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Ethanol: Toxicity and Dangers in Women of Child-Bearing Age

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Abstract

The World Health Organisation estimates that alcohol abuse by adults accounts for about 5% of global disease burden. Additionally, prenatal alcohol exposure (PAE) causes 'fetal alcohol spectrum disorder' (FASD). Depending on severity, FASD is characterised by low birth weight, small head size at birth and growth retardation. There are also facial features of narrow eyes, flat upper lip and midface and impaired fine motor skills, hearing loss, poor hand-eye coordination and cognitive impairment. World-wide, up to 10% of children may be affected by PAE. It is unclear what dose or pattern of drinking results in these damaging effects, but animal models suggest that high, acute doses of ethanol ('binge drinking') in early pregnancy can result in the facial changes of FASD, whilst sustained, lower dose intake in later pregnancy produces anxiety and depression-like symptoms and deficits of learning and memory. The mechanisms underlying the deleterious effects of PAE are also unresolved, but evidence exists of long-lasting damage due to oxidative stress, increases in inflammatory mediators and changes to the brain renin-angiotensin system. There is also evidence of epigenetic changes. There is a need to prevent or limit the potential adverse effects of ethanol on the unborn child. It is highly unlikely, however, that all sexually-active women of child-bearing age not using reliable contraception will abstain from alcohol. There is therefore a need to research methods of reducing ethanol toxicity for the unborn child and / or develop therapeutic strategies to reverse the deleterious effects of ethanol on the unborn child.

According to the American Addiction Centers (Alcohol.org), the people of Belarus consume the highest amount of alcohol per head of population (over 15 years old) per year at the equivalent of 14.4 l of pure ethanol per annum; this equates to over fifty bottles of vodka per person per year. Russia lies fifth at 11.5 l of pure ethanol per person per year and USA is 25th at 8.7 l per person per year. Such alcohol consumption is known to have deleterious effects on health and to decrease lifespan and 'healthspan', and much effort has been invested in public health initiatives to raise awareness of these risks. The World Health Organisation estimates that harmful use of alcohol accounts for 7.1% of global disease burden in males and 2.2% in females.

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Several countries issue guidelines for 'safe limits' of alcohol consumption, for example, USA recommends no more than one standard drink (=14 g pure ethanol) per day whilst the UK recommends no more than 14 "units" of alcohol (=112 g pure ethanol) per week (16 g per day). US recommendations equate to 22 bottles of vodka per year. Other countries, for example Kazakhstan have decreased alcohol consumption by introducing regulations concerning minimum prices for alcohol and restrictions in that hours that it can be sold.

One particular, often under-publicised, risk of alcohol consumption is the risk of damage to the unborn fetus when alcohol is drunk by pregnant women. A recent study from Germany suggests that 18.6% of non-pregnant young women may drink alcohol at harmful levels, but that this is only slightly lower in pregnant women at 13.8% [1].

Prenatal alcohol exposure (PAE) can cause adverse effects on the developing fetus and can result in a wide range of anatomical and neurobehavioural deficits typically labelled 'fetal alcohol spectrum disorder' (FASD). The severity of FASD ranges from the most severe foetal alcohol syndrome (FAS) with its facial dysmorphology, through partial foetal alcohol syndrome (pFAS) to alcohol-related birth defects (ARBD). Being the most severe, FAS is characterized by anatomical features such as low birth weight, small head size at birth, lack of weight gain over time and growth retardation. There are also the characteristic facial features of narrow eyes, flat upper lip/filtrum and flat midface. Less obvious, but equally real, are the features of impaired fine motor skills, hearing loss, poor hand-eye coordination and cognitive impairment.

A recent study in the UK estimated that 79% of pregnant women consumed alcohol at some point during pregnancy, and that up to 17% of off-spring exhibited at least some of the features of FASD [2]. Children with FASD typically perform poorly in school and often fail to secure good employment. Prevalence of FASD is greater within the prison population than within the general population.

