REVIEW ARTICLE

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Fetal alcohol syndrome and developing craniofacial and dental structures – a review

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Structured abstract

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Objectives – Fetal alcohol syndrome (FAS) is a collection of signs and symptoms seen in children exposed to alcohol in the prenatal period. It is characterized mainly by a distinct pattern of craniofacial malformations, physical and mental retardation. However, with the increased incidence of FAS, there is a great variation in the clinical features of FAS.

Design – Narrative review.

Results – This review describes data from clinical and experimental studies, and *in vitro* models. Experimental studies have shown that alcohol has a direct toxic effect on the ectodermal and mesodermal cells of the developing embryo, particularly in the cells destined to give rise to dentofacial structures (i.e. cranial neural crest cells). Other effects, such as, abnormal pattern of cranial and mandibular growth and altered odontogenesis are described in detail. The exact mechanism by which alcohol induces its teratogenic effects remains still unknown. The possible mechanisms are outlined here, with an emphasis on the developing face and tooth. Possible future research directions and treatment strategies are also discussed.

Conclusion – Early identification of children affected by prenatal alcohol exposure leads to interventions, services, and improved outcomes. FAS can be prevented with the elimination of alcohol consumption during pregnancy. We need to provide education, target high-risk groups, and make this issue a high priority in terms of public health.

Key words: alcohol-induced disorders; craniofacial abnormalities; embryogenesis; fetal alcohol syndrome; odontogenesis; prenatal exposure

Introduction

Maternal ingestion of high levels of ethanol during pregnancy can cause serious birth defects in children due to disruption of normal embryonic development, of which fetal alcoholic syndrome (FAS) is a most devastating sequel (1). FAS was first formally reported by Lemoine *et al.* (2) in 1968. However, since the article was in French, it did not generate much interest till the report of Jones and Smith (3) in 1973. FAS is characterized by retardation of the pre- and postnatal craniofacial growth, central nervous system (CNS) deficiencies and a characteristic set of facial anomalies (Fig. 1). There are lifelong consequences, and the behavioral and learning difficulties are often greater than the degree of neurocognitive impairment (4).

Alcohol probably acts through multiple mechanisms and a range of disabilities has been observed in the absence of dysmorphic features reflecting varying degrees of damage during fetal development; undoubtedly, timing and degree of exposure are important variables that contribute to the variation (5). Thus, the term 'suspected fetal alcohol effects' (FAE) was created (6). These effects were delineated by the United States' Institute of Medicine (IOM), which published recommendations in 1996 for diagnosis of FAS in consultation with a panel of experts. The diagnostic categories presented were FAS with and without a confirmed history of alcohol exposure, partial FAS, alcohol-related births defects (ARBD), and alcohol-related neurodevelopmental disorder (ARND) (7). The term fetal alcohol spectrum disorder (FASD) was recently adopted at a meeting convened by National Organization on Fetal Alcohol Syndrome (NOFAS), a non-profit advocacy group that included representatives from families, researchers, and several governmental agencies (8). FASD is an umbrella term describing the range of effects that can occur in an individual whose mother drank during pregnancy, including physical, mental, behavioral and learning disabilities with possible lifelong implications. As this definition indicates, multiple diagnostic categories (e.g. FAS, ARND, and ARBD) are subsumed under the term FASD. However, FASD is not a diagnostic category and should only be used when referring to the collection of diagnostic terms resulting from prenatal exposure to alcohol (9).

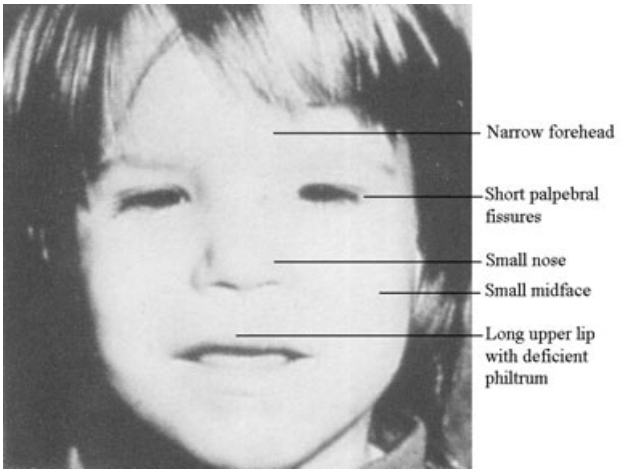

Fig. 1. Child with typical craniofacial characteristics. Source: Sulik KK (2005). Reprinted with permission.

