA. Different patterns of regional Purkinje cell loss in the cerebellar vermis as a function of the timing of prenatal ethanol exposure in an ovine model. Neurotoxicol Teratol. 2013; 35:7–13. DOI: 10.1016/j.ntt.2012.11.001 [PubMed: 23195754]

Servais L, Hourez R, Bearzatto B, Gall D, Schiffmann SN, Cheron G. Purkinje cell dysfunction and alteration of long-term synaptic plasticity in fetal alcohol syndrome. Proc Natl Acad Sci USA. 2007; 104(23):9858–9863. DOI: 10.1073/pnas.0607037104 [PubMed: 17535929]

Shen L, Ai H, Liang Y, Ren X, Anthony CB, Goodlett CR, et al. Effect of prenatal alcohol exposure on bony craniofacial development: a mouse MicroCT study. Alcohol. 2013; 47(5):405–415. DOI: 10.1016/j.alcohol.2013.04.005 [PubMed: 23809873]

Shibley IA Jr, McIntyre TA, Pennington SN. Experimental models used to measure direct and indirect ethanol teratogenicity. Alcohol Alcohol. 1999; 34(2):125–140. DOI: 10.1093/alcalc/34.2.125 [PubMed: 10344772]

Skorput AGJ, Yeh HH. Effects of ethanol exposure in utero on Cajal-Retzius cells in the developing cortex. Alcohol Clin Exp Res. 2015; 39(5):853–862. DOI: 10.1111/acer.12696 [PubMed: 25845402]

Sliwowska JH, Barker JM, Barha CK, Lan N, Weinberg J, Galea LA. Stress-induced suppression of hippocampal neurogenesis in adult male rata is altered by prenatal ethanol exposure. Stress. 2010; 13(4):301–313. [PubMed: 20536332]

Smiley JF, Saito M, Bleiwas C, Masiello K, Ardekani B, Guilfoyle DN, et al. Selective reduction of cerebral cortex GABA neurons in a late gestation model of fetal alcohol spectrum disorder. Alcohol. 2015; 49(6):571–580. DOI: 10.1016/j.alcohol.2015.04.008 [PubMed: 26252988]

Sokol RJ, Clarren SK. Guidelines for use of terminology describing the impact of prenatal alcohol on the offspring. Alcohol Clin Exp Res. 1989; 13(4):597–598. DOI: 10.1111/j.1530-0277.1989.tb00384.x [PubMed: 2679217]

Sokol RJ, Delaney-Black V, Nordstrom B. Fetal alcohol spectrum disorder. J Am Med Assoc. 2003; 290(22):2996–2999. DOI: 10.1001/jama.290.22.2996

Sowell ER, Mattson SN, Kan E, Thompson PM, Riley EP, Toga AW. Abnormal cortical thickness and brain-behavior correlation patterns in individuals with heavy prenatal alcohol exposure. Cerebral Cortex. 2008; 18(1):136–144. DOI: 10.1093/cercor/bhm039 [PubMed: 17443018]

Spadoni AD, McGee CL, Fryer SL, Riley EP. Neuroimaging and fetal alcohol spectrum disorders. Neurosci Biobehav Rev. 2007; 31(2):239–245. DOI: 10.1016/j.neubiorev.2006.09.006 [PubMed: 17097730]

Spong CY, Abebe DT, Gozes I, Brenneman DE, Hill JM. Prevention of fetal demise and growth restriction in a mouse model of fetal alcohol syndrome. J Pharmacol Exp Ther. 2001; 297(2): 774–779. [PubMed: 11303069]

Stade B, Bailey C, Dzendoletas D, Sgro M, Dowswell T, Bennett D. Psychological and/or educational interventions for reducing prenatal alcohol consumption in pregnant women and women planning pregnancy. Cochrane Database Syst Rev. 2009; 2:CD004228.doi: 10.1002/14651858.CD004228.pub2

Staisey NL, Fried PA. Relationships between moderate maternal alcohol consumption during pregnancy and infant neurological development. J Stud Alcohol. 1983; 44(2):262–270. [PubMed: 6645511]

Stratton, K., Howe, C., Battaglia, F. Fetal alcohol syndrome: diagnosis and clinical evaluation of fetal alcohol syndrome. National Academy Press; Washington, D.C: 1996.

