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## REVIEW

# Placental Hypoxia and Foetal Development Versus Alcohol Exposure in Pregnancy

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**Abstract — Aims:** To examine the causes of variability in the effect of maternal drinking on the foetus, with particular reference to the pattern, frequency and duration of the period of drinking, differences in maternal, foetal and placental metabolism of ethanol/acetaldehyde, and genetic factors. **Methods:** Narrative review of published studies of the pathogenesis of foetal alcohol syndrome (FAS) with emphasis in the development of the central nervous system. **Results:** Animal models suggest that acetaldehyde, the primary hepatic oxidative metabolite of ethanol, reaches the foetus either by placental production or by placental transference, which in turn could affect foetal growth and development. The most likely hypothesis regarding the decrease of foetal growth is via hypoxia and increased oxidative/nitrative stress, which interfere with cellular processes that require oxygen in order to function adequately, such as placental transport. **Conclusion:** There seems to be an association between the teratogenic effect, hypoxia and oxidative stress, the molecular mechanism involved (e.g. apoptosis) and the range of effects. The review sums ups the evidence that could explain some of the abnormalities in the brain development that could be related to behavioural problems observed in individuals with FAS/foetal alcohol spectrum disorder. This suggests that alcohol consumption produces failures in the normal migration of radial cells, from which the rest of the brain cells would eventually develop.

## INTRODUCTION

Upon recognition of pregnancy, most women spontaneously reduce their alcohol intake. However, the recognition of the pregnancy does not occur until 4–6 weeks of gestation (Floyd *et al*., 1999) and thus, many women may drink alcohol prior to realizing they are pregnant.

Alcohol is one of the commonest teratogenic agents and its uncontrolled consumption by pregnant women can give rise to an irreversible condition in the child, foetal alcohol syndrome (FAS). This syndrome, described by Jones and Smith (1973), is characterized by intrauterine growth retardation (IUGR), craniofacial malformations, physical and mental retardation, and cardiac septal defects. However, FAS represents only a part of the spectrum of foetal alcohol effects. The term foetal alcohol spectrum disorders (FASDs), is currently used to describe a lesser degree of deformities associated to FAS, sometimes unnoticeable except by close examination, as well as a variable degree of mental retardation (Rossett, 1980; Chaudhuri, 2000). While some offspring of mothers who drink heavily during pregnancy develop FAS and evidence all the symptoms described earlier, some others show no symptoms at all, a condition known as ethanol resilience (Gemma *et al*., 2006, 2007). Also, a considerable number show only partial FAS-related phenotypes.

It has been proposed that maternal ethanol ingestion may cause foetal injury, particularly the impairment of somatic and brain growth, by at least two mechanisms: (a) directly, by fetotoxicity from ethanol and/or acetaldehyde; (b) indirectly, by ethanol-induced placental injury and selective foetal malnutrition (Fisher and Karl, 1988; Bosco, 2005). The wide variety of cellular/biochemical effects of alcohol on foetal tissues is in itself a puzzle, and this fact strongly suggests that the fetotoxic responses to alcohol may reflect a multifactorial setting.

This review will focus on recent insights into normal process of placental and human development that are susceptible to the mother's alcohol consumption.

### *Embryonic developmental processes*

After fertilization, the nuclei of the sperm and the oocyte fuse to form the zygote. As this cell travels to the uterus, it divides, forming a cluster of cells, the morula, by about 3 days after fertilization. As the morula enters the uterine cavity, fluid penetrates into it to form the blastocyst. The epithelial outer wall of this structure is the trophoblast, and the inner cell mass is the embryoblast. During implantation, the trophoblast differentiates into two layers: the syncytiotrophoblast and the cytotrophoblast. The former is external and has many nuclei and a continuous cytoplasm forming a syncytium. The cytotrophoblast consists of a layer of ovoid cells immediately under the syncytiotrophoblast. Both structures contribute to the formation of the villi and ultimately the placenta. Villous cytotrophoblast fuse to form the syncytiotrophoblast layer that contributes to the exchange of gas and nutrient, as well as to the process of waste elimination. In anchoring villi, cytotrophoblast generates a multilayered column of highly invasive extravillous trophoblast (EVT) that later migrates into the decidua and remodels the endometrial spiral arteries to produce the low-resistance vascular system that is essential for foetal growth (Pijnenborg *et al*., 1981a,b; Aplin, 1991).

Meanwhile, the embryoblast cell mass organizes itself into a flat disc-shaped structure, the bilaminar germ disc, which consists of two layers of cells (the epiblast and the hypoblast), with the primitive streak forming ulterior on the surface of the epiblast. During the gastrulation process, the cells from the epiblast layer migrate towards the primitive streak to form the mesoderm layer between the epiblast and the hypoblast layers—forming thus the trilaminar germ disc, composed by ectoderm, mesoderm and endoderm. These three layers are responsible for the formation of all the body organ systems.

An important but yet unresolved question is when exactly the critical periods for ethanol exposure are manifested during embryogenesis, and which of the molecular

components that are expressed during such periods are ethanol-sensitive (Chudley *et al*., 2005; Zhou *et al*., 2011).

*Placental functions*

The passage of nutrients from the maternal blood to the foetus is mediated by the placenta, an organ that establishes critical functional connections for embryonic survival (Cross *et al*., 1994). The placenta transports oxygen, nutrients and antibodies to the foetus and removes carbon dioxide and metabolic waste; serves as a protective barrier against harmful effects produced by certain drugs and microorganisms; acts as a partial barrier between the mother and foetus in order to prevent foetal and maternal blood from mixing; and produces steroid and peptide hormones essential for maintaining the pregnancy (Bosco, 1995, 2005). It is essential then that for a normal foetal metabolism and growth, an adequate exchange across the placenta must occurs.

Epigenetic regulation of the placenta evolves during the preimplantation developmental period and further gestation. Epigenetic modification refers to heritable changes in gene expression that are not mediated by alterations in the DNA sequence (Jaenisch and Bird, 2003). These expression patterns, including the important parent-of-origin-dependent gene expression resulting from genomic imprinting, play a pivotal role in proper foetal and placental development. Imprinted genes are those whose expression depends on their parental origin (Delaval and Feil, 2004). Imprinting is also controlled by epigenetic mechanisms under the control of environmental factors and nutrients (Reik *et al*., 2003). This may provide a linkage between maternal nutrition and foetal placental growth (Myatt, 2006; Nelissen *et al*., 2011).

It is known that the number of placental genes altered by moderate ethanol exposure plays critical roles in pattern formation during the development of the nervous system. For example, bone morphogenic proteins coordinate the acquisition of pattern information and the stimulation of proliferation in the embryonic spinal neural tube (Chesnutt *et al*., 2004). A substantial amount of work remains to be done in order to confirm the utility of placental gene alterations as a biomarker system, both for detecting ethanol consumption and for its potential use as a prognostic indicator of adverse neurobehavioral outcomes in the absence of morphological alterations.

Systematic examination of altered gene expression as a function of different levels and patterns of ethanol consumption, as well as the persistence of gene alterations after the last drinking episode are critical questions that remain to be addressed. Further, how the presence of other common pregnancy risk factors impacts ethanol-induced alterations in placental gene expression patterns needs to be determined. For example, how will concomitant exposure to factors such as nicotine (Aliyu *et al*., 2009), other drugs of abuse, stress, malnutrition or heavy metals exposure modify a biomarker signature pattern? Data from such studies would be critical for interpreting altered patterns of placental gene expression in clinical studies (Rosenberg *et al*., 2010).