Fetal alcohol spectrum disorder occurs in about 10 per 1000 live births or about 40 000 babies per year. FAS, the most recognized in the spectrum, is estimated to occur in 0.5–2 per 1000 live births. It now outranks Down syndrome and autism in prevalence (10). Although the simplest prevention strategy is for women to avoid alcoholic consumption when pregnant or planning to conceive, one-half of all pregnancies are unplanned. By the time, these pregnancies are confirmed, major embryonic events already have occurred (11). The consequences of fetal exposure to maternal alcohol consumption, therefore, is a serious problem for the individual and for society, in terms of human suffering, lost productivity and medical and social monetary costs (12). Early diagnosis is essential to allow appropriate intervention for children affected by prenatal alcohol exposure and can reduce their risk of facing social difficulties later in life (e.g. problems with employment or trouble with the law resulting from impulsive behavior and lack of inhibition) (13).

To help define the risks associated with maternal alcohol use during pregnancy, researchers are seeking to understand the mechanisms of alcohol's teratogenic effects on various tissues. It has been demonstrated that the effects of FAS in mouse, rat and chick models, are comparable with those in humans and these organisms serve as valuable mechanistic models to study alcohol's teratogenic effects on tissue development (14). In addition, recent findings in molecular embryology have greatly advanced scientific knowledge about the signals and agents that govern normal craniofacial development. Applying this knowledge can help elucidate how prenatal alcohol exposure results in

birth defects and promote the future treatment of this preventable condition (11). Although the basis for craniofacial defects seen in FAS/FAE is not entirely clear, one known target of prenatal alcohol exposure are craniofacial precursor cells, most prominently the cranial neural cells. Many of the structures affected in FAS involve cranial neural crest-derived tissues (facial bone and cartilage, teeth, conotruncal heart defects, and thymic aplasia), giving rise to the hypothesis that alcohol may exert its teratogenic effects by altering the normal development of cranial neural crest cells (14–17). Likewise, tooth development is controlled by specific interactions between its epithelial and neural crest – derived mesenchymal components (18,19) and it is under genetic control but is subject to environmental modification, such as, teratogens (20).

Accurate information regarding the risks of alcohol consumption during pregnancy is necessary for the implementation of health promotion and prevention strategies (21). This article first reviews the clinical features and experimental findings regarding FAS. Then, the article explores current understanding about the mechanisms underlying alcohol's effects on the fetus, particularly for craniofacial and dental defects, discusses the relevance of FAS to clinical dental practice and concludes with a discussion of possible future research directions and treatment strategies.

Clinical studies

The Centers for Disease Control and Prevention diagnostic criteria for FAS require three specific facial findings (i.e. smooth philtrum, thin vermilion border of the upper lip, and short palpebral fissures), growth deficits, and CSN abnormalities. In the absence of characteristic facial findings, the diagnosis of FASD still should be considered in children with growth problems, CSN abnormalities, and a history of prenatal alcohol exposure (4).

Heavy prenatal alcohol exposure can severely affect the physical and neurobehavioral development of a child. Autopsy and brain imaging studies indicate reductions and abnormalities in overall brain size and shape, significantly in structures such as the cerebellum, basal ganglia, and corpus callosum. A wide range of neuropsychological deficits have been found in children prenatally exposed to alcohol, including impairments in overall IQ, memory, language, attention, reaction time (RT), visualspatial abilities, executive functioning, fine and gross motor skills, and social and adaptive functioning (8).

The commonest deformity seen in FAS is a moderate to severe growth retardation during the prenatal and postnatal period. Prenatal and postnatal growth deficiencies are manifested as a decreased birth weight for gestational age and lack of catch-up growth in spite of adequate nutrition, respectively (21). Children with FAS are usually below the third percentile in height, weight, and head circumference. As they develop, the reduced adipose tissue becomes more pronounced, and as children they are often very thin (22). Compensatory growth in stature, weight, and head circumference can take place in some instances (23,24). There is a long-term tendency to gain weight more than height. Much less catch-up growth was recorded for head circumference, and microcephaly persisted in 65% of children at follow-up. All aspects of growth deficiency were worse in boys than girls. As affected children reach puberty, many of the physical characteristics of FAS become less prominent, although a number of signs and symptoms persists. In many cases, eye anomalies, short palpebral fissures, abnormalities of the philtrum and lips remain useful diagnostic features in adolescents and adults (Fig. 2). However, behavioral, emotional, and social problems become even more pronounced (25).