Streissguth AP, Clarren SK, Jones KL. Natural history of the fetal alcohol syndrome: a 10-year follow-up of eleven patients. Lancet. 1985; 326(8446):85–91. DOI: 10.1016/S0140-6736(85)90189-8

Sulik K, Johnston M. Sequence of developmental alterations following acute ethanol exposure in mice: craniofacial features of the fetal alcohol syndrome. Am J Anat. 1983:257–269.

Sulik KK, Schoenwolf GC. Highlights of craniofacial morphogenesis in mammalian embryos, as revealed by scanning electron microscopy. Scanning Electron Microsc. 1985; 4:1735–1752.

Sulik KK, Johnston MC, Webb MA. Fetal alcohol syndrome: embryogenesis in a mouse model. Science. 1981; 214(4523):936–938. DOI: 10.1126/science.6795717 [PubMed: 6795717]

Sulik KK, Cook CS, Webster WS. Teratogens and craniofacial malformations: relationships to cell death. Development. 1988; 103:213–231. [PubMed: 3074910]

Sulik KK, Johnston MC, Daft PA, Russell WE, Dehart DB. Fetal alcohol syndrome and DiGeorge anomaly: critical ethanol exposure periods for craniofacial malformations as illustrated in an animal model. Am J Med Gen (Suppl). 1986; 2:97–112.

Suttie M, Foroud T, Wetherill L, Jacobson JL, Molteno CD, Meintjes EM, et al. Facial dysmorphism across the fetal alcohol spectrum. Pediatrics. 2013; 131(3):e779–e788. DOI: 10.1542/peds.2012-1371 [PubMed: 23439907]

Tan CH, Denny CH, Cheal NE, Sniezek JE, Kanny D. Alcohol use and binge drinking among women of childbearing age — United States, 2011–2013. MMWR – Morbid Mortal Wkly Rep. 2015; 64(37):1042–1046. DOI: 10.15585/mmwr.mm6437a3

Tateno M, Ukai W, Yamamoto M, Hashimoto E, Ikeda H, Saito T. The effect of ethanol on cell fate determination of neural stem cells. Alcohol Clin Exp Res. 2005; 29:225S–229S. [PubMed: 16385227]

Taylor, A., Branch, B., Van Zuylen, J., Redei, E. Maternal alcohol consumption and stress responsiveness in offspring. In: Chrousos, GP.Loriaux, DL., Gold, PW., editors. Mechanisms of physical and emotional stress. Springer; Boston: 1988. p. 311-317.

Thomas JD, Idrus NM, Monk BR, Dominguez HD. Prenatal choline supplementation mitigates behavioral alterations associated with prenatal alcohol exposure in rats. Birth Defects Res, Part A. 2010; 88(10):827–837. DOI: 10.1002/bdra.20713

Uban KA, Comeau WL, Ellis LA, Galea LAM, Weinberg J. Basal regulation of HPA and dopamine systems is altered differentially in males and females by prenatal alcohol exposure and chronic variable stress. Psychoneuroendocrinology. 2013; 38(10):1953–1966. DOI: 10.1016/j.psyneuen.2013.02.017 [PubMed: 23579081]

Volgin DV. Perinatal alcohol exposure leads to prolonged upregulation of hypothalamic $GABA_A$ receptors and increases behavioral sensitivity to gaboxadol. Neurosci Lett. 2008; 439(2):182–186. DOI: 10.1016/j.neulet.2008.05.011 [PubMed: 18514412]

Wagner JL, Klintsova AY, Greenough WT, Goodlett CR. Rehabilitation training using complex motor learning rescues deficits in eyeblink classical conditioning in female rats induced by binge-like neonatal alcohol exposure. Alcohol Clin Exp Res. 2013; 37(9):1561–1570. DOI: 10.1111/acer.12122 [PubMed: 23647404]

Wahlsten D, Bachmanov A, Finn DA, Crabbe JC. Stability of inbred mouse strain differences in behavior and brain size between laboratories and across decades. Proc Natl Acad Sci USA. 2006; 103(44):16364–16369. DOI: 10.1073/pnas.0605342103 [PubMed: 17053075]

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(3):269e1–269.e4. DOI: 10.1016/j.ajog.2007.06.031 [PubMed: 17826415]

Webster WS, Walsh DA, Lipson A, McEwen SE. Teratogenesis after acute alcohol exposure in inbred and outbred mice. Neurobehav Toxicol Terat. 1980; 2:227–234.