It is also well known that the placenta generates reactive oxygen species (ROS), which may contribute to the oxidative stress observed even in normal pregnancy, but this is increased in pregnancies complicated by preeclampsia (PE), IUGR and pre-gestational diabetes, where oxidative and nitrative stress are increased (Webster *et al*., 2008; Myatt, 2010). Recently, it has been demonstrated that identical maternal ethanol exposure levels produced different levels of foetal exposure in a dizygotic human twin pair (Gareri *et al*., 2009). This was also observed in guinea pig littermates through concentration of fatty acid ethyl ester meconium analysis (Chan *et al*., 2004; Brien *et al*., 2006).

*Oxidative stress in the placenta*

Placental pathology has long been associated with IUGR and PE in humans. The development of placenta is a highly regulated process and is therefore quite susceptible to perturbation. Foreign compounds may interfere with placental functions at many levels, including signalling, production and release of hormones and enzymes, transport of nutrients and waste products, implantation, cellular growth and maturation, and finally in the delivery. Placental responses may also be due to alcohol/toxicodynamic responses, e.g. hypoxia (Myllynen *et al*., 2005; Cross, 2006; Salihu *et al*., 2011). On the other hand, oxidative stress constitutes a unifying mechanism of injury of many types of disease processes. It occurs when there is an imbalance between the production of ROS and the ability of the biological system to readily detoxify these intermediate reactives or easily repair the resulting damage (Rodrigo *et al*., 2005; Wells *et al*., 2009).

The mechanism of oxidative stress seems to operate in alcohol ingest pregnancy (Ornoy, 2007). It has been demonstrated that ethanol exposure induces oxidative stress in the human placental villi (Kay *et al*., 2000). This may account for the decreased nitric oxide (NO) release because NO may be shunted towards scavenging free radicals (Dotsch *et al*., 2001). Decreased NO availability could adversely affect placental blood flow regulation, which could, in turn, account for the restriction of growth observed in ethanol-exposed foetuses.

The defence mechanisms against ROS damage are also enhanced in pregnancy. Thus, a progressive increase in free radical scavengers, such as glutathione and bilirubin, as well as in the activity of antioxidant enzymes in placenta has been reported (Watson *et al*., 1997; Qanungo and Mukherjea, 2000). In the same line of evidence, Gundogan *et al*. (2010) have demonstrated the impact of ethanol-mediated oxidative stress on placental trophoblast function and its potential impact on pregnancy loss. They found that rat chronically exposed to ethanol during gestation evidenced placental apoptosis/necrosis. Ethanol mediates its adverse effect on pregnancy maintenance via inhibition of the prolactin family hormones (Soares, 2004).

*Peroxynitrite mediates lipid oxidation and nitration*

As the human foetal-placental vasculature lacks autonomic innervation, autocrine and/or paracrine agents such as NO radical play an important role in the regulation of foetal-placental blood flows (Myatt and Cui, 2004). Conversely, the activity of NO is prolonged by the presence of superoxide dismutase, which removes superoxide. Nevertheless, when tissues are induced to simultaneously produce both NO and superoxide in a concentrated and localized manner by inflammatory stimuli, sepsis and ischemia/reperfusion, both NO and superoxide react to produce the peroxynitrite anion ($ONOO^-$), a powerful oxidant of a variety of biomolecules (Beckman *et al*., 1994) that has cytotoxic activity and

inhibits mitochondrial electron transport, resulting in the inhibition of cellular respiration (Radi *et al*., 1994; Szabó, 2003) that cause lipid peroxidation and nitrate tyrosine residues (Brown and Borutaite, 1999; Palacios-Callender, 2004). In addition, Myatt *et al.* (1996, 2002) observed increased expression of nitrotirosine residues in the foetal vasculature and villous stroma of preeclamptic and diabetic placentas.

Peroxynitrite has also been shown to disorganize actin polymerization through actin nitration (Kasina *et al*., 2005). These effects have been associated with disruption of both intestinal (Banan *et al*., 2001) and endothelial barrier function (Neumann *et al*., 2006). It is important to note that the placenta plays a function similar to that of the intestine in order to absorb metabolites and it has also been demonstrated that peroxynitrite produce intestinal barrier dysfunction mediate by ethanol-induced microtubule disruption (Banan *et al*., 2000). Once the level of cellular damage inflicted by peroxynitrite supersedes any possibility of repair, the cell eventually dies via one of the two main pathways of cell demise, necrosis or apoptosis (Bonfoco *et al*., 1995; Virag *et al*., 2003).

On the other hand, Olney *et al.* (2002) reported that a single episode of ethanol intoxication, lasting for several hours, can trigger a massive wave of apoptotic neurodegeneration in the developing rat or mouse brain. The window of vulnerability coincides with the developmental period of synaptogenesis, also known as the brain growth-spurt period, which in rodents is a postnatal event, but in humans extends from the 6 month of gestation to several years after birth (Ikonomidou *et al*., 2000).

### *Placental apoptosis*

The morphological architecture and function of the human placenta depends upon an adequate balance in the process of proliferation, differentiation and apoptosis. An imbalance between them may result in spontaneous abortion, PE, preterm delivery and reduced foetal growth (Myatt, 2002; Crocker *et al*., 2003). In the last decade Huppertz *et al.* (2006) have characterized the role of the apoptosis cascade in villous trophoblast turnover and syncytium formation. Apoptosis has been observed to naturally occur in placentas of normal human pregnancies, but as expected, placentas from women with PE or IUGR show enhanced apoptosis when compared with those from normal pregnancy (Allaire *et al*., 2000).

### *Oxidative stress in the foetal development*

Exposure of the developing placenta, embryo or foetus to environmental agents such as alcohol or acetaldehyde is known to produce anatomical anomalies leading to *in utero* death or structural birth defects (Gundogan *et al*., 2008), a process commonly termed teratogenesis (Wells *et al*., 2009). The cellular mechanisms underlying ethanol-induced damage *in utero* are not yet well understood, although induction of oxidative stress is believed to be a putative mechanism due to an imbalance in prooxidant and antioxidant levels (Cohen-Kerem and Koren, 2003). Ethanol can induce oxidative stress both directly and indirectly. The direct effect is achieved by the formation of free radicals, which react with various cellular components. Formation of free radicals in the presence of ethanol has been demonstrated in cell lines such as rat hepatocytes (Henderson *et al*., 1995) as well as in the rat animal model (Reinke *et al*., 1987). Another direct oxidative stress effect of ethanol is the formation of ROS (Dong *et al*., 2010), which probably plays a role in mediating programmed cell death (Jacobson, 1996). Oxidative stress mechanisms seem to operate in teratogenicity caused by hypoxia as well as alcohol abuse (Ornoy, 2007). It has also been reported in rat hepatocytes that ethanol can induce oxidative stress indirectly by reducing the intracellular antioxidant capacity, such as the levels of glutathione peroxidase (Oh *et al*., 1998; Bailey *et al*., 2001).