Hearing and vestibular disturbances are also seen in FAS. The hearing disorders are both conductive and sensorineural and at times associated with recurrent episodes of secretory otitis media. Since the development of language in a child is dependent on an intact hearing apparatus, children with FAS often exhibit language disorders (26), such as poor receptive and expressive language skills, slurred and monotonous speech, articulation, and fluency problems (27). The vestibular damage commonly manifests as postural disturbances in children with FAS (26). Central and peripheral hearing disorders, as well as dentofacial defects and mental impairment may contribute to these language and speech disorders (28).

Cardiac malformations are frequently seen in FAS. They include ventricular septal defects, pulmonary artery hypoplasia and interruption of aortic arch, type A (1,29). Other less common associated abnormalities

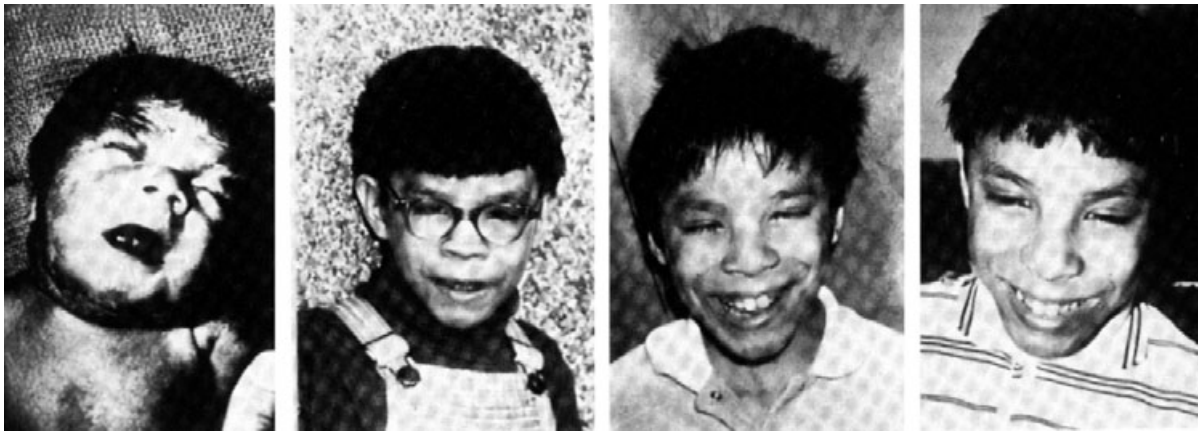

Fig. 2. Severely retarded American Indian adolescent diagnosed with fetal alcohol syndrome at birth and photographed as a neonate and at ages 5, 10 and 14 years. He has been growth deficient and microcephalic throughout his life. With increasing age, there is a considerable relative growth of the nose, resulting in a high, wide nasal bridge. Note persistence of smooth philtrum. Source: Streissguth AP (1991). Reprinted with permission.

are also reported in FAS, such as skeletal (30), urinary (31), ocular anomalies (32) and immune system impairment (33). Thus, it can be seen that alcohol has the capacity to affect different organ systems in developing fetus. The commonest disturbances observed however, continue to be physical and mental retardation, craniofacial anomalies and cardiac malformations (1).

Craniofacial features

The most common facial anomalies associated with FAS include short palpebral fissures, smooth philtrum, thin upper lip, midfacial hypoplasia and/or misaligned teeth (3,25). These specific anomalies were identified in 80% of patients with FAS (25). In the most pronounced form, the face in FAS can be an important aid in diagnoses when coupled with other key features such as developmental delay and documented prenatal exposure to alcohol (3,25).

The craniofacial features observed include midfacial underdevelopment with gross shortage of bone, cranial base sloping to an extreme degree with backward-facing displacement of the cranial base, delayed dental development and enamel anomalies. Maxillary incisors were proclined in dental compensation of maxillary retrusion (23). Church *et al.* (34) demonstrated high incidences of dentofacial and temporomandibular joint disorders, including small teeth, absent teeth, hypoplastic enamel, displaced or rotated teeth, cross bite, overbite, delayed emergence of the adult dentition, high-arched palate and cleft palate or cleft lip.

