Postnatal nutritional treatment of neurocognitive deficits in Fetal Alcohol Spectrum Disorder (FASD)

Bastons-Compta A[1], Astals M[1], Andreu V[1], Navarro E[1], Garcia-Algar O[1]

[1]Neonatology Unit, Hospital Clinic-Maternitat, ICGON, Grup de Recerca Infancia i Entorn (GRIE), BCNatal, Barcelona, Spain

Corresponding author:

Oscar Garcia-Algar

Neonatology Unit, Hospital Clinic-Maternitat

C/Sabino Arana 1

08028 Barcelona (Spain)

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Abstract

Ethanol is the most important teratogen agent in humans. Prenatal alcohol exposure can lead to a wide range of adverse effects, which are broadly termed as Fetal Alcohol Spectrum Disorder (FASD). The most severe consequence of maternal alcohol abuse is the development of Fetal Alcohol Syndrome (FAS), defined by growth retardation, facial malformations and central nervous system impairment expressed as microcephaly and neurodevelopment abnormalities. These alterations generate a broad range of cognitive abnormalities such as learning disabilities and hyperactivity, and behavioural problems.

Socioeconomic status, ethnicity, differences in genetic susceptibility related to ethanol metabolism, alcohol consumption patterns, obstetric problems and environmental influences like maternal nutrition, stress, and other co-administered drugs are all factors that may influence FASD manifestations.

Recently, much attention has been paid to the role of nutrition as a protective factor against alcohol teratogenicity. There are a great number of papers related to nutritional treatment of nutritional deficits due to several factors associated with maternal consumption of alcohol and with eating and social disorders in FASD children. Although research showed the clinical benefits of nutritional interventions, most of work was in animal models, in a preclinical phase or in the prenatal period. However, a minimum number of studies refer to postnatal nutrition treatment of neurodevelopmental deficits.

Nutritional supplementation in children with FASD has a dual objective: to overcome nutritional deficiencies and to reverse or improve the cognitive deleterious effects of prenatal alcohol exposure. Further research is necessary to confirm positive results, to determine optimal amounts of nutrients needed in supplementation, and to investigate the collective effects of simultaneous multiple-nutrient supplementation.

Keywords: Fetal Alcohol Spectrum Disorder (FASD); Fetal Alcohol Syndrome (FAS); Nutrition; Neurocognitive deficit; Nutritional treatment

Background

Ethanol is the most common teratogen agent in humans and its consumption can lead to a wide range of adverse effects, which are broadly termed as Fetal Alcohol Spectrum Disorder (FASD). The most severe consequence of maternal alcohol abuse is the development of Fetal Alcohol Syndrome (FAS), defined by growth retardation, a characteristic facial pattern, and central nervous system (CNS) impairment manifested by microcephaly and neurodevelopment abnormalities. These alterations generate a broad range of cognitive abnormalities such as learning disabilities and hyperactivity, and behavioural problems (Dörrie et al. 2014).

Prenatal alcohol exposure is considered a major public health concern. In fact, FASD is the first cause of preventable intellectual disability worldwide. The last published systematic review about the prevalence of FAS indicated the five countries with the highest estimated prevalence of alcohol use during pregnancy which were Ireland (60.4% of pregnant women consume alcohol during pregnancy), Belarus (46.6%), Denmark (45.8%), UK (41.3%), and Russia (36.5%). In Spain, the prevalence of alcohol consumption during pregnancy, through maternal hair analysis at the delivery is about 60% (Joya et al. 2016). In the general population of Europe, about a quarter of women drink alcohol during pregnancy. In relation to the prevalence of FAS, the review indicates that the five countries with the highest prevalence of FAS are South Africa (58.5 per 1,000), Croatia (11.5 per 1,000), Ireland (8.9 per 1,000), Italy (8.2 per 1,000) and Belarus (6.9 per 1000). In Spain there is no data about FASD prevalence in general population nor in children adopted from East Europe countries, who represent the most risk population nowadays. The global prevalence of FAS among the general population is estimated to be 1.4 per 1,000 (Popova et al. 2017).

Despite public health efforts to reduce or eliminate alcohol consumption during pregnancy, i.e. specific public health programs or clinical guidelines, an important number of pregnant women continues consuming alcohol (Tsai et al. 2010). The adequate strategy for FASD prevention remains ethanol abstention, but effective and evidence-based interventions and treatment against ethanol damage is needed to ameliorate adverse consequences in children who are affected by FASD.

More interesting than the symptomatic treatment of clinical disorders due to neurodevelopmental deleterious effects of prenatal alcohol exposure is specific neurovedelopmental disorders treatment.