The effects of ROS and oxidative stress on placental, embryonic and foetal development may adversely alter the development by causing oxidative damage on the cellular lipids, proteins and DNA, and/or by altering signal transduction. The latter can adversely alter the cellular function or trigger apoptotic or necrotic cellular death (Wells *et al*., 2009).

Additionally, the low antioxidant defences in foetal tissues and accumulation of toxic aldehyde products of lipid peroxidation predispose the foetus to oxidative damage. Wentzel *et al.* (2006) found that vitamin E treatment administered to pregnant chronic ethanol-consuming rats diminishes foetal malformations. This study was in agreement with the study of Heaton *et al*. (2000), who were able to block the effects of ethanol on Purkinje cells by using a very high dose of vitamin E.

However, some authors have not found a clear effect of antioxidant vitamin E supplementation (Tran *et al*., 2005), thereby raising the issue of the importance of dosage and variable sensitivity in different tissues towards the deleterious effects of ethanol. Wentzel and Eriksson (2008), using two different rat strains exposed to ethanol for 48 h in early pregnancy (9–11 days of gestation), have proposed a role for genetic predisposition, oxidative stress and apoptosis in ethanol teratogenicity. These authors suggest that the teratogenic predisposition of the more susceptible rat strain may reside, at least in part, in the regulation of the ROS-scavenging enzymes. Folic acid also acts as an antioxidant, and Cano *et al.* (2001) studied its effects on oxidative stress induced by ethanol in the rat. This study showed an association between FAS and oxidative stress measurements and the mitigation of those measures by administering the antioxidant folic acid (Oh *et al*., 1998; Bailey *et al*., 2001). In addition, the study of Naseer *et al.* (2010) suggested that the antioxidant vitamin C can effectively reduce the severity of ethanol-induced brain injury and growth retardation by the modulation of γ-aminobutiric acid receptor B ($GABA_B$ R) and protein kinase A-α (PKA) expression during early rat foetal development.

### *Alcohol and pregnancy hypertension*

It has been demonstrated that pregnancy-induced hypertension contribute to low weight at birth and preterm delivery. Although alcohol-associated hypertension is common among women who drink heavily (Ascherio *et al*., 1996; Seppa *et al*., 1996), the effect of alcohol abuse on the course of hypertensive pregnancies has not been sufficiently studied. Mankes *et al.* (1985) showed that alcohol administration during pregnancy increased hypertension, caused multiple birth defects, and increased foetal mortality in both normotensive and spontaneously hypertensive rats.

*Neuroactive steroid responses to hypoxic stress*

Compromised pregnancies can have serious effects on foetal brain development, the nature of which depends, in large part, on the time of gestation when they occur. Maternal stress, infections or problems with placentation can affect brain development due to changes in the delivery of oxygen and glucose to the brain, or the cytokine environment, leading to acute cell death (Inder *et al*., 1999; du Plessis and Volpe, 2002; Hirst *et al*., 2009). In addition, chronically suboptimal conditions during pregnancy dramatically increase the sensitivity of the foetal brain to further episodes of hypoxia/ischemia around the time of birth and in the immediate neonatal period. The resulting damage can lead to severe neuropathologies, including intellectual impairment and cerebral palsy (Inder *et al*., 1999).

Hirst *et al*. (2009) used the term neuroactive steroids to refer to steroids that influence foetal or maternal nervous system function and may be synthesized in both the nervous system and/or other peripheral organs. Pregnancy is characterized by elevated neuroactive steroid levels both in the maternal circulation and brain, as well as in the foetal brain. The placenta is a source of considerable amounts of progesterone that enter not only into the maternal circulation, but also into the foetal blood and the foetal brain, where it is actively converted into allopregnanolone (Crossley *et al*., 1997). It has also been reported that severe foetal hypoxia produces greater damage when the synthesis of allopregnanolone is suppressed (Yawno *et al*., 2007; Hirst *et al*., 2009). Evidence from animal studies indicates that elevated neuroactive steroid levels in pregnancy suppress excitability and increase lethargy (Paoletti *et al*., 2006). These alterations are resolved at birth with the removal of the placenta, suggesting that placental progesterone production is mostly responsible for the elevated neuroactive steroid levels. In addition, progesterone has been found to act on gene expression in neural cells to promote myelination by interaction with associated Schwann cells. As progesterone receptors were not detectable in Schwann cells, these observations suggest a possible interaction between neuronal and glial cells in order to promote myelination (Schumacher *et al*., 2007).

In another line of evidence, it has been demonstrated that human and animals foetus prenatally exposed to alcohol have smaller brain size and thinner cerebral cortex, leading to significant decreases in the total cell number (Miller and Potempa, 1990; Ashwell and Zhang, 1996; Miller, 1996; Archibald *et al*., 2001). Amino-acid-activated ion channels of excitatory (glutamate) and inhibitory (GABA) neurotransmitters in the prenatal rat brain have been shown to be altered in these fetuses (Lee *et al*., 1994; Allan *et al*., 1998; Naseer *et al*., 2010). Additionally, Ikonomidou (2000) demonstrated that ethanol effects on the developing rat brain trigger a widespread neuronal death by blocking the *N*-methyl-d-aspartate (NMDA)—glutamate receptor and activating $GABA_A$ receptors. It has also been reported that neuroactive steroids reduce NMDA-induced excitotoxicity both *in vitro* (Lockhart *et al*., 2002) and *in vivo* models of brain injury (Djebaili *et al*., 2005). These excitatory pathways contribute to brain injury in the neonate following acute hypoxic episodes that may occur around the time of birth (Gilbert Evans *et al*., 2005).

*Impaired placentation in rat FAS*

IUGR is a key feature of FAS, and recently Gundogan *et al*. (2008, 2010) have demonstrated in the rat that chronic gestational exposure to ethanol causes increased foetal resorption as well as impairment in placental development, and placentation. Since ethanol in maternal blood reaches the foetus and/or the placenta, its toxic effects on the foetus can be directly or indirectly mediated. The direct effect of foetal exposure to ethanol was demonstrated by Chu *et al*. (2007) on rat foetal brain development and the indirect effect is related to placental pathology, especially within the rat placental barrier. The ischemia or infarction observed in ethanol-exposed placentas reduced the thickness of the organ by an increase in cellular necrosis. Since the exchange of nutrients between the mother and the foetus occurs within the placental barrier, ethanol-induced reductions in the mass of this layer could impair the delivery of nutrients to the rat foetus and thereby resulting in IUGR.

Gundogan *et al*. (2008, 2010) described that a second major placental abnormality associated with chronic gestational exposure to ethanol was failure of maternal uterine spiral arteries to remodelling by EVT. This compromises both placental blood flow as well as the nutrients exchange (Pijnenborg *et al*., 1981b). Therefore, the motile and invasive properties of EVT are critical for the establishment and maintenance of pregnancy, and ensuring adequate blood and nutrient delivery to the foetus in order to support growth and development (Gundogan *et al*., 2008).