It has been documented that alcohol use during pregnancy may be a cause of isolated cleft lip with or without cleft palate (28). Munger *et al.* (35) reported that maternal alcohol use during pregnancy was found to be associated with an increased risk of isolated cleft lip with or without cleft palate. When compared with women who did not drink alcohol during pregnancy, the relative odds of isolated offspring having a cleft lip with or without cleft palate rose with increasing level of maternal drinking. Adjustment for maternal smoking, vitamin use, education, and household income did not substantially alter these results.

Naidoo *et al.* (28) analyzed the oral health status of 90 children with FAS, and compared them to matched controls. FAS patients had significantly more dentofacial anomalies than the controls. The most frequently occurring anomalies were crowded incisors, maxillary overjet, and open bite. Levels of plaque and gingival bleeding were high, which was not an unexpected finding since these children come from backgrounds where oral hygiene is not a high priority.

In addition to the cleft palate, cleft lip, malocclusion, and dentition anomalies there are malformed noses (small, upturned, cleft, and flat nasal bridge) and deformed ears (narrow canals, prominent/deformed pinnae, and otosclerosis) (34). The eye is also frequently affected in FAS (1). The common abnormalities include microphthalmos, epicanthus, strabismus, ptosis, corneal opacities, retinal defects and hypoplastic opit disk (32,34).

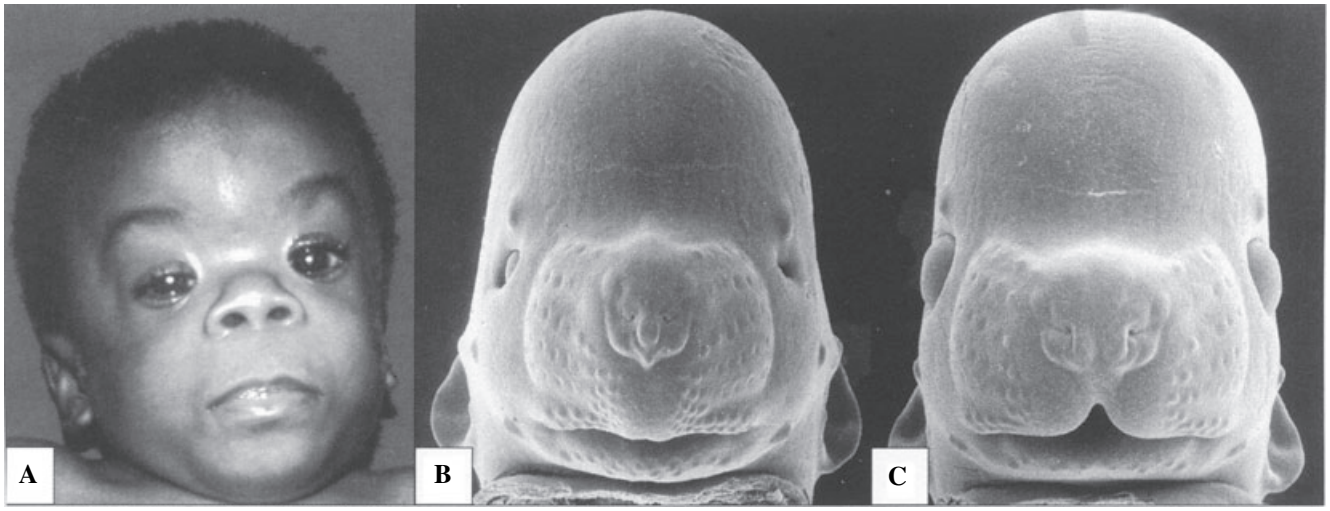

Fig. 3. A child with FAS (A) shares the typical craniofacial features, including microcephaly, short palpebral fissures, a small nose, and long (from nose to mouth) upper lip with a deficient philtrum, with a mouse fetus whose mother was treated with alcohol on her seventh day of pregnancy (B). Illustrated for comparison is a normal mouse fetus of the same developmental stage (C). Source: Sulik KK (2005). Reprinted with permission.

Experimental studies

As with other teratogenic agents, the amount and frequency of alcohol consumed by the pregnant mother, as well as the stage(s) of pregnancy during which an unborn child is exposed to alcohol, are critical in determining the pattern of abnormal development (36). Various animal models (e.g. chicks, mice, rats or primates) have been chosen to study the teratogenic effects of alcohol (37–39), where it has been possible to duplicate many of the features of FAS (Fig. 3). In most cases, it is possible to extrapolate these findings to human beings (1).