Webster MJ, Knable MB, O'Grady J, Orthmann J, Weickert CS. Regional specificity of brain glucocorticoid receptor mRNA alterations in subjects with schizophrenia and mood disorders. Mol Psychiatry. 2002; 7(9):985–994. DOI: 10.1038/sj.mp.4001139 [PubMed: 12399952]

Weinberg J. Nutritional issues in perinatal alcohol exposure. Neurobehav Toxicol Teratol. 1984; 6(4):261–269. [PubMed: 6514087]

Weinberg J. Effects of ethanol and maternal nutritional status on fetal development. Alcohol Clin Exp Res. 1985; 9(1):49–55. DOI: 10.1111/j.1530-0277.1985.tb05049.x [PubMed: 3887968]

Weinberg J. Prenatal ethanol exposure alters adrenocortical development of offspring. Alcohol Clin Exp Res. 1989; 13(1):73–83. DOI: 10.1111/j.1530-0277.1989.tb00287.x [PubMed: 2538092]

Weinberg J. Prenatal ethanol effects: sex differences in offspring stress responsiveness. Alcohol. 1992; 9:219–223. DOI: 10.1016/0741-8329(92)90057-H [PubMed: 1605890]

Weinberg J. Neuroendocrine effects of prenatal alcohol exposure. Ann N Y Acad Sci. 1993; 697:86–96. DOI: 10.1111/j.1749-6632.1993.tb49925.x [PubMed: 8257025]

Weinberg J, Bezio S. Alcohol-induced changes in pituitary-adrenal activity during pregnancy. Alcohol Clin Exp Res. 1987; 11:274–280. DOI: 10.1111/j.1530-0277.1987.tb01307.x [PubMed: 3307493]

Weinberg J, Gallo P. Prenatal ethanol exposure: pituitary-adrenal activity in pregnant dams and offspring. Neurobehav Toxicol Teratol. 1982; 4:515–520. [PubMed: 7177301]

Weinberg J, Sliwowska JH, Lan N, Hellemans KGC. Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. J Neuroendocrinol. 2008; 20:470–488. DOI: 10.1111/j.1365-2826.2008.01669.x [PubMed: 18266938]

Wellen KE, Thompson CB. Cellular metabolic stress: Considering how cells respond to nutrient excess. Mol Cell. 2010; 40(2):323–332. DOI: 10.1016/j.molcel.2010.10.004 [PubMed: 20965425]

West JR, Chen WA, Pantazis NJ. Fetal alcohol syndrome: the vulnerability of the developing brain and possible mechanisms of damage. Metab Brain Dis. 1994; 9(4):291–322. DOI: 10.1007/BF02098878 [PubMed: 7898398]

Wieczorek L, Fish EW, O'Leary-Moore SK, Parnell SE, Sulik KK. Hypothalamic–pituitary–adrenal axis and behavioral dysfunction following early binge-like prenatal alcohol exposure in mice. Alcohol. 2015; 49(3):207–217. DOI: 10.1016/j.alcohol.2015.01.005 [PubMed: 25709101]

Wilkemeyer MF, Menkari CE, Spong CY, Charness ME. Peptide antagonists of ethanol inhibition of L1-mediated cell-cell adhesion. J Pharmacol Exp Ther. 2002; 303(1):110–116. DOI: 10.1124/jpet.102.036277 [PubMed: 12235240]

Wilkemeyer MF, Chen S-y, Menkari CE, Brenneman DE, Sulik KK, Charness ME. Differential effects of ethanol antagonism and neuroprotection in peptide fragment NAPVSIPQ prevention of ethanol-induced developmental toxicity. Proc Natl Acad Sci. 2003; 100(14):8543–8548. DOI: 10.1073/pnas.1331636100 [PubMed: 12808140]

Wilson DA, Masiello K, Lewin MP, Hui M, Smiley JF, Saito M. Developmental ethanol exposure-induced sleep fragmentation predicts adult cognitive impairment. Neuroscience. 2016; 322:18–27. DOI: 10.1016/j.neuroscience.2016.02.020 [PubMed: 26892295]

Young C, Klocke BJ, Tenkova T, Choi J, Labruyere J, Qin YQ, et al. Ethanol-induced neuronal apoptosis in vivo requires BAX in the developing mouse brain. Cell Death Differ. 2003; 10(10): 1148–1155. DOI: 10.1038/sj.cdd.4401277 [PubMed: 14502238]