*Alcohol-induced apoptosis of neural crest cells*

Although it is clear that exposure to alcohol during gestation can have profound consequences, not all the cells within the embryo are equally affected. Studies in mice and chicks models have demonstrated that alcohol exposure at specific stages of early embryonic development results in significant death among cells destined to give rise to facial structures, such as the cranial neural crest cells (cNCC). Alcohol triggers apoptosis in retinoic acid (RA, a type of vitamin A) deficient cells from the neural crest, and also reduces levels of antioxidant compounds such as free radicals scavengers (Smith, 1997). Furthermore, Wentzel and Eriksson (2009) demonstrated on NCC of 10-day rat embryos that ethanol causes a shift towards apoptosis in both neural cells from cNCC as well as trunk (tNCC) portion, which is diminished by treatment with the antioxidant *N*-acetylcysteine. Oxidative defence genes as well as genes involved in NCC development are differently affected in cNCC compared with tNCC upon ethanol exposure: the cNCC Hox genes are down-regulated, whereas tNCC are up-regulated. Normal craniofacial morphology develops as a consequence of interactions between embryonic tissues such as cNCC, mesoderm and ectoderm and requires precise regulation of cell movement, growth, patterning and differentiation of craniofacial tissue (SanťAnna and Tosello, 2006). Alcohol exposure during the gastrulation period decrease the rate of cell division in mice embryos (Sulik, 1984), and the process of migration of mesodermal cells towards the primitive streak (Nakatsuji and Johnsson, 1984). Deficiencies in gastrulating mesodermal cells are responsible for inducing and maintaining neuroepithelial differentiation, an adverse effect on the mesoderm that

could result in size reduction in the neural plate, which was particularly noticeable in the forebrain region (Sulik *et al*., 1984).

Interestingly, alcohol exposure causes cCNN apoptosis only if alcohol is administered before the CNN migrate from their birthplace into the neuroectoderm. Once the cells began the migration towards the site where the face will develop, they become resistant to alcohol-induced apoptosis (Cartwright and Smith, 1995). Studies in animal models have linked the characteristic facial abnormalities in FAS to cell death by apoptosis of cCNN during very well defined periods of vulnerability such as gastrulation or neurulation (Cartwright *et al*., 1998). One mechanism by which this may occur is thought to be the formation of free radicals (Chen and Sulik, 1996). Others described mechanisms are deficiency in RA, altered expression of homeobox genes, intracellular communication and alterations in the activity of growth factors. Because neural crest cells differentiate into several neuronal lineages, these findings offer novel insights into how ethanol disrupts the processes of early neurogenesis (Garic *et al*., 2011).

The fact that alcohol also has an effect on NCC of odontogenesis makes the tooth a good representative organ model to study developmental toxicity of ethanol (Bowden *et al*., 1983; Campos and Duranza, 1988; Kattainen *et al*., 2001).

### *Foetal alcohol exposure and alterations in brain and behaviour*

Ethanol has a neuroteratogenic effect on *in vitro* generation of neural cells from human embryonic stem cells (Talens-Visconti *et al*., 2011). These authors found that ethanol exposure affects: (a) cell differentiation into neurons and astrocytes, (b) disrupts the actin cytoskeleton and (c) affects expression of different genes associated with neural differentiation, resulting in the FASD condition (Kumada *et al*., 2007).

Sustained exposure to alcohol during the period of gastrulation has a negative impact on the developing brain (Fig. 1), reducing the neural cell progenitor pool (Rubert *et al*., 2006) and causing long-term effects on the forebrain (Ashwell and Zhang, 1996). The cerebral cortex formation as well as the increase in both cortical surface area and neural cell number seems to be critical for the emergence of complex cognitive functions. The neural cell number is in turn dependent on the number of neural progenitor cells, their rate of proliferation and the mitotic cycles they undergo (Rubert *et al*., 2006). It has been reported that ethanol can induce microcephaly and deficits in cognitive functions and in some behavioural responses (Mattson and Riley, 1998). A critical period for ethanol-induced microcephaly and teratogenesis has been shown to occur during early embryogenesis in the rat brain, a period encompassing the first 10 days of gestation, the equivalent to the first trimester in human (Maier *et al*., 1997; Guerri, 2002). During this period, the neural progenitor cells, radial glia (RG) are generated, and dysfunctions in its proliferation and survival could lead to an important reduction in the final number of neural cells (Cameron and Rakic, 1994).

The establishment of RG cells from the neuroepithelium precedes the generation and migration of neurons in the cerebral cortex. During early corticogenesis, RG cells generate neurons and then guide the daughter neurons in their migration towards the developing cortical plate (Rakic, 1972;

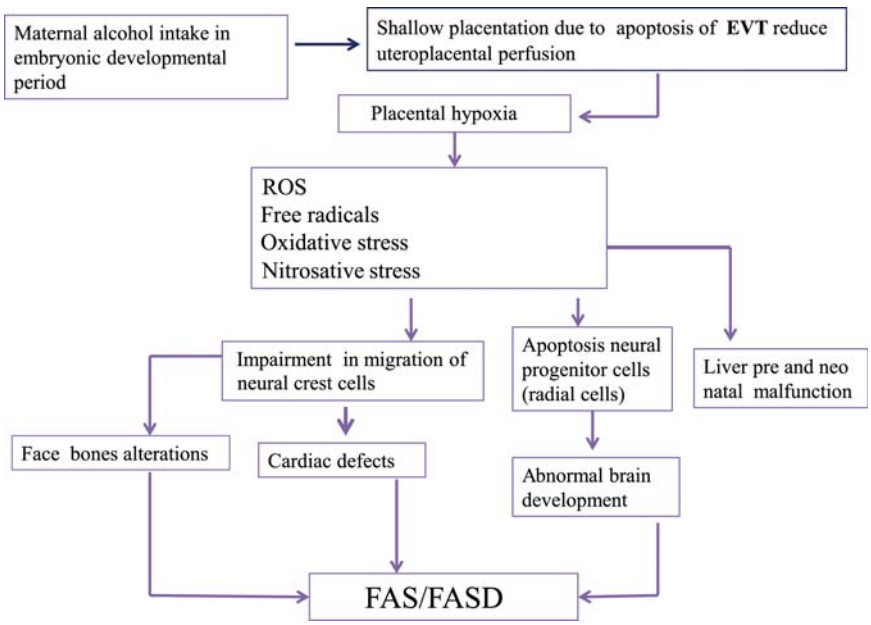


Fig. 1. Model representation of the effect of maternal alcohol consumption on first stages of placental development. During the first weeks of gestation (Weeks 10–12), placental development occurs in a normal hypoxic environment. Exposure to alcohol by maternal consumption during this period will trigger the hypoxic effect of alcohol or acetaldehyde, which in turn will increase the hypoxic environment. As a result, hypoxia will be higher than normal and the response will depend on constitutional maternal factors, as well as on the ability of the placenta to react. If this hypoxic situation persists beyond Weeks 10–12, the differentiation of cytotrophoblast (CTF) to ETV will decrease, producing a shallow placentation, in addition to a diminished uterine irrigation of the organ. The latter will induce an oxidative stress in the placenta, altering consequently the normal development of the embryo.

Komuro and Rakic, 1998; Hatten, 1999). Once neuronal migration is completed, most RG disappear by differentiating into astrocytes (Rakic, 1995), the cells that play critical roles in the metabolic processes linked to neuronal activity such as blood flow, energy and glucose utilization (Magistretti, 2006). In addition, it has been recently suggested that the metabolic anomalies observed in different brain structures of individuals with FAS disorder are consistent with abnormalities in the glial cell pool, rather than in the neurons (Guerri *et al*., 2009). Disruptions in these mechanisms and/or in the gradients of signalling molecules might affect neural progenitor cells and consequently central nervous system (CNS) development such as cerebellar hypoplasia (Chu *et al*., 2007).