As can be seen, in the UK up to 79% of women may drink at least 'some' alcohol during pregnancy, and in Germany, 13% drink harmful amounts of alcohol during pregnancy. If 79% of unborn babies are exposed to alcohol, but only 17% develop FASD, what is it causes the problem? Dose of ethanol, timing and frequency of exposure or some form of genetic predisposition? The big question is "what is a safe limit of alcohol consumption during pregnancy?"

FAS was first identified by Jones and Smith in the USA in 1973 [3]. Importantly the mothers were 'alcoholic', often drinking more than one bottle of spirits per day (240 g ethanol per day). Associated with such high alcohol consumption it might also be expected that there would be nutritional deficiencies, social deprivation, etc. It is difficult, therefore, to separate the many factors in order to estimate a safe level of alcohol consumption.

I and many other researchers have utilized animal models to determine possible 'safe' levels of prenatal alcohol exposure [4]. Such models have significant advantages over clinical studies in that they allow controlled exposure to ethanol, at fixed doses, at specific periods during pregnancy and in particular patterns (e.g., constant low-level versus binge-drinking). Animal studies also remove the confounding factors of nutritional status, educational status, socio-economic status, income, etc. seen in human populations. The animals used are typically of 'inbred' strains, generated by sibling mating, and are considered to be > 99% genetically identical with similar, and controlled prior experiences and nutritional status. On the other hand, however, animal studies are potentially misleading because of the different rates of development of mice and humans. For example the mouse gestation period is 20 days and the pups are born blind and relatively undeveloped; development continues postnatally. Thus days 1–10 of a mouse pregnancy are generally seen as being equivalent to the human first trimester, days 11–20 are the equivalent of second trimester and post-natal days 1–10 are equivalent to trimester 3.

Another big difference between humans and rodents is the way in which ethanol is distributed around the body and metabolised. For example, the body water content per kilogram of body weight is different thus apparent volumes of distribution for ethanol are different. It is also known that mice metabolise and excrete ethanol more rapidly than humans [5]. Thus, for example Schambra et al. [6] gave pregnant mice 2.4 g/kg ethanol, twice, 4 h apart, and reported peak blood alcohol concentrations of 104 mg/dl. For a 65 kg Human, 2.4 g/kg ethanol equates to approximately 1.5 l of table wine in a single bout. Alternatively some researchers put ethanol in the drinking water, as a model of chronic low dose exposure. An intake of approximately 10 g ethanol per kg body weight per day in mice is required to give a peak blood alcohol concentration of 80 mg/l [4]. 10 g ethanol per kg bodyweight per day in mice would equate to 6.5 l of table wine over 24 h in a 65 kg Human.

Notwithstanding the seemingly unfeasible doses of ethanol used and the differences between mice and Humans, animal models of prenatal alcohol exposure have generated invaluable data. A review of animal data by Petrelli et al. [4] clearly demonstrates that high, acute doses of ethanol (two doses of 2.9 g/kg) on gestational days 7, 8 or 9 (first trimester equivalent) result in the characteristic facial changes of FAS, as does chronic lower dose ethanol exposure (20 g/kg/day) from days 1 to 8. The situation changes, however, later in pregnancy. Lower dose, consistent, prenatal ethanol exposure (10–20 g/kg/day) was seen to produce anxiety and depression-like symptoms and deficits of learning and memory, without the facial changes. It is thus suggested that early binge drinking by the mother may result in the facial features of FAS, whilst

later sustained lower dose prenatal ethanol exposure may result in the cognitive changes. These animal studies, however, have not been able to identify a 'safe' level of alcohol consumption during pregnancy. The public health advice therefore has to be that the only safe behaviour is to abstain from drinking throughout the whole of pregnancy, even before pregnancy is confirmed. Thus sexually-active women not using contraception should be advised to abstain from alcohol in order to protect the unborn child.