Recently, much attention has been paid to the role of nutrition as a protective factor against alcohol's teratogenicity. There are a great number of papers related to nutritional treatment of nutritional deficits due to several factors associated with maternal consumption of alcohol and with eating and social disorders in FASD children. Although research has shown the clinical potential of nutritional interventions, the majority of work has been in the preclinical phase or has been focused on the prenatal period. However, a minimum number of studies refer to postnatal nutrition treatment of neurodevelopmental deficits.

The aim of this review was to provide an overview of the existing studies on the use of nutrients (vitamins, minerals, choline, omega-3 fatty acids and antioxidants such EGCG) in the postnatal period to prevent or alleviate the development of neurodevelopment symptomathology due to FASD.

Fetal Alcohol Spectrum Disorder (FASD)

Prenatal exposure to alcohol causes a dysfunction of central nervous system due to structural damage to the brain. These structural alterations can be visualized using image tests such as MRI. The most common alterations are: microcephaly, reduced volume of white matter and gray matter, malformation or agenesis of the corpus callosum, reduction of the volumes of the caudate nucleus, hippocampus and frontal temporal and parietal lobes (Label et al. 2011).

FASD diagnosis includes validated clinical criteria supported by scientific literature and various international institutions (WHO, DSM classification, Institute of Medicine (IOM), Canadian Medical Association, etc.). The last published reviewed version of IOM criteria, still classified FASD into 4 specific disorders: FAS, partial FAS, alcohol-related birth defects (ARBD), alcohol-related neurodevelopment disorders (ARND) (Hoyme et al. 2016). FAS is the most severe form of FASD and it includes growth deficiency, a characteristic pattern of facial anomalies which comprises short palpebral fissures, broad philtrum and thin upper lip, and a delay on the development of CNS, which include microcephaly and other brain anomalies. Although a single cognitive profile pattern that may be considered prototypical of FASD has not been identified,

some of the more common alterations are mental retardation, memory impairment, behavioural abnormalities, and impaired development of social, mental, executive function and motor skills (Mattson et al. 2011).

The prognosis in each case does not depend exclusively on the final diagnosis within the spectrum of FASD, but mainly on the symptomatology that the affected person presents, and therefore, on the ability to adapt to daily life.

Alcohol exposure and consumption pattern

The severity of fetal damage due to prenatal ethanol exposure depends on several factors such as dose of alcohol consumed and timing of consumption during pregnancy, differences in genetic susceptibility related to ethanol metabolism, and environmental influences like maternal nutrition, socioeconomic status, ethnic origin, stress, and other co-administered drugs.

The drinking patterns are crucial to determine the effects of maternal alcohol consumption on the offspring. Binge drinking pattern refers to a high consumption of alcohol for short periods of time, whereas a Mediterranean drinking pattern refers to a lower consumption during a long period of time. Several researches based on animal models found that binge drinking patterns are particularly harmful even if the overall alcohol amount consumed in short times is less than those of more continuous drinking patterns. Consistently, long-term studies in humans have confirmed that children of binge drinking mothers exhibited especially severe cognitive and behavioural deficits, as they were exposed to high blood alcohol concentrations during critical periods of fetal development (NIAAA 2017).

Toxic damage due to alcohol

The mechanisms underlying ethanol-induced damage during fetal development show a high complexity, involving different cell types and molecular pathways. All periods of fetal development are equally “critical” and vulnerable to alcohol, it is just that the target differs. Alcohol toxicity implies alterations in cell cycle, cellular proliferation and migration, interferences in cell signaling and gene expression, epigenetic modifications and finally cell death mechanisms activation. At present, a growing body of evidence suggests that the oxidative

stress generated after alcohol consumption and the production of reactive oxygen species (ROS) play a pivotal role in FASD (Sulik 2014).

Alcohol toxicity is produced both directly by ethanol itself and also by its metabolic products, including acetaldehyde and ROS produced during its biotransformation. Oxidative stress from alcohol is caused by a redox imbalance due to the excess production of NADH during alcohol oxidation and the need to rebalance NAD/NADH ratios thereafter through lactate dehydrogenase. Also, an important increase of the reactive oxygen species (ROS) can be generated (Figure 1). Besides, ethanol not only increases ROS species but also promotes a decrease in the levels of antioxidant pathways and molecules. ROS are highly reactive molecules whose excess affects the integrity of cellular membranes, cause alterations in RNA and protein functions, lipid peroxidation, and promote the activation of cell death pathways as apoptosis (Brocardo et al. 2011).

The role of placenta

Placenta is a crucial structure during fetal development acting as a protective barrier against harmful effects produced by certain drugs as well as a partial barrier between the mother and fetus in order to prevent fetal and maternal blood from mixing. Therefore, an adequate exchange across the placenta is essential for a normal fetal metabolism and growth (Bosco and Diaz 2012).