A number of studies in animal models revealed that the ethanol effects on the CNS are not uniform and that some brain areas or cell populations are more vulnerable than others (Guerri, 1998, 2002; Tran and Kelly, 2003). Bonthius *et al*. (2008) found in cultured cerebellar granule cells from mouse pups that neuronal NO synthase protects developing neurons against alcohol toxicity by activating the NO and the cyclic GMP/protein kinase G system on $Ca^{2+}$ signalling and nuclear factor kappaB pathway. In addition, Kumar *et al*. (2010) demonstrated that high doses of ethanol affect the expression and activation of RA receptors, which could impair the signalling events and induce harmful effects on the survival and differentiation in rat cerebellar granule cells. Additionally, the study of Rout and Dhossche (2010) showed that pregnant Long-Evans rats consumed significantly less of a protein fortified liquid-diet when the alcohol present in the diet is gradually increased and that the expression of molecules involved in the integrin pathway signalling are significantly altered in the cerebral cortex of foetuses exposed to alcohol during gestation, even in the absence of obvious morphological defects in the offspring. Indeed, it has been well established that ethanol is a highly toxic substance for the developing foetal brain and, in fact, it is one of the leading preventable causes of birth defects and neurobehavioral disorders (American Academy of Pediatrics, 2000).

### *Effect of alcohol on cardiac embryo development*

Cardiac malformations are frequently seen in FAS, the most common being ventricular septal defects (Sardor *et al*., 1981), pulmonary artery hypoplasia, interruption of the aortic arch (Terrapon *et al*., 1977), atrio-ventricular defects, patent ductus arteriosus and Fallot teratology (Loser *et al*., 1992). Recently, it has been found that exposure of zebrafish embryos to ethanol during development results in structural and functional changes in the heart that mimic malformations that occur in patients with FAS (Dlugos and Rabin, 2010).

In the previous paragraphs, it has been described that alcohol alters the migration of neural crest cells. It is important to emphasize that in both avian and mammalian embryos, a migration pathway is present from the occipital neural crest cells to the cardiac outflow tract, and that a disturbance in this process can result in cardiac septation defects such as aortic pulmonary septum (Carlson, 2005), similar to that observed in cardiac malformations of FAS babies.

### *Effect of alcohol on liver embryo development*

The liver is also affected in FAS, and the characteristic deformities observed are similar to those evidenced in alcoholic liver disease in adult. The more commonly seen features include hepatomegaly and raised levels of serum transaminases. Light microscopy revealed increased parenchymal fat with portal and perisinusoidal spaces containing deposits of intermediated and large size collagen fibres, myofibroblast and occasional Ito cells, as well as subendothelial basement membrane-like material (Lefkowitch *et al*., 1983). The presence of thick sclerotic central veins in the hepatic lobule has also been evidenced, in conjunction with the occurrence of extrahepatic biliary atresia (Daft *et al*., 1986). Furthermore, Renaul-Piqueras *et al*. (1997) demonstrated that prenatal exposure to ethanol affects the morphological, structural and functional features of the Golgi apparatus, thus altering the glycosylation process in foetal hepatocytes, causing finally an accumulation of hepatic proteins. Additionally, Fofana *et al*. (2010) demonstrate that prenatal alcohol exposure alters protein phosphorylation in rat offspring liver and that the principal pathway affected by these protein alterations includes cell signalling, cellular stress, protein synthesis, as well as glucose, aminoacids, adenosine and energy metabolism.

## SUMMARY AND CONCLUSIONS

We have reviewed the principal evidence pointing to the teratogenic effects of prenatal ethanol exposure on placentation, placenta growth, placenta function and foetal development, with emphasis in the development of the central nervous system. These evidences strongly suggest an association between alcohol teratogenic effect, hypoxia and oxidative stress, pinpointing molecular mechanism involved (e.g. apoptosis) and the range of effects. The present review also sums up evidence that could explain some of the abnormalities observed in brain development that could be related to behavioural problems observed in individuals with FAS/FASD.

Some of this evidence suggests that alcohol consumption might produce failures in the normal migration of radial cells, from which the rest of the brain cells would eventually develop.

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## REFERENCES

Aliyu M, Wilson R, Zoorob R *et al*. (2009) Prenatal alcohol consumption and fetal growth restriction: potentiation effect by concomitant smoking. *Nicotine Tob Res* **11**:36–43.

Allaire AD, Ballenger KA, Wells SR *et al*. (2000) Placental apoptosis in preeclampsia. *Obstet Gynecol* **96**:271–6.

Allan AM, Wu H, Paxton LL *et al*. (1998) Prenatal ethanol exposure alters the modulation of the gamma-aminobutyric acid A1 receptor-gated chloride ion channel in adult rat offspring. *J Pharmacol Exp Ther* **284**:250–7.

American Academy of Pediatrics. Committee on Substance Abuse and Committee on Children with Disabilities (2000) Fetal