Animal studies have also explored the possible chemical aetiology of FASD in order to explore potential prophylactic or corrective therapies. Abate et al. [7] dosed pregnant rats via a feeding tube with an acute ethanol dose of 2 g/kg on days 17–20 of pregnancy. The gestation period for a rat is 21.5 days. The adolescent offspring were tested at 30 days of age, which is considered pre-pubertal. The ethanol exposed animals exhibited decreased rearing behaviour, which is indicative of increased response to stress, and increased met-enkaphalin in several brain areas but not all. This finding is interesting in the light of the work of Banks et al. [8] who studied the effects of ethanol consumption (5% in drinking water) in mice over 56 days. Met-enkephalin was measured in the brain and serum. Brain concentrations of Met-enkaphalin were increased in ethanol-consuming mice in comparison to controls after 7, 10, and 14 days of ethanol drinking. Values then declined below those of controls after days 28 days. There were no significant differences in serum met-enkephalin concentrations between ethanol-treated and control animals at any of the time points. This finding indicates that ethanol disrupts brain met-enkephalin which may explain some changes in response to stress, plus some addictive behaviours.

There is also a significant body of literature that indicates that prenatal alcohol exposure elevates oxidative stress [9]. Brocardo et al. [10], for example, treated pregnant rats with ethanol, via drinking water, to give peak blood alcohol concentrations of 90–100 mg/dl. There were three experimental groups, rats receiving ethanol only for days 1–10 of gestation, those receiving ethanol for days 11–21 of gestation and those receiving ethanol on post-partum days 4–10. Offspring were tested at 60 days of age when lipid peroxidation was assessed in several brain areas using formation of malondialdehyde (MDA). Those offspring exposed to ethanol during the second and third trimester equivalent were found to have evidence of oxidative damage (i.e., increased lipid peroxidation) in the hippocampus, thus demonstrating that prenatal ethanol exposure can have long-term consequences in the adult brain by dysregulation of its redox status, possibly by depletion of the antioxidant glutathione [9].

Hormones of the Hypothalamic-Pituitary-Adrenal (HPA) axis have also been shown to be influenced by prenatal alcohol exposure. Xia et al. [11] dosed pregnant rats orally with ethanol (4 g/kg/day) from day 11 of pregnancy until delivery. The male offspring were then fed a high fat diet until postnatal week 16 when blood samples were taken for determination of adrenocorticotrophic hormone (ACTH) and corticosterone (the rat equivalent of human cortisol). The results indicated that both ACTH and corticosterone were significantly decreased, by about 30%, in the rats exposed to ethanol prenatally. This might suggest that components of the HPA may be suppressed in children exposed to ethanol in utero, although there is no clinical evidence that this is the case.

There is also a body of literature concerning the effects of prenatal alcohol exposure on inflammatory mediators. Raineki et al. [12], for example, dosed pregnant rats on gestational days 1–21 in a manner similar to that described for Brocardo et al. [10] above (6.6% ethanol in drinking water). Biochemical parameters were determined on postnatal day 12. The results showed that pups exposed to ethanol prenatally had significantly (30%) elevated serum C-reactive protein, a non-specific hepatic protein marker of inflammation, infection and trauma. These results supported the previous findings of the same group in that they had previously reported elevated serum TNF-α, interleukin-13 and interferon-γ, all inflammatory mediators (cytokines), on post-natal day 8 which had returned to control values by post-natal day 22.