Effects of alcohol on craniofacial and dental structures

Although maternal exposure to alcohol is teratogenic at many if not all stages of embryonic and fetal development, alcohol-induced damage at specific early stages of development may be particularly devastating (40). Alcohol has been shown to have a direct toxic effect on the ectodermal and mesodermal cells. Gastrulation is a period of intense mitotic activity (particularly in the developing mesoderm) in mammalian embryos. Alcohol exposure during this period suppresses rates of cell division in mice embryos (40) and the process of migration of mesodermal cells towards the primitive streak (41). Since gastrulation is occurring at the time of alcohol exposure and mesoderm is responsible for inducing and maintaining neuroepithelial differentiation, an adverse effect on the mesoderm could result in size reduction in the neural plate, which was particularly noticeable in the forebrain region. Scanning electron microscopic analysis of later embryonic stages revealed deficiencies in the medial nasal prominences (area responsible for forming the philtral region of the upper lip, the alveolar ridge containing the upper incisors, and the anterior portion of the hard palate). Cleared skeletal preparations of these affected newborns had marked premaxillary bone deficiencies and rudimentary and malpositioned upper incisors (42).

Keeping in mind the mental retardation associated with FAS, the brain has been particularly well studied. The hallmark of CNS damage in FAS is neuronal damage and loss (1). Although selective programmed neuronal death is a normal aspect of CNS development, excessive neuronal death disrupts the development of normal neural networks and may lead to cognitive and behavioral dysfunctions. Alcohol causes a reduction of cerebellar Purkinge cells, cells of the olfactory bulb, and pyramidal cells in a part of the hippocampus known as the CA1 region (43). Miller (44) demonstrated that prenatal alcohol exposure delays the migration of neurons from the zone where they are produced to their final destinations, and the rate of migration of these cortical neurons was decreased as well. Such errors in proliferation and migration disrupt synchronized developmental events, which results in

ectopic clusters of cortical neurons and abnormal neuronal circuitry.

Prenatal ethanol exposure produces a pattern of abnormal cranial and mandibular growth in the rat. Direct skull measurements of adult rats revealed a significant and persistent decrease in cranial and mandibular size resulting from exposure to ethanol between days 6 and 20 of gestation (45,46). Linear measurements of mandibles showed that the initial target of alcohol was the posterior region of the mandible (47). This may be explained by the teratogenic action of alcohol on the mitotic activity of the condyle playing an important role in the mandibular growth.

Among the effects of alcohol in the odontogenesis, cellular alterations in the basal layer of the epithelium of the tooth germ in the bud stage and in the inner enamel epithelium were described (48). Römert and Matthissen (49) found ultra structural changes in secretory ameloblasts from tooth germs of mini-pig fetuses after exposure to ethanol in pregnant mini-pigs. Ingestion of a 20% ethanol solution before and during gestation caused retardation in cell differentiation within the tooth germ and in calcification of the dentin matrix (50). Previously, we had shown that ethanol during pregnancy caused reduction in development of tooth germ and secretion of the dentine and enamel matrices of rat molars at postnatal day 5 (50). Retardation of tooth eruption has been shown in offspring of macaque monkeys, whose mothers were exposed to ethanol (51). In rat molar retardation of the tooth eruption was seen on postnatal day 14.5 (47). Another study demonstrated a delay in eruption and post-eruptive growth of the incisors in offspring whose mothers had been given ethanol intraperitoneally on the seventh day of pregnancy (gastrulation period) (52) (Fig. 4).

Studies using mice (53) and chicks (14) show that alcohol exposure at specific stages of early embryo development results in significant death among the cells destined to give rise to facial structures (i.e. cranial