Alcohol syndrome and alcohol-related neurodevelopmental disorders. *Pediatrics* **106**:358–61.
Aplin JD. (1991) Implantation, trophoblast differentiation and haemochorial placentation: mechanistic evidence *in vivo* and *in vitro*. *J Cell Sci* **99**:681–92.
Archibald SL, Fennema-Notestine C, Gamst A *et al*. (2001) Brain dysmorphology in individuals with severe prenatal alcohol exposure. *Dev Med Child Neurol* **43**:148–54.
Ascherio A, Hennekens C, Willett WC *et al*. (1996) Prospective study of nutritional factors, blood pressure, and hypertension among US women. *Hypertension* **27**:1065–72.
Ashwell KW, Zhang LL. (1996) Forebrain hypoplasia following acute prenatal ethanol exposure: quantitative analysis of effects on specific forebrain nuclei. *Pathology* **28**:161–6.
Bailey SM, Patel VB, Young TA *et al*. (2001) Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver. *Alcohol Clin Exp Res* **25**:726–33.
Banan A, Fields JZ, Decker H *et al*. (2000) Nitric oxide and its metabolites mediate ethanol-induced microtubule disruption and intestinal barrier dysfunction. *J Pharmacol Exp Ther* **294**:997–1008.
Banan A, Fields JZ, Zhang Y *et al*. (2001) iNOS upregulation mediates oxidant-induced disruption of F-actin and barrier of intestinal monolayers. *Am J Physiol Gastrointest Liver Physiol* **280**: G1234–46.
Beckman JS, Ye YZ, Anderson PG *et al*. (1994) Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. *Biol Chem Hoppe Seyler* **375**:81–8.
Bonfoco E, Krainc D, Ankarcrona M *et al*. (1995) Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with *N*-methyl-d-aspartate or nitric oxide/superoxide in cortical cell cultures. *Proc Natl Acad Sci USA* **92**:7162–6.
Bonthius DJ, Bonthius NE, Karacay B. (2008) The protective effect of neuronal nitric oxide synthase (nNOS) against alcohol toxicity depends upon NO-cGMP-PKG pathway and NF-kappaB. *Neurotoxicology* **29**:1080–91.
Bosco C. (1995) The cytotrophoblast: the first human amine precursor uptake and decarboxylation (APUD) cell. *Med Sci Res* **23**:205–7.
Bosco C. (2005) Alcohol and xenobiotics in placenta damage. In Preedy VR, Watson RR (eds). *Comprehensive Handbook of Alcohol Related Pathology*, **Vol. 2**. London: Elsevier Science, 921–35.
Bowden DM, Weathersbee PS, Clarren SK *et al*. (1983) A periodic dosing model of fetal alcohol syndrome in the pig-tailed macaque (*Macaca nemestrina*). *Am J Primatol* **4**:143–57.
Brien JF, Chan D, Green CR *et al*. (2006) Chronic prenatal ethanol exposure and increased concentration of fatty acid ethyl esters in meconium of term fetal Guinea pig. *Ther Drug Monit* **28**:345–50.
Brown GC, Borutaite V. (1999) Nitric oxide, cytochrome *c* and mitochondria. *Biochem Soc Symp* **66**:17–25.
Cameron RS, Rakic P. (1994) Identification of membrane proteins that comprise the plasmalemmal junction between migrating neurons and radial glial cells. *J Neurosci* **14**:3139–55.
Campos RM, Duranza MC. (1988) Efectos del consumo prolongado de etanol sobre las etapas tempranas del desarrollo dentario en ratones. *Rev Cubana Invest Bioméd* **7**:30–5.
Cano MJ, Ayala A, Murillo ML *et al*. (2001) Protective effect of folic acid against oxidative stress produced in 21-day postpartum rats by maternal-ethanol chronic consumption during pregnancy and lactation period. *Free Radic Res* **34**:1–8.
Carlson MB (2005) Cresta neural. In Carlson BM (ed). *Embriología Humana y Biología del Desarrollo*. Madrid: Elsevier, 277–90.
Cartwright MM, Smith SM. (1995) 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* **19**:378–86.
Cartwright MM, Tessmer LL, Smith SM. (1998) Ethanol induced neural crest apoptosis is coincident with their endogenous death, but is mechanistically distinct. *Alcohol Clin Exp Res* **22**:142–9.
Chan D, Klein J, Karaskov T *et al*. (2004) Fetal exposure to alcohol as evidence by fatty acid ethyl esters in meconium in the absence of maternal drinking history in pregnancy. *Ther Drug Monit* **26**:474–81.
Chaudhuri J. (2000) Alcohol and the developing foetus- a review. *Med Sci Monit* **6**:1031–41.
Chen S, Sulik KK. (1996) Free radicals and ethanol-induced cytotoxicity in neural crest. *Alcohol Clin Exp Res* **20**:1071–6.
Chesnutt C, Burrus LW, Brown AM *et al*. (2004) Coordinate regulation of neural tube patterning and proliferation by TGFbeta and WNT activity. *Dev Biol* **274**:334–47.
Chu J, Tong M, de la Monte SM. (2007) Chronic ethanol exposure causes mitochondrial dysfunction and oxidative stress in immature central nervous system neurons. *Acta Neuropathol* **113**:659–73.
Chudley AE, Conry J, Cook JL *et al*. (2005) Fetal alcohol spectrum disorder: Canadian guidelines for diagnosis. *Canad Med Assoc J* **172**:S1–21.
Cohen-Kerem R, Koren G. (2003) Antioxidants and fetal protection against ethanol teratogenicity I. Review of the experimental data and implications to humans. *Neurotoxicol Teratol* **25**:1–9.
Crocker IP, Cooper S, Ong SC *et al*. (2003) Differences in apoptotic susceptibility of cytotrophoblasts and syncytiotrophoblasts in normal pregnancy to those complicated with preeclampsia and intrauterine growth restriction. *Am J Pathol* **162**:637–43.
Cross CJ. (2006) Placental function in development and disease. *Reprod Fertil Dev* **18**:71–6.
Cross CJ, Werb Z, Fisher SJ. (1994) Implantation and the placenta: key pieces of the development puzzle. *Science* **26**:1508–17.
Crossley KJ, Nicol MB, Hirst JJ *et al*. (1997) Suppression of arousal by progesterone in fetal sheep. *Reprod Fertil Dev* **9**:767–73.
Daft PA, Johnston MC, Sulik KK. (1986) Abnormal heart and great vessel development following acute ethanol exposure in mice. *Teratology* **33**:93–104.
Delaval K, Feil R. (2004) Epigenetic regulation of mammalian genomic imprinting. *Curr Opin Genet Dev* **14**:188–95.
Djebaili M, Guo Q, Pettus EH *et al*. (2005) The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma* **22**:106–18.
Dlugos CA, Rabin RA. (2010) Structural and functional effects of developmental exposure to etanol on the zebrafish heart. *Alcohol Clin Exp Res* **34**:1–9.
Dong J, Sulik KK, Chen SY. (2010) The role of NOX enzymes in ethanol-induced oxidative stress and apoptosis in mouse embryos. *Toxicol Lett* **193**:94–100.
Dotsch J, Hogen N, Nyul Z *et al*. (2001) Increase of endothelial nitric oxide synthase and endothelin-1 mRNA expression in human placenta during gestation. *Eur J Obstet Gynecol Reprod Biol* **97**:163–7.
du Plessis AJ, Volpe JJ. (2002) Perinatal brain injury in the preterm and term newborn. *Curr Opin Neurol* **15**:151–57.
Fisher SE, Karl PI. (1988) Maternal ethanol use and selective fetal malnutrition. *Recent Dev Alcohol* **6**:277–89.
Floyd RL, Decofle P, Hungerfond DW. (1999) Alcohol use prior to pregnancy recognition. *Am J Prev Med* **12**:101–7.
Fofana B, Yao XH, Rampitsch C *et al*. (2010) Prenatal alcohol exposure alters phosphorylation of proteins in rat offspring liver. *Proteomics* **10**:417–34.
Gareri J, Brien J, Reynolds J *et al*. (2009) Potential role of the placenta in fetal alcohol spectrum disorder. *Paediatr Drugs* **11**:26–9.
Garic A, Flentke G, Amberger E *et al*. (2011) CaMKII activation is a novel effector of alcohos neurotoxicity in neural crest stem/progenitor cells. *J Neurochem* **118**:646–57.
Gemma S, Vichi S, Testai E. (2006) Individual susceptibility and alcohol effects: biochemical and genetic aspects. *Ann Ist Super Sanita* **42**:8–16.
Gemma S, Vichi S, Testai E. (2007) Metabolic and genetic factors contributing to alcohol induced effects and fetal alcohol syndrome. *Neurosci Biobehav Rev* **31**:221–9.
Gilbert Evans SE, Ross LE, Sellers EM *et al*. (2005) 3 alpha-reduced neuroactive steroids and their precursors during pregnancy and the postpartum period. *Gynecol Endocrinol* **21**:268–79.