Zhang X, Sliwowska JH, Weinberg J. Prenatal alcohol exposure and fetal programming: effects on neuroendocrine and immune function. Exp Biol Med. 2005; 230:376–388. DOI: 10.1177/15353702-0323006-05

Zhang X, Lan N, Bach P, Nordstokke D, Yu W, Ellis L, et al. Prenatal alcohol exposure alters the course and severity of adjuvant-induced arthritis in female rats. Brain, Behav, Immun. 2012; 26:439–450. DOI: 10.1016/j.bbi.2011.11.005 [PubMed: 22155498]

Zhang CR, Ho MF, Vega MC, Burne TH, Chong S. Prenatal ethanol exposure alters adult hippocampal VGLUT2 expression with concomitant changes in promoter DNA methylation, H3K4 trimethylation and miR-467b-5p levels. Epigen Chrom. 2015; 8(1):40.doi: 10.1186/s13072-015-0032-6

Table 1

FASD-like outcomes in mouse models of prenatal alcohol exposure (PAE): craniofacial malformations, brain abnormalities, and behavioral deficits seen by varying the dosage, duration, and gestational timing of PAE.

Exposure Paradigm					Model			Effects									Reference
	Level	Treatment		Time	Species	Age Assessment	Sex	FD	ED	SH	RV	RA	CA	AL	LD	IC	
1st Trimester	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse C57Bl6	GD 14	M/F	■	■	■							(Sulik et al. 1981)
	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse C57Bl6	GD17	M/F	■	■	■	■	■					(Godin et al. 2010)
	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse 129S6	GD10–19	M/F	■		■	■	■					(Hong and Krauss 2012)
	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse C57Bl6	PN 45	M/F					■					(Cao et al. 2014)
	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse C57Bl6 Shh & Gli2 +/−	GD 17	M/F	■	■		■	■					(Kietzman et al. 2014)
	A	2.9g/kg (2X/day)	I.P.	GD 7	Mouse C57Bl6	GD17	M/F	■		■	■	■					(Lipinski et al. 2012)
				GD 8				■			■	■					
	A	2.8g/kg (2X/day)	I.P.	GD 8	Mouse C57Bl6	GD17	M/F		■	■	■	■					(Parnell et al. 2009)
	A	2.9g/kg (2X/day)	I.P.	GD 9	Mouse C57Bl6	GD 17	M/F				■	■					(Parnell et al. 2013)
	A	2.9g/kg (2X/day)	I.P.	GD 10	Mouse C57Bl6	GD 17	M/F				■	■					(O'Leary et al. 2010)
	C	10% v/v EtOH	AdL	GD 0–8.5	Mouse C57Bl6	PD 21–30	M/F	■		■			■				(Kaminen-Ahola et al. 2010a)
	C	10% v/v EtOH	AdL	GD 0–8.5	Mouse C57Bl6	GD 16.5	M/F						■				(Kaminen-Ahola et al. 2010b)
1st to 2nd Trimester	C	4.2% v/v EtOH	LD	GD7–16	Mouse C57Bl6	PN7	M/F			■							(Shen et al. 2013)
						PN21		■		■							
	C	10% v/v EtOH	LD	GD 0–19	Mouse C57Bl6	PN60	M/F					■			■		(Akers et al. 2011)
	C	4.8% v/v EtOH	LD	GD 0–19	Mouse C57Bl6	GD17	M/F	■									(Anthony et al. 2010)