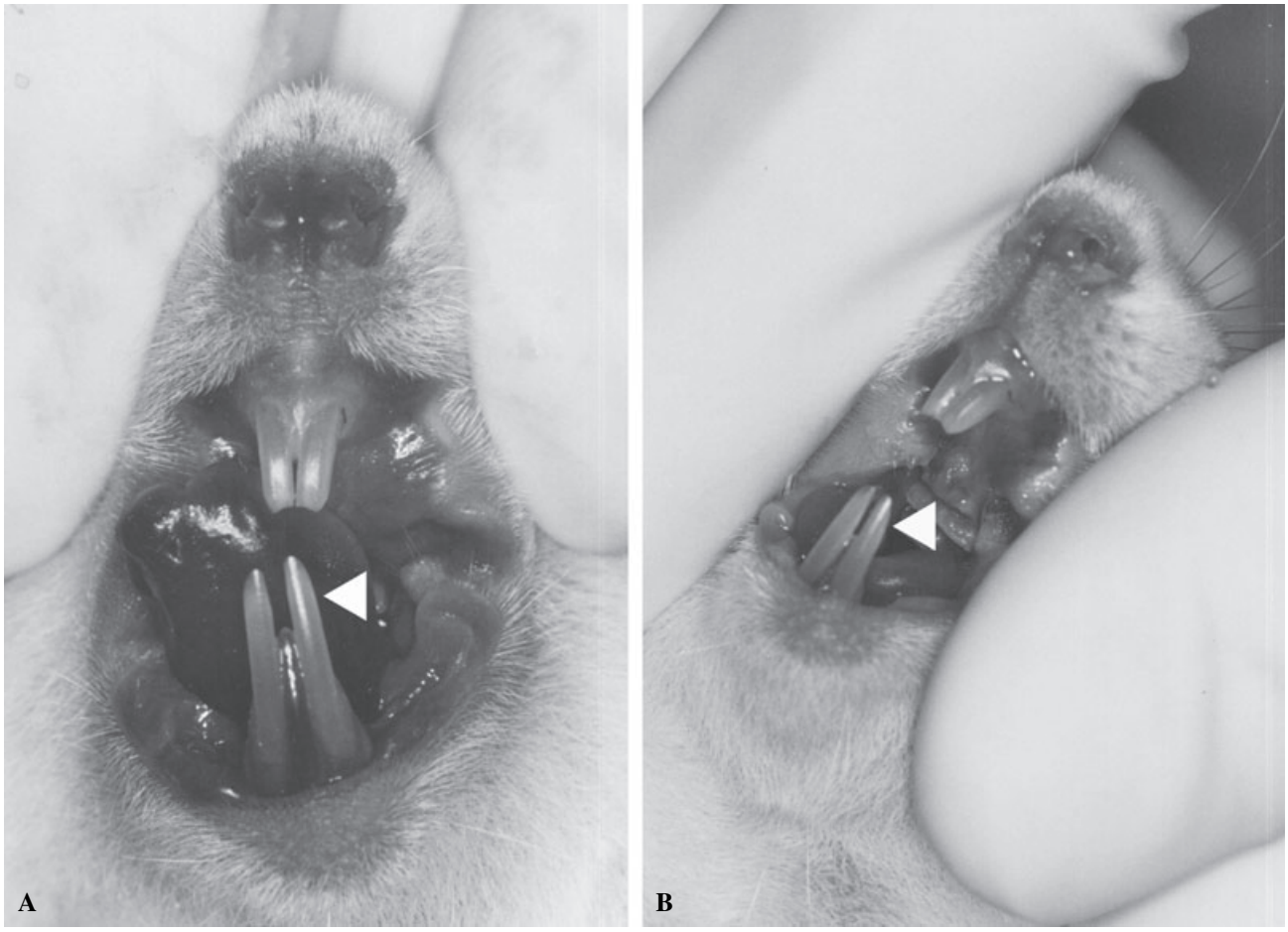

Fig. 4. Photographs of incisive teeth (arrows) of rats at postnatal day 30: (A) control group; (B) ethanol group-delayed in the post-eruptive growth. Source: Silva et al. (1999).

neural crest cells) (11). Alcohol exposure caused cell death only if alcohol was administered before the neural crest cells emigrated from their birthplace in the neurectoderm. Once the cells began migrating toward the site where the face would develop, the neural crest cells were resistant to alcohol-induced death (14). Cranial neural crest cells migrate from the neural tube and differentiate into a wide variety of structures. The specific body structure that cranial neural crest cells will generate (i.e. their positional identity, such as whether the cells will contribute to the upper or lower jaw, for example) is determined before they leave the neurectoderm. Similarly, their differentiation fate (e.g. whether they develop into nerve or bone tissue) is determined before or shortly after the cells begin migration. Because of this fact, the premigratory neural crest cells are particularly vulnerable to alcohol-induced death (11).

Dunty *et al.* (54) have defined location of cell death in the embryonic murine brain and face resulting from in utero ethanol exposure at time of development ranging from gestational day 6.5 to 11 (corresponds to a period late in the second through early in the sixth week of human gestation). Ethanol-induced apoptosis was notable in neurectoderm, neural plate, primitive streak, mesencephalon/rhombencephalon junction, in the ectoderm of the frontonasal prominence, cranial nerve sensory ganglia, and in the maxillary and mandibular prominences of the first branchial arch. The loss of neural crest cells destined to populate the frontonasal and maxillary prominences resulted in midface hypoplasia and dysmorphology of the mandible. Furthermore, the severity of ethanol-induced neural crest apoptosis (55) was influenced by genetic background in an avian model of FAS. Recently, it has been shown that an acute dose of ethanol given during synaptogenesis (GD 19 to postnatal day 14) triggered massive apoptotic neurodegeneration in several regions of the postnatal rat forebrain (56). However, other factors contribute to the selective cellular vulnerability for apoptosis. They will be detailed in the following sections.

Mechanisms of alcohol's action

The fetotoxic effects of maternal ethanol consumption have been documented for over two decades, yet the mechanisms of alcohol's teratogenesis are not fully understood. The wide variety of cellular/biochemical effects of alcohol on fetal tissues is itself a puzzle and strongly suggests that fetotoxic responses to alcohol reflect multifactorial setting. Scientists believe that a number of distinct mechanisms work simultaneously along different biochemical pathways and at different physical sites in the developing embryo. The ways in which these alcohol-induced mechanisms produce damage to the fetus depend on several variables including the timing, frequency, and amount of maternal drinking during pregnancy; the mother's health status and habits; and the genetic make up of the mother and fetus (57).

Animal studies have linked the characteristic facial abnormalities in FAS to cell death by apoptosis of neural crest cells during a very defined period of vulnerability (gastrulation or neurulation) (38,58). One mechanism by which this occurs is thought to be the formation of free radicals (59,60). Four other possible mechanisms, described in more detail below, are a deficiency in retinoic acid (RA), altered expression of homeobox genes, intracellular communication and alteration with the activity of growth factors. All five of these mechanisms are likely interrelated, since RA is a key regulator of gene expression, and both free-radical toxicity and altered gene expression can produce apoptosis (1,11,57).

Free radical damage

Maternal alcohol exposure can increase the levels of highly reactive oxygen molecules known as free radicals as well as deplete cells of the protective compounds (i.e. antioxidants) that normally scavenge these toxic molecules. In particular, free radicals can damage cell membranes through a process called lipid peroxidation. This process may interfere with many important cell regulatory processes, including control over substances entering and leaving the cell, intercellular communication balance of calcium and protein synthesis. Damage from such interference could be fatal to cells (11). Free radical damage may explain regional differences in sensitivity to fetal alcohol because some tissues such as neural crest cells, express lower levels of antioxidants (e.g., superoxide dismutase) than other tissues. Malformations associated with FAS, such as

facial and cardiovascular defects, may arise from the damaging effects of free radicals because craniofacial and visceral structures derive from neural crest cells (59). In addition, fetal cells, on the whole, may be more sensitive to the damaging effects of free radicals because these cells have lower levels of antioxidants (57).

Chen and Sulik (60) have been critical on the hypothesis relating free radical damage to neural crest apoptosis. They cultured alcohol-exposed neural crest cell together with superoxide dismutase and other free radical scavengers. The results showed that the addition of antioxidants modestly reduced alcohol-induced cell death. Because free radical mechanisms also participate in endogenous apoptosis, however, it is unclear whether antioxidants interfere with alcohol's action or with the apoptosis process itself. In a more recent study, Chen and Sulik (61) confirm the role of free radical-mediated damage in ethanol-induced cytotoxicity and suggest that a major factor underlying ethanol-induced neural crest cells death is iron overloading, which initiates the formation of free radicals.

Retinoic acid deficiency

Retinoic acid, an active form of vitamin A, is essential for neural crest survival and is a key regulator of body form and structure development (i.e. morphogenesis) (62). RA affects the positional identity of cells according to the structure that they will form within diverse embryonic regions, including the cranial neural crest, face, CNS, limbs, and urogenital tract. In addition, RA is a potent mediator of differentiation in many cell lineages (13). Retinoic acid was shown to be necessary for the development of neural crest cells into craniofacial features (63,64). Alcohol exposure at specific period of embryonic development can reduce the production of RA (65). Ultimately deficiencies or abnormalities in RA or its receptor cause neural crest cells to die by apoptosis leading to craniofacial defects (64,66).

Altered gene expression

Normal craniofacial morphology develops as a consequence of interactions between embryonic tissues (e.g. cranial neural crest derived cells, mesoderm and ectoderm), and requires precise regulation of cell movement, growth, patterning, and differentiation of craniofacial tissues. Genetic studies have revealed the involvement of numerous genes in these processes, including genes encoding a variety of transcription factors, growth factors and receptors (67). Alterations in genes that influence any of these processes would cause craniofacial abnormalities. Among the critical factors involved in craniofacial development are members of the *Msx* homeobox gene family (68). In alcohol research, scientists are particularly interested in the expression of *Msx*1 and *Msx*2, which regulate the timing and coordination of craniofacial development (35). In the mouse, transcripts of the *Msx*2, are localized in premigratory and migratory cephalic neural crest, neural crest derived mesenchyme of the first through fourth branchial arches, Meckle's cartilage, osteogenic tissue of the mandible and maxilla, eye, ear, membranous bone of the calvaria and developing teeth (69–72). Contrary to *Msx*1, whose expression is confined to the mesenchyme throughout tooth development, *Msx*2 expression can be detected in both the epithelial and mesenchymal compartments of the developing tooth germs (68).

Expression of *Msx*2 was found to decrease in mouse embryos exposed to a binge pattern of alcohol exposure, during key development periods leading to growth retardation, altered craniofacial morphogenesis and cardiac defects (73). This finding contrasts with another study using chick embryos in which expression of the homeobox gene *Msx*2 was not affected by alcohol (58). Although species differences may explain the discrepancies, possibly the lack of homeobox gene expression in the mouse cells was a result of massive apoptotic cell death following administration of alcohol (57). In 2002, Ahlgren *et al.* (17) demonstrated that ethanol exposure in chick embryos at stage 9–10 results in a loss of frontonasal mass tissue and death of cranial neural crest cells that are caused by altered expression of regulatory genes involved in craniofacial patterning. Ethanol exposure causes a dramatic loss of *Sonic hedgehog* (*Shh*), as well as a loss of transcripts involved in *Shh* signaling pathways, leading to subsequent cranial neural crest cell death, then resulting in craniofacial growth defects seen in FAS.

A more recent study using Xenopus embryo found that alcohol dose-dependently reduced the expression of several key neural genes (xPax6, xOtx2, xSox3, and xSox2), of which xPax6 was most vulnerable. This Pax6

suppression produced microcephaly and growth retardation (74). Future research on FAS mechanisms will need to fill the gaps in our understanding of alcohol-induced changes in gene expression (57).

Altered cell membrane

The cell membrane and its surface molecules have an important role in cell proliferation, intracellular communication, adhesion, migration and cell maturation. Ethanol can interrupt these physiologic processes by modifying the morphology of the cell membrane. This effect occurs by inserting into the lipid bilayer, expanding the membrane surface area, and causing membrane fluidization (75). Therefore, ethanol disrupts the function of a selective number of proteins, such as growth factors, their receptor and ion channels, that transfer critical chemical signals from the cell surface to its interior (i.e. transmembrane or intracellular communication). Interference with this process could cause aberrant activation or inhibition of the communication pathways vital to cell survival (11).

Ethanol differs from other psychotropic drugs in that it does not act through a specific membrane receptor but rather affects the function of many different membrane proteins (57).

Interference with growth factors

Ethanol can interfere with the activity of a number of chemicals called growth factors that regulate cell proliferation, differentiation and survival. Loss of normal growth factor signaling can also interfere with or prevent normal growth and craniofacial development. Growth factors acting through surface receptors on cells powerfully stimulate the transcription of the genetic material and the synthesis of many proteins (76). Ethanol interference with growth factor action could occur at three levels: ligand production, receptor expression, and/or signal transduction.

Numerous growth factors are needed for cell division to precede normally, including two factors called insulin-like growth factors (IGF) I and II. Ethanol can interfere with the activity of the IGF-I receptor. As a result, IGF-I still binds to its receptor, but the receptors signaling function is blocked, and IGF-I-mediated cell division cannot proceed (77). This example demonstrated that alcohol could prevent the normal production of CNS cells by interfering with the growth factors that regulate cell division. Ethanol may induce cell death by inhibiting several growth factors that support cell that have attained their final function (i.e. that are differentiated) and no longer divide. For example, IGF-I and the IGF-I receptor also play a role in the survival of non-dividing cells and can prevent apoptosis in several models of cell death. Similar to the situation in dividing cells described above, alcohol can inhibit the IGF-I receptor in non-dividing cells, thereby preventing the survival