Guerri C. (1998) Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. *Alcohol Clin Exp Res* **22**:304–12.
Guerri C. (2002) Mechanisms involved in central nervous system dysfunctions induced by prenatal ethanol exposure. *Neurotox Res* **4**:327–35.
Guerri C, Bazinet A, Riley EP. (2009) Foetal alcohol spectrum disorders and alterations in brain and behaviour. *Alcohol Alcohol* **44**:108–14.
Gundogan F, Elwood G, Longato L *et al*. (2008) Impaired placentation in fetal alcohol syndrome. *Placenta* **29**:148–57.
Gundogan F, Elwood G, Mark P *et al*. (2010) Ethanol-induced oxidative stress and mitochondrial dysfunction in rat placenta: relevance to pregnancy loss. *Alcohol Clin Exp Res* **34**:415–23.
Hatten ME. (1999) Central nervous system neuronal migration. *Annu Rev Neurosci* **22**:511–39.
Heaton MB, Mitchell JJ, Paiva M. (2000) Amelioration of ethanol induced neurotoxicity in the neonatal rat central nervous system by antioxidant therapy. *Alcohol Clin Exp Res* **24**:512–8.
Henderson GI, Devi BG, Perez A *et al*. (1995) In utero ethanol exposure elicits oxidative stress in the rat foetus. *Alcohol Clin Exp Res* **19**:714–20.
Hirst JJ, Walker DW, Yawno T *et al*. (2009) Stress in pregnancy: a role for neuroactive steroids in protecting the fetal and neonatal brain. *Dev Neurosci* **31**:363–77.
Huppertz B, Kadyrov M, Kingdom JC. (2006) Apoptosis and its role in the trophoblast. *Am J Obstet Gynecol* **195**:29–39.
Ikonomidou C, Bittigau P, Ishimaru MJ *et al*. (2000) Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science* **287**:1056–60.
Inder TE, Huppi PS, Warfield S *et al*. (1999) Periventricular white matter injury in the premature infant is followed by reduced cerebral cortical gray matter volume at term. *Ann Neurol* **46**:755–60.
Jacobson MD. (1996) Reactive oxygen species and programmed cell death. *Trends Biochem Sci* **21**:83–6.
Jaenisch R, Bird A. (2003) Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* **33**:245–54.
Jones KL, Smith DW, Ulleland CN *et al*. (1973) Pattern of malformations in offspring of chronic alcoholic mothers. *Lancet* **1**:267–71.
Kasina S, Rizwani W, Radhika KV *et al*. (2005) Nitration of profilin effects its interaction with poly (L-proline) and actin. *J Biochem* **138**:687–95.
Kattainen H, Tuukkanen J, Simanainen U *et al*. (2001) In utero/lactation 2,3,7,8-tetrachlordibenzo-p-dioxin exposure impairs molar tooth development an rats. *Toxicol Appl Pharmacol* **174**:216–24.
Kay HH, Grindle KM, Magness RR. (2000) Ethanol exposure induces oxidative stress and impairs nitric oxide availability in the human placental villi: a possible mechanism of toxicity. *Am J Obstet Gynecol* **182**:682–8.
Komuro H, Rakic P. (1994) Identification of membrane proteins that comprise the plasmalemmal junction between migrating neurons and radial glial cells. *J Neurosci* **18**:1478–90.
Komuro H, Rakic P. (1998) Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular $Ca2^{+}$ fluctuations. *J Neurobiol* **37**:110–30.
Kumada T, Jiang Y, Cameron DB *et al*. (2007) How does alcohol impair neuronal migration? *J Neurosci Res* **85**:465–70.
Kumar A, Singh CK, DiPette DD *et al*. (2010) Ethanol impairs activation of retinoic acid receptors in cerebellar granule cells in a rodent model of fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* **34**:928–37.
Lee YH, Spuhler-Phillips K, Randall PK *et al*. (1994) Effects of prenatal ethanol exposure on N-methyl-Daspartate-mediated calcium entry into dissociated neurons. *J Pharmacol Exp Ther* **271**:1291–8.
Lefkowitch JH, Rushton AR, Feng-Chen KC. (1983) Hepatic fibrosis in fetal alcohol syndrome. Pathologic similarities to adult alcoholic liver disease. *Gastroenterology* **85**:951–7.
Lockhart EM, Warner DS, Pearlstein RD *et al*. (2002) Allopregnanolone attenuates N-methyl-D-aspartate-induced excitotoxicity and apoptosis in the human NT2 cell line in culture. *Neurosci Lett* **328**:33–6.
Loser H, Pgeferkorn JR, Themann H. (1992) Alcohol in pregnancy and fetal heart damage. *Klin Padiatr* **204**:235–9.
Magistretti PJ. (2006) Neuron-glia metabolic coupling and plasticity. *J Exp Biol* **209**:2304–11. Review
Maier SE, Chen WJ, Miller JA *et al*. (1997) Fetal alcohol exposure and temporal vulnerability regional differences in alcohol-induced microencephaly as a function of the timing of binge-like alcohol exposure during rat brain development. *Alcohol Clin Exp Res* **21**:1418–28.
Mankes RF, LeFevre R, Fieseher J *et al*. (1985) Effects of ethanol on reproduction and arterial hypertension in spontaneously hypertensive and normotensive rats: a preliminary communication. *Alcohol Clin Exp Res* **9**:284–90.
Mattson SN, Riley EP. (1998) A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. *Alcohol Clin Exp Res* **22**:279–94.
Miller MW. (1996) Effect of early exposure to ethanol on the protein and DNA contents of specific brain regions in the rat. *Brain Res* **734**:286–94.
Miller MW, Potempa G. (1990) Numbers of neurons and glia in mature rat somatosensory cortex: effects of prenatal exposure to ethanol. *J Comp Neurol* **293**:92–102.
Myatt L. (2002) Role of placenta in preeclampsia. *Endocrine* **19**:103–11.
Myatt L. (2006) Placental adaptive responses and fetal programming. *J Physiol* **572**:25–30.
Myatt L. (2010) Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. *Placenta* **31**:S66–9.
Myatt L, Cui X. (2004) Oxidative stress in the placenta. *Histochem Cell Biol* **122**:369–82.
Myatt L, Rosenfield LD, Eis AL *et al*. (1996) Nitrotirosine residues in placenta. Evidence of peroxinitrite formation and action. *Hypertension* **28**:488–93.
Myllynen P, Pasanen M, Pelkonen O. (2005) Human placenta. A human organ for developmental toxicology research and biomonitoring. *Placenta* **26**:361–71.
Nakatsuji N, Johnsson KE. (1984) Effects of ethanol on the primitive streak stage mouse embryo. *Teratology* **29**:269–75.
Naseer MI, Lee HI, Kim MO. (2010) Neuroprotective effect of vitamin C against the ethanol and nicotine modulation of $GABA_B$ receptor and PKA-α expression in prenatal rat brain. *Synapse* **64**:467–77.
Nelissen EC, van Montfoort AP, Dumoulin JC *et al*. (2011) Epigenetics and the placenta. *Hum Reprod Update* **17**:397–417.
Neumann P, Gertzberg N, Vaughan E *et al*. (2006) Peroxynitrite mediates TNF-α-induced endothelial barrier dysfunction and nitration of actin. *Am J Physiol Lung Cell Mol Physiol* **290**: L674–84.
Oh SI, Kim CI, Chun HJ *et al*. (1998) Chronic ethanol consumption affects glutathione status in rat liver. *J Nutr* **128**:758–63.
Olney JW, Wosniak DF, Farber NB *et al*. (2002) The enigma of fetal alcohol neurotoxicity. *Ann Med* **34**:109–19.
Ornoy A. (2007) Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. *Reprod Toxicol* **24**:31–41.
Palacios-Callender M, Quintero M, Hollis VS *et al*. (2004) Endogenous NO regulates superoxide production at low oxygen concentrations by modifying the redox state of cytochrome *c* oxidase. *Proc Natl Acad Sci USA* **101**:7630–5.
Paoletti AM, Romagnino S, Contu R *et al*. (2006) Observational study on the stability of the psychological status during normal pregnancy and increased blood levels of neuroactive steroids with GABA-A receptor agonist activity. *Psychoneuroendocrinology* **31**:485–92.
Pijnenborg R, Bland JM, Robertson WB *et al*. (1981a) The pattern of interstitial trophoblastic invasion of the myometrium in early human pregnancy. *Placenta* **2**:303–16.
Pijnenborg R, Robertson WB, Brosens I *et al*. (1981b) Review article: trophoblast invasion and the establishment of haemochorial placentation in man and laboratory animals. *Placenta* **2**:71–91, Review.
Qanungo S, Mukherjea M. (2000) Ontogenic profile of some antioxidants and lipid peroxidation in human placental and fetal tissues. *Mol Cell Biochem* **215**:11–9.
Radi R, Beckman JS, Bush KM *et al*. (1994) Inhibition of mitochondrial electron transport by peroxinitrite. *Arch Biochem Biophys* **308**:89–95.

Rakic P. (1972) Mode of cell migration to the superficial layers of fetal monkey neocortex. *J Comp Neurol* **145**:61–83.
Rakic P. (1995) Radial versus tangential migration of neuronal clones in the developing cerebral cortex. *Proc Natl Acad Sci USA* **92**:11323–7.
Reik W, Constancia M, Fowden A *et al*. (2003) Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *J Physiol* **547**:35–44.
Reinke LA, Lai EK, DuBose CM *et al*. (1987) Reactive free radical generation *in vivo* in heart and liver of ethanol-fed rats: correlation with radical formation *in vitro*. *Proc Natl Acad Sci USA* **84**:9223–7.
Renaul-Piqueras J, Guasch R, Azorin I *et al*. (1997) Prenatal alcohol exposure affects galactosyltransferase activity and glycoconjugates in the Golgi apparatus of fetal rat hepatocytes. *Hepatology* **25**:343–50.
Rodrigo R, Parra M, Bosco C *et al*. (2005) Pathophysiological basis for the prophylaxis of preeclampsia through early supplementation with antioxidant vitamins. *Pharmacol Ther* **107**: 177–97.
Rosenberg MJ, Wolff CR, El-Emawy A *et al*. (2010) Effects of moderate drinking during pregnancy on placental gene expression. *Alcohol* **44**:673–90.
Rosset HL. (1980) A clinical perspective of the fetal alcohol syndrome. *Alcohol Clin Exp Res* **4**:119–22.
Rout UK, Dhossche JM. (2010) Liquid-diet with alcohol alters maternal, fetal and placental weights and the expression of molecules involved in integrin signaling in the fetal cerebral cortex. *Int J Environ Res Public Health* **7**:4023–36.
Rubert G, Miñana R, Pascual M *et al*. (2006) Ethanol exposure during embryogenesis decreases the radial glial progenitor pool and affects the generation of neurons and astrocytes. *J Neurosci Res* **84**:483–96.
Salihu HM, Kornosky JL, Lynch O *et al*. (2011) Impact of prenatal alcohol consumption on placenta-associated syndromes. *Alcohol* **45**:73–9.
SantÁnna LB, Tosello DO. (2006) Fetal alcohol syndrome and developing craniofacial and dental structures-a review. *Orthod Craniofac Res* **9**:172–85.
Sardor GG, Smith DF, Mac Leod PM. (1981) Cardiac malformations in the fetal alcohol syndrome. *J Pediatr* **98**:771–3.
Schumacher M, Guennoun R, Stein DG *et al*. (2007) Progesterone: therapeutic opportunities for neuroprotection and myelin repair. *Pharmacol Ther* **116**:77–106.
Seppa K, Laippala P, Sillanaukee P. (1996) High diastolic blood pressure: common among women who are heavy drinkers. *Alcohol Clin Exp Res* **20**:47–51.
Smith SC, Baker PN, Symonds EM. (1997) Placental apoptosis in normal human pregnancy. *Am J Obstet Gynecol* **177**:57–65.
Soares MJ. (2004) The prolactin and growth hormone families: pregnancy-specific hormones/cytokines at the maternal fetal interface. *Reprod Biol Endocrinol* **2**:51–66.
Sulik KK. (1984) Critical period for alcohol teratogenesis in mice, with special reference to the gastrulation stage of embryogenesis. *Ciba Found Symp* **105**:124–41.
Szabó C. (2003) Multiple pathways of peroxynitrite cytotoxicity. *Toxicol Lett* **140**:105–12.
Taléns-Visconti R, Sanchez Vera I, Kostic J *et al*. (2011) Neural differentiation from human embryonic stem cells as a tool to study early brain development and the neuroteratogenic effects of ethanol. *Stem Cells Dev* **20**:327–39.`
Terrapon M, Schneider P, Friedli B *et al*. (1977) Aortic arch interruption type A with aortopulmonary fenestration in an offspring of chronic alcoholic mother ('fetal alcohol syndrome'). *Helv Paediatr Acta* **32**:141–8.
Tran TD, Kelly SJ. (2003) Critical periods for ethanol-induced cell loss in the hippocampal formation. *Neurotoxicol Teratol* **25**:519–28.
Tran TD, Jackson HD, Hom KH *et al*. (2005) Vitamin E does not protect against neonatal ethanol-induced cerebellar damage or deficits in eyeblink classical conditioning in rats. *Alcohol Clin Exp Res* **29**:117–29.
Virag L, Szabo E, Gergely P *et al*. (2003) Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. *Toxicol Lett* **140**:113–24.
Watson AL, Palmer ME, Jauniaux E *et al*. (1997) Variations in expression of copper/zinc superoxide dismutase in villous trophoblast of the human placenta with gestational age. *Placenta* **18**:295–9.
Webster RP, Roberts VH, Myatt L. (2008) Protein nitration in placenta: functional significance. *Placenta* **29**:985–94.
Wells PG, Ma Callum GP, Chen CS *et al*. (2009) Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. *Toxicol Sci* **108**:4–18.
Wentzel P, Eriksson UJ. (2008) Genetic influence on dysmorphogenesis in embryos from different rat strains exposed to ethanol *in vivo* and *in vitro*. *Alcohol Clin Exp Res* **32**:874–87.
Wentzel P, Eriksson UJ. (2009) Altered gen expression in neural crest cells exposed to ethanol *in vitro*. *Brain Res* **1305**: S50–60.
Wentzel P, Rydberg U, Eriksson UJ. (2006) Antioxidative treatment diminishes ethanol-induced congenital malformations in the rat. *Alcohol Clin Exp Res* **30**:1732–60.
Yawno T, Yan EB, Walker DW *et al*. (2007) Inhibition of neurosteroid synthesis increases asphyxia-induced brain injury in the late gestation fetal sheep. *Neuroscience* **146**:1726–33.
Zhou F, Zhao Q, Liu Y *et al*. (2011) Alteration of gene expression by alcohol exposure at early neurulation. *BMC Genomics* **12**:124–41.