	C	25% v/v EtOH	LD	GD 0–19	Mouse CD1	P0–P20	M/F										(El Shawa et al. 2013)
	C	2.0g/kg	G	GD 5–19	Mouse C57Bl6	PD 0–30	M/F										(Cui et al. 2010)
		4.0g/kg															
3rd Trimester	A	2.5g/kg (2X/day)	G	PN 7	Mouse C57Bl6	PN 72–89	M/F										(Smiley et al., 2015)
	A	2.5g/kg (1X/day)	S.C.	PN 7	Mouse C57Bl6	PN82	M/F										(Coleman et al. 2012)
	C	2.2g/kg	I.P.	PN 4–9	Mouse F2 129SVJ x C57Bl6	PD 85–90	M/F										(Karacay et al. 2016)
		4.4g/kg															
		2.2 & 4.4 g/kg			Mouse (nNos −/−)												
	C	4g/kg (1X/day)	G	PN 4–9	Mouse C57Bl6	PN10	M/F										(Drew et al. 2015)
1st to 3rd Trimester	C	10% v/v EtOH	LD	GD0-PN6	Mouse C57Bl6	PN 90–150	M										(Brady et al. 2012)
	C	10% v/v EtOH	LD	GD0-PN6	Mouse C57Bl6	PN 40–50	M										(Allan et al. 2014)
	C	5% v/v EtOH	LD	GD0-PN6	Mouse C57Bl6	PN 60–90	M										(Caldwell et al. 2008)
	C	10% v/v EtOH	LD	GD 0 – PN 10	Mouse C57Bl6	PN 2–21	M/F										(Kleiber et al. 2011)
						PN 25–70											

Abbreviations:

Exposure Paradigm:
C - Chronic PAE
A - Acute PAE
I.P. - Intraperitoneal Inj.
AdL -*Ad Libitum*
LD - Liquid Diet
G - Oral Gavage
S.C. - Sub-Cutaneous Inj.
GD - Gestational Day
PN - Postnatal Day

Effects:
FD - Facial Dysmorphologies
ED - Eye Defects
SH - Small Head
VR - Volume Reduced
RA - Region Aberrations

CA - Cellular Anomalies
AL - Anxiety & Depression-Like
LD - Learning Deficits
IC - Impaired Coordination

Table 2

Mouse model of prenatal alcohol exposure (PAE) showing standardization of blood alcohol concentration (BAC): variations in dosage depending on delivery or timing (liquid diet/ad libitum or gavage/intraperitoneal injection) of alcohol exposure in mouse models of PAE.

PAE dosage	PAE dosage†	BAC (per day)‡	BAC (per hour)‡‡	BAC (human)
4.2% *v*/*v*	8.14	1480.00	61.67	
4.8% *v*/*v*	9.30	1690.91	70.45	
5.0% *v*/*v*	9.69	1761.82	73.41	80 mg/dL LDL *
10.0% *v*/*v*	19.38	3523.64	146.82	200 mg/dLAIBO **
25.0% *v*/*v*	48.45	8809.09	367.05	
2.0 g/kg	2.00	363.64	363.64	400 mg/dL AID ***
2.2 g/kg	2.20	400.00	400.00	
2.5 g/kg	2.50	454.55	454.55	
2.8 g/kg	2.80	509.09	509.09	
2.9 g/kg	2.90	527.27	527.27	
4.0 g/kg	4.00	727.27	727.27	
4.4 g/kg	4.40	800.00	800.00	

Note: The purpose of this table was to standardize the PAE dosage paradigms using the most common PAE dosages discussed in this review. First, PAE dosages were standardized to grams per kilogram of body weight (instead of volume of alcohol/distilled water). Mouse weight was standardized to 0.025 kg (25 g). The PAE dosage was then divided by the amount of blood in the average mouse and provides theBAC equivalent (per day). The BAC equivalent was then divided by the number of hours a day alcohol was provided using those paradigms (*v*/*v* dosage paradigms are ad libitum and all day voluntary drinking). As a result *v*/*v* dosages were divided by 24 h where g/kg gastric gavage techniques were divided by 1 h to get the maximum blood alcohol concentration possible in that mouse model paradigm. As a result a 25% *v*/*v* alcohol dosage paradigm mimics the 2.0 g/kg gastric gavage alcohol dosage paradigm using blood alcohol concentration per hour equivalent.

† Standardized to grams (g) of ethanol per kilograms (kg) of mouse weight (avg. mouse = 25 g).

‡ Total amount of ethanol consumed over a 24 h period (chronic ad libitum paradigms are higher due to all day voluntary drinking compared with binge acute paradigms (avg. mouse blood conc. = 1.375 mL). Units are mg/dL.

‡‡ Amount of ethanol approximately consumed per hour of the day (binge acute paradigms are administered by i.p., s.c., or oral gavage producing high (peak) BACs as it is in the blood system for 1 hr (or 2) after exposure). Units are mg/dL.

* Legal driving limit (LDL) in humans (0.08 or 80 mg/dL).

** Alcohol induced black out (AIBO) in humans (200 mg/dL).

*** Alcohol induced death (AID) in humans (400 mg/dL).