Once alcohol is consumed, ethanol readily crosses the placental barrier and enters to the fetal circulation. Kinetics of ethanol metabolism differs between pregnant and non-pregnant women and also between the maternal and fetal circulations. Remarkably, a dose of ethanol consumed by a pregnant women reach 40% of concentration in amniotic fluid compared to the concentration found in maternal blood; however, the clearance of ethanol is slower in amniotic fluid than in maternal blood. As a result, maternal ethanol consumption generates prolonged periods of alcohol exposure in the fetus, causing a severe damage to developing structures (Mancinelli 2014).

The development of placenta is a highly regulated process and it is therefore susceptible to toxic compounds as alcohol. It is well known that after alcohol consumption placenta generates oxidative stress (ROS) which modify several placental functions such as signaling, production

and release of hormones and enzymes, transport of nutrients and waste products, implantation, cellular growth, and maturation (Bosco and Diaz 2012; Mancinelli 2014).

Alcohol also alters placental blood flow. On the one hand, ethanol impairs the physiological conversion of uterine vessels required for expansion of maternal circulation into placenta. This effect diminishes the production of the vascular system essential for ensuring adequate blood and nutrient delivery. On the other hand, ethanol affects fetal-placental vasculature. As fetal-placental vasculature lacks autonomic innervations, autocrine and/or paracrine agents such as nitric oxide (NO) radical play an important role in the regulation of fetal-placental blood flow. Ethanol decreases NO availability as part of it is used towards scavenging free radicals, thus causing a deregulation of fetal blood flow. This process is demonstrated in ethanol exposed placentas showing ischemia, infarction and reduced thickness due to increased cellular necrosis and apoptosis. It has also been demonstrated that moderate ethanol exposure can alter the expression of several placental genes playing critical roles in fetus development. All these abnormalities described above difficult the delivery of nutrients to fetus and generate intrauterine growth retardation and other anatomical anomalies that can lead to structural birth defects seen in FASD (Bosco and Diaz 2012; Mancinelli 2014). (Figure 2)

The importance of nutrition in FASD: Maternal nutrition status

Research clearly shows that nutrition is an important risk factor for FASD. Nutrition interacts with alcohol in various ways that may potentially exacerbate or protect against alcohol's teratogenicity. There are increasing evidence that poor maternal nutrition can compromise the healthy fetus development and moreover it is known that women who drink alcohol during pregnancy often have poor nutritional status (May et al. 2004; Guerrini et al. 2007). A population-based study the Western Cape Province of South Africa showed that mothers with FASD children had major nutritional deficiencies, with significantly lower intake of vitamins A, C, D, E, B_2, calcium, omega-3 fatty acids and choline compared to nondrinking mothers (May et al. 2014). Another study with pregnant women in Ukraine and Russia showed that the mothers that consumed alcohol during pregnancy had lower levels of plasma zinc that non-drinking mothers (Keen et al. 2010). Some researches tried to treat women with choline, minerals and

antioxidants in order to reverse nutritional deficiencies commonly seen in FAS mothers (Cohen-Kerem and Koren 2003; Keen et al. 2010; Kable et al. 2015).

Prenatal nutritional treatment of neurocognitive disorders related to FASD

Some studies found that nutritional supplementation during pregnancy may attenuate alcohol teratogenic effects. The studies focused on intervening on the toxicological mechanisms of alcohol such as oxidative stress or changes in DNA methylation.

Studies with rodent models of FASD have shown that the supplementation with antioxidants to pregnant females at the time they were given alcohol, reduced oxidative stress and cell loss of offspring. Other studies demonstrated that nutrients such as choline, betaine, folic acid, methionine, and zinc can attenuate alcohol-induced changes to the epigenome. Despite these results, none of prenatal methods are currently approved for clinical use (Werts et al. 2014; Gupta et al. 2016).

Feeding behaviours and postnatal nutritional treatment of neurocognitive disorders related to FASD

Prenatal alcohol exposure affects several behavioural domains. It is thought that alcohol can also affect the dynamics surrounding nutrition and eating behaviours and, in consequence, nutritional status of the affected person. Werts et al tried to identify possible abnormalities in food and eating behaviours among FASD children. The results were that about 50% of girls were overweight or obese and there were recurring feeding problems included constant snacking, lack of satiety, and picking eating/poor appetite (Murawski et al. 2015). All this feeding problems have a direct impact on the quality and quantity of nutrients in the diet, with deficits in vitamins, minerals, and essential acids. Some studies analyzed the dietary nutrient intake of FASD children, concluding that these children were vulnerable to nutritional inadequacies with clearly deficits in vitamins C, D, E and K, magnesium, potassium, zinc, calcium, and choline (Suresh et al. 1999; Martinez and Egea 2007; Fuglestad et al. 2013; Nguyen et al. 2016a). (Figure 3)

Following this research line, some authors tried to improve these deficits with nutritional supplements in order to reverse or improve neurocognitive disorders related to FASD. Most

authors studied the role of vitamin E, choline, and antioxidants. All this nutrients will be discussed in the following sections, including studies in animal models and some in humans. Despite most of the studies used animal models, in order to facilitate the following discussion, the experimental parameters, and the main findings are summarized in Table 1.

The role of oxidative stress in FASD has been corroborated by numerous studies which show the positive effects of antioxidant molecules therapy upon prenatal ethanol exposure, but nearly every one of those studies looks at oxidative endpoints, no to the assessment of brain function. A large body of clinical studies consistently fail to show benefit for antioxidant intervention in a range of diseases having a strong oxidative damage component, but positive results have been found in Down syndrome patients. Several *in vivo* studies have indicated that antioxidant treatment could prevent or reduce growth retardation and/or the occurrence of malformations upon ethanol exposure during development. As expected, the use of compounds with antioxidant properties has also been shown to reduce oxidative stress level and/or to increase the endogenous antioxidants capacity in different rodent models of FASD (Brocardo et al. 2011).

However, several of antioxidant compounds could act as reactive chemicals and create false-positives on assays for oxidative stress, interfering in assay endpoints and perhaps in clinical results, and this problem needs to be addressed in future studies.

Vitamin E

Vitamin E has been extensively demonstrated to prevent alcohol-induced cell loss using animal models. Although it has been shown in animal models exposed to alcohol that vitamin E normalizes fetal development (Wentzel et al. 2006), in humans it was found that vitamin E supplementation of pregnant women consuming alcohol apparently decreased mean birth weight (Boskovic et al. 2005; Kable et al. 2015). At postnatal time, vitamin E decreased oxidative stress in different models of FASD. In offspring rat pups exposed prenatally to ethanol, vitamin E reversed the levels of protein and lipid oxidation in both hippocampus and cerebellum and amplified the g-GCS and total GSH protein expression levels. Vitamin E also decrease DNA damage and restores the elevated level of homocysteine to control level (Heaton et al. 2004; Marino et al. 2004; Siler-Marsiglio et al. 2004; Siler-Marsiglio et al. 2005; Shirpoor et al.

2009). Moreover, vitamin E could a positive impact at the neuroanatomical level. In this sense, embryos prenatally exposed to ethanol and treated with vitamin E attenuated the reduction and damage in the number of Purkinje cells in the cerebellum, but a clear benefit from a clinically relevant vitamin E intervention was not found (Heaton at al. 2000a; Heaton et al. 2000b; Tran et al. 2005).

Resveratrol

Resveratrol is a natural compound, an antioxidant with antiapoptotic, free radical-scavenging and antilipoprotein peroxidation properties (Shakibaei et al. 2009). Using a rat model of FASD, the treatment with resveratrol before ethanol exposure decreased ROS levels because of its antioxidant properties and also restored nuclear factor (Nrf) which has been demonstrated to be a critical transcription factor that regulates the induction of antioxidant enzymes detoxifiying and antioxidant genes (Zhang 2006; Nguyen et al. 2009; Kumar et al. 2011). Resveratrol also protected and recovered Swann cell viability and increased Brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) expression (Yuan et al. 2013).

Omega-3

DHA (docosahexaenoic acid) is an essential polyunsaturated fatty omega-3 acid. DHA is important during fetal development because of it is one of the most easily influenced and relevant nutrient for the correct neuronal development, so playing a significant role in the development of CNS (Horrocks and Yeo 1999; Sheppard and Cheatham 2017). A study published in 2013 using rat model revealed that prenatal ethanol exposure caused a decrease in glutathione concentrations in the brain and the long-terme potentiation (LTP), increasing lipid peroxidation, leading to oxidative stress. The supplementation with DHA increased glutathione concentrations, reversed the deficits of LTP and decreased lipid peroxidation, thereby partially reversing the negative effects of prenatal ethanol exposure This study suggested that DHA supplementation could reduce oxidative stress and enhance antioxidant capacity in fetus exposed to ethanol (Patten et al. 2013a; Patten et al. 2013b).

There are no known studies on DHA supplementation in pregnant mothers who consumed alcohol. Intervention studies are needed to understand the potential therapeutic effect of DHA

on infants with FASD and determine how these damage effects may be alleviated o reduced with DHA.

Choline

Choline is an essential nutrient and together with its metabolites participates in an important number of metabolic pathways involved in neurotransmission, structural integrity of cell plasma membranes, and cell signaling. This nutrient is the most-studied nutrient related to brain development and memory function. Literature illustrates that pre and postnatal choline supplementation leads to long-lasting morphological, electrophysiological, and neurochemical changes in the CNS that contribute to long-lasting cognitive enhancements. Perinatal choline supplementation can also enhance cognitive improvements to later environmental manipulations, such as enriched environment (Ryan et al. 2008; Young et al. 2014).

Animal and human studies suggested the given potential effects of choline, specifically with prenatal ethanol exposure.

Using animal models, some studies showed that perinatal choline supplementation could mitigate alcohol related deficits, in some brain domains such as working memory and spatial memory but there is no agreement in the effects on the hyperactivity (Ryan et al. 2008; Young et al. 2014; Bearer et al. 2015; Schneider and Thomas 2016). Other studies examined the effects of developmental alcohol exposure and perinatal choline supplementation on hippocampal M1 and M2/4 muscarinic receptors in rats, which would indicate alcohol-related behavioural affects. Choline mitigates the increase in M2/4 receptors density but did not prevent decrease in muscarinic M1 receptor density in dorsal hippocampus (Monk et al. 2012).

There are limited research studies on choline supplementation in human subjects prenatally exposed to alcohol. There are specifically two studies that observed the effects of supplementation with choline at the postnatal level. The first wanted to determine if choline supplementation had the potential to improve neurocognitive functioning, particularly hippocampal-dependent memory in children with FASD aged 2.5 to 5 years. They observed that treatment effect on elicited imitation items recalled was significant in the younger participants and there was an inverse relation between choline dose (in mg/kg) and memory improvement, suggesting that there were potential sensitive periods (Wozniak et al. 2015). The other study

explored the effectiveness of a choline intervention in children with FASD aged 5 to 10 years. The main result was that choline group did not improve cognitive performance in any domain (Nguyen et al. 2016b).

In conclusion, there is a need to continue studying the role of the supplementation with choline in children with FASD.

Epigallocatechin gallate (EGCG)

Polyphenols are natural antioxidants, including EGCG, epigallocatechin, epicatechin gallate, and epicatechin. EGCG is the major constituent and it is also the component with the highest antioxidant and free radical scavenging activity. It is believed to be responsible for most of the health benefits attributed to green tea consumption. EGCG is able to reduce lipid peroxidation, decrease the activity of CYP2E1 (which process ethanol generating ROS) and decreases free radical protein adducts formation. For all that, the use of EGCG antioxidant have been postulated as a promising treatment in order to attenuate the adverse effects of oxidative stress increase (Cohen-Kerem and Koren 2003; Oliva et al. 2011; Joya et al. 2015). The effectiveness of EGCG on the promotion of adult hippocampal neurogenesis has been reported, mainly in vitro research and invites its application in clinical neurodevelopmental and neurodegenerative disorders. In this context, previous reports also suggested the beneficial effects of EGCG in animal models of Down syndrome and Alzheimer disease (Wang et al. 2012; de la Torre et al. 2014). It is known that Down syndrome and FASD share common dysmorphologies in humans and animals models. Moreover, there exist common mechanisms at cellular and molecular levels that are disrupted by trisomy or alcohol consumption during pregnancy and lead to craniofacial and neurological phenotypes associated with Down syndrome or FASD (Solzak et al. 2013).

A study published in 2010, using a murine model of FASD, aimed to assess the role of ROS in FASD and to determine the effects of EGCG in ameliorating the effects of ethanol. The results were that EGCG ameliorated ethanol-induced growth retardation and also inhibited the increase in H_2O_2 and MDA. They concluded that EGCG can prevent some of the embryonic injuries caused by ethanol (Long et al. 2010).

To our knowledge, there is only one study, registered in clinicaltrials.gov (NCT02558933), which aims to determine the efficacy of EGCG as a therapeutic candidate to improve cognitive performance in children diagnosed with FASD (Garcia-Algar 2014). This study included children aged 7 to 15 years and treated with 9 mg/kg/day of EGCG for one year. Preliminary results after 6 months of treatment showed that there was an improvement in the recent and delayed memory and also in working memory of children treated with EGCG. These results are very promising since, for the first time, a treatment would have been achieved that would improve the cognitive performance of children with FASD, so far nonexistent. Actually the only treatments that exist are at a symptomatic level to alleviate the associated symptoms that children suffer, focusing on neurocognitive rehabilitation.

Conclusion

FASD is due to prenatal exposure to alcohol and it functions as a clinical complex and multifaceted disorder, being the leading cause of preventable mental delayed and birth defects in Western countries. Despite public health efforts to reduce or eliminate alcohol consumption during pregnancy, an important number of pregnant women continue consuming alcohol, so nowadays FASD is a major public health problem. The simplest method for the prevention of FASD remains abstinence from ethanol, but effective and evidence-based interventions and treatment against ethanol damage are needed to ameliorate adverse consequences in children who are affected by FASD.

Based on the mechanisms involved in the ethanol induced damage, nutritional supplementation appears as an innovative and promising alternative. Nutritional supplementation in children with FASD could have a dual objective: to overcome nutritional deficiencies and to reverse or to improve specific neurocognitive deleterious effects of prenatal alcohol exposure.

Although robust information on the role of nutrients and intervention is limited in FASD, mainly at postnatal level, potential nutrients, such as vitamin A, DHA, resveratrol, choline, and EGCG have been suggested and clinical trials are going to start in the next future.

Further research is necessary to confirm positive results, to determine optimal amounts of nutrients needed in supplementation, and also to investigate the collective effects of simultaneous multiple-nutrient supplementation.

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Figure legends

Figure 1. Ethanol metabolism involves the mitochondrial respiratory chain, the xanthine oxidase and the NADPH oxidase pathways producing oxidant agents as acetaldehyde and ROS. Superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH-) increase inside the cell (green arrows). Ethanol exposure also decrease antioxidants enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (SPx) and glutathione reductase (GR) as well as the intracellular levels of reduced glutathione (GSH) (red arrows).

Figure 2. Effect of maternal alcohol consumption on the placental development. Alcohol generates oxidative stress promoting a shallow placentation and a diminished uterine irrigation of the placenta. ROS alters the normal development of the embryo by different mechanisms such as impairment in migration of neural crest cells (which can cause face bones alterations and cardiac defects)and apoptosis of neural progenitor cells (causing abnormal brain development).

Figure 3. Current strategies for the prevention and treatment of FASD.

TABLE 1: Studies of specific nutrient supplementation to alleviate postnatal ethanol exposure effects

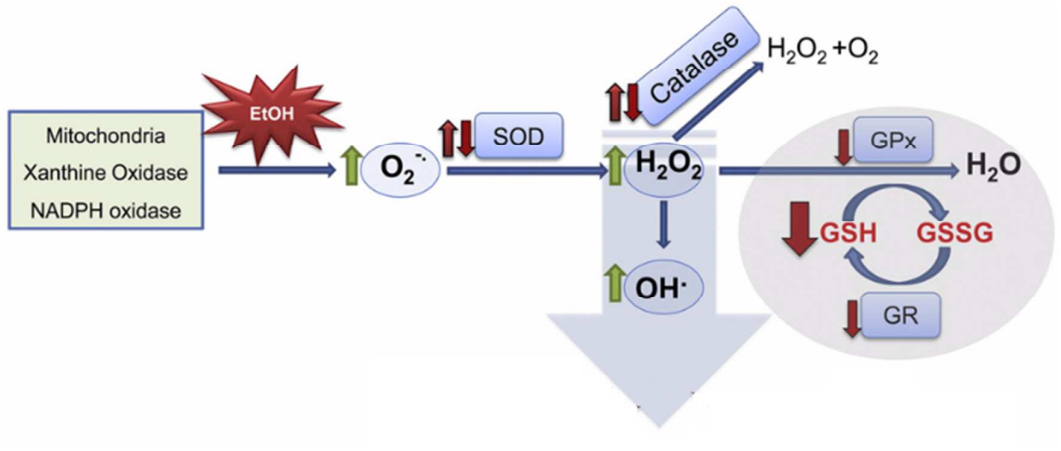
Nutrient or Drug	Animal model	Range of drug concentrations/ Exposure period	Range of EtOH concentrations/ Exposure period	Results and Observations	Reference
Vitamin C	Guinea pigs	250 mg/kg	9g/kg/day	Reduces lipid peroxidation in the liver Enhances the activities of glutathione peroxidase and reductase Reduces the activity of GGT	Suresh et al. 1999
Vitamin E	Long-Evan rats	30-60 IU/100ml PD 4-5	12% PD 4-5	Prevents the loss of Purkinje cells High dose completely prevented Purkinje cell damage and loss	Heaton et al. 2000a; Heaton et al. 2000b
	Long-Evan rats	0.05mM/24h PD8	86.7-346.6mM/24h PD8	Restores the expression of NTFs (BDNF and neurotrophin-3) Diminishes the cellular disturbances in oxidative processes	Heaton et al. 2004
	Long-Evan rats	2000 g/kg PD6	5.25g/kg PD 7-9	Alleviates the increase in protein carbonyls Does not improve spatial learning in the ethanol-exposed animals	Marino et al. 2004
	Long-Evan rats	12.26 mg/kg/day PD 4-9	2.625 g/kg/day PD 4-9	Fails to protect against reduction of cerebellar Purkinje cells	Tran et al. 2005
	Long-Evan rats	0.05mM/24h PD9	86.7- 346.6mM/24h PD9	Amplifies the g-GCS and total GSH protein expression levels	Siler-Marsiglio et al. 2005
	Wistar rats	300mg/day GD7-PD21	4.5g/kg/day GD7-PD21	Decrease DNA damage Restores the elevated level of Hcy to control levels	Shirpoor et al. 2009
Vitamin E + Pycnogenol	Long-Evan rats	25-100 µg/ml/5s-24h PD9	86.7-346.6mM/5s-24h PD9	Decreases cell death and reduces the activation of caspase-3	Siler-Marsiglio et al. 2004
Resveratrol	Long-Evan rats	2-100 mg/kg/1-24h before EtOH treatment	80mM/5h PD7	Decreases ROS levels Restores the	Kumar et al. 2011

		PD7		expression levels of Nrf in the nucleus Retains the expression and activity of NADPH quinine oxidoreductase 1 and SOD	
	Wistar rats	30mM/96h PD3	1500 mg/dl/96h	Recovers cell viability Increases the BDNF and GDNF expression	Yuan et al. 2009
Curcumin	Wistar rats	30-60 mg/kg PD6-28	5g/kg PD7-9	Ameliorates neuroinflamation (oxidative nitrosative stress, TNFα, IL-1β and TGF-β1) Decreases neuronal apoptosis (NF-κβ and caspase 3) in both cerebral cortex and hippocampus	Tiwari and Chopra. 2013
Melatonin	Sprague-Dawley rats	20 mg/kg/day PD4-9	6 g/kg/day PD4-9	Does not decrease the apoptotic Purkinje cell number Does not change BAD measured on PD6	Grisel and Chen 2005
Alpha-lipoic acid	Wistar rats	100 mg/kg GD7-PD21	4.5 g/kg GD7-PD21	Decrease DNA damage Restores the elevated protein carbonyl and lipid hydroperoxide levels	Shirpoor at al. 2008
Selenium + folic acid	Wistar rats	0.5µg/g of selenium and 8µg/g of folic acid PD21	5% w1, 10%w2, 15%w3, 20% from w8 before pregnancy until end of lactation period	Reduces selenium loss Improved balance among oxidative enzymes	Ojeda et al. 2009
Omega-3	Sprague-Dawley rats	34.2% Dams: GD21-PD22 Pups: PD22-60	35.5% GD1-21	Increased glutathione concentrations Decreased lipid peroxidation Reduced oxidative stress Enhanced antioxidant capacity	Patten et al. 2013a
	Sprague-Dawley rats	34.2% PD1-65	36% GD1-22	Ethanol-exposed adult males exhibited reduction in long-term potentiation (LTP) Omega-3 reversed the deficit of LTP	Patten et al. 2013b

Choline	Sprague-Dawley rats	100mg/kg/day PD11-20, 21-30 or 11-30; measured after PD45	5.25g/kg/day PD4-9	Mitigated deficits in spatial memory	Ryan et al. 2008
	Sprague-Dawley rats	100mg/kg/day PD4-30; measured at PD30-33	5.25g/kg/day PD4-9	Attenuated hyperactivity Mitigated increase in M2/4 receptor density Did not prevent decrease in muscarinic M1 receptor density in dorsal hippocampus	Monk et al. 2012
	C57B16/J mice	18.8 mg/ml PD1-5, PD6-20	13.6% (6g/kg) P5	Choline treatment increased total distance crossed for females and males No effect of choline in no-alcohol exposed mice	Bearer et al. 2015
	Sprague-Dawley rats	100mg/kg/day PD40-60	5.25g/kg/day PD4-9	Choline supplementation mitigate alcohol-related deficits in working memory Choline treatment failed to attenuate alcohol-related hyperactivity	Schneider and Thomas 2016
	Children with FASD aged 2.5-5y	500mg choline during 9 month		Treatment effect on elicited imitation items recalled was significant in the younger participants (2.5- to ≤4.0y) Inverse relation between choline dose (in mg/kg) and memory improvement	Wozniak et al. 2015
	Children with FASD aged 5-10y	625 mg/day during 6 weeks		Choline group did not improve in cognitive performance in any domain	Nguyen et al. 2016b
EGCG	C57B16/J mice	200,300 or 400 mg/kg/day GD7-8	0.005-0.02 ml/g GD8	EGCG ameliorated ethanol-induced growth retardation EGCG inhibited the increase in H_2O_2 and MDA	Long et al. 2010
	Children with FASD aged 7-15 y	9mg/kg/day during 12 month		After 6 months of treatment with EGCG, there was an improvement in the recent and	Garcia-Algar 2014

				delayed memory and working memory	

(EGCG) Epigallocatechin-3-gallate; (FASD) fetal alcohol spectrum disorder; (GD) gestational days; (GGT) Gammaglutamyltransfere; (MDA) malondialdehyde; (Nrf) nuclear factor; (PD) postnatal days; (SOD) superoxide dismutase



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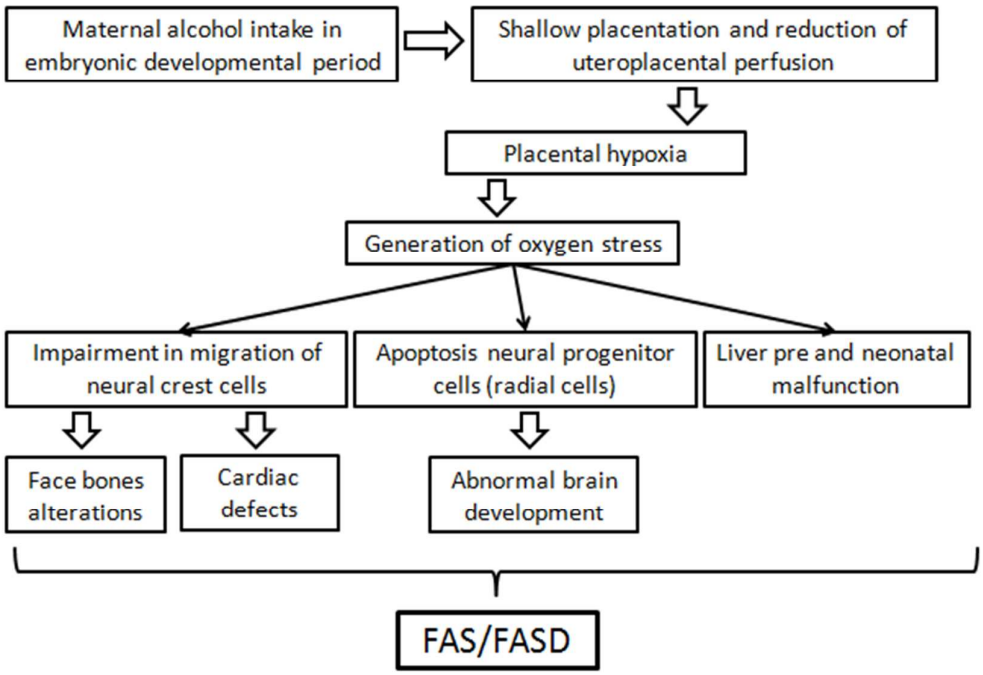

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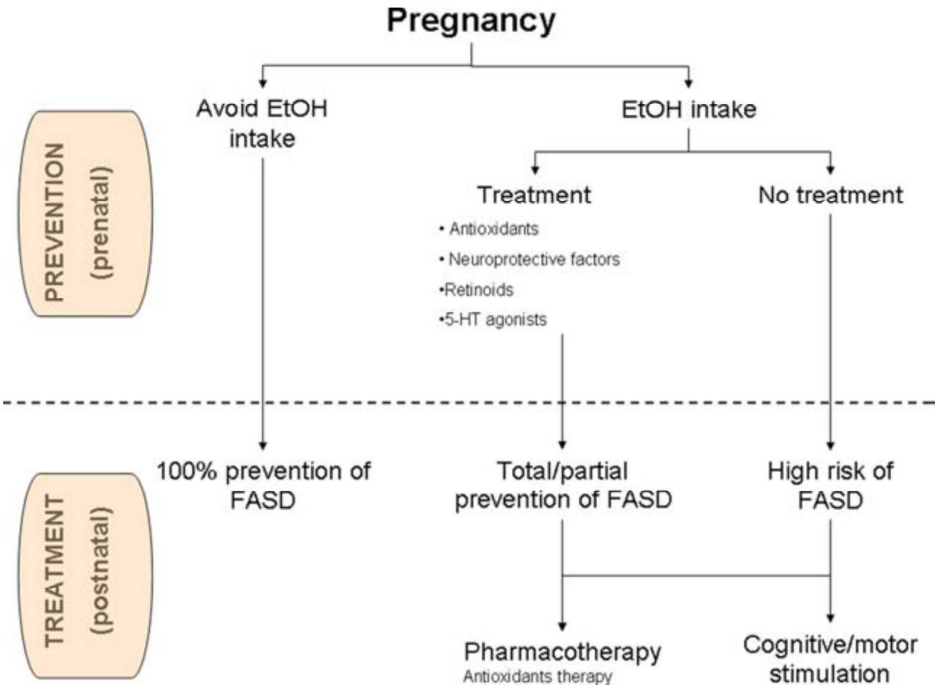

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