My own unpublished, preliminary research suggests that prenatal alcohol exposure may also induce long-lived changes in brain derived neurotrophic factor (BDNF). Changes in this parameter may be reflected by changes in plasma or serum. BDNF is a nerve growth factor important in neuronal development, neuronal repair and learning and memory. It is known to be elevated at times of stress and other forms of neuronal trauma (e.g., infection and inflammation). Terasaki and Schwarz [13] reported that prenatal exposure of rats to ethanol 2 g/kg twice daily from gestational days 10–16, which resulted in a maternal blood alcohol concentration of 70 mg/dl, resulted in increased BDNF gene expression in the perirhinal area of the brain

at 90 days of age. Our own data in mice supports these findings in that prenatal exposure to ethanol (5%) via drinking water throughout pregnancy and until weaning resulted in significantly elevated BDNF in the cerebrocortex, cerebellum and hippocampus at 124 days of age. Such elevated brain BDNF may indicate a prolonged neurochemical response to the foetal neuronal trauma of the alcohol exposure. Plasma BDNF has, however, also been seen to be elevated 48 h post-delivery in infants with neonatal opiate abstinence syndrome [14] and other workers have suggested that prenatal alcohol exposure decreases brain expression of BDNF and its receptor [9]; the potential role of BDNF in the aetiology of FASD is therefore uncertain.

Our work has also highlighted another set of possible factor in FASD. Angiotensin IV, a component of the renin-angiotensin system, is able to improve learning and memory in animal models [15]. Furthermore it has been shown that administration of drugs known to interfere with the renin-angiotensin system, for example the anti-hypertensive angiotensin receptor blockers (ARBs), can improve cognition in both young and old Human volunteers, possibly as a consequence of elevated endogenous brain angiotensin IV [16]. Acting on the hypothesis that administration of angiotensin IV may be able to reverse the deficits of learning and memory caused by prenatal alcohol exposure, we tested its effects in control mice and mice exposed to ethanol in utero at the age of 2–3 months. The pro-cognitive effect of angiotensin IV was abolished by prenatal alcohol exposure [17], suggesting some alcohol-induced, long-lasting perturbation of the brain renin-angiotensin system [18].

Genetric and epigenetic changes in FASD have also been explored. These are important as the condition has a origin in the foetus which generally only manifests some years later in the young child. For this to occur there must be some "molecular memory" of the events occurring in the foetus. Since the majority of proteins have half-lives less than a few days, it is unlikely that changes in protein conformation or covalent modification to protein molecules would persist in the body for years. However, epigenetic changes are copied as part of the normal DNA replication process and thus inherited by daughter cells. Therefore, changes to the DNA in utero may still be present after several years. The methylation of DNA can affect the expression of affected genes. These covalent alterations can also be preserved through DNA replication and thus passed to daughter cells. Such epigenetic changes provide a mechanism by which developmental or environmental changes can be "remembered" through cellular lineages [19]. Critically for FASD, it provides a mechanism by which the consequences of exposure to alcohol in utero can persist into childhood and beyond [20, 21]. A number of epigenetic changes have been associated with FASD [22]. Over 25 years ago, it was observed that ethanol consumption by pregnant mice reduced the methylation of DNA in the foetus. This was considered to be partly due to the inhibition of DNA methyltransferase enzymes by acetaldehyde (a product of alcohol metabolism) [23]. Recently, a number of studies have attempted to provide a global picture of epigenetic modifications which occur as a consequence of foetal alcohol exposure. One study on Canadian children identified 658 sites in the genome which are differently methylated in patients with FASD. Many of the affected genes are ones which are expressed in neuronal tissues [24]. Another study identified 259 sites and highlighted genes encoding procadherins (which assist in the proper cell-cell adhesion of neurons) among those affected [25].

It is, therefore, still unclear by what mechanism pre-natal alcohol exposure adversely affects the unborn infant. Whether is neurochemical changes in the brain, inflammatory changes, oxidative damage, or genetic changes remains to be resolved. It is, however, unlikely that young women, of child-bearing age, will persistently abstain from consuming alcohol in order to protect the unborn child. There is therefore a need to:

- Develop more alcohol-free alternative drinks to offer young people
- Research methods of reducing ethanol toxicity for the unborn child
- Develop therapeutic strategies to reverse the deleterious effects of ethanol on the unborn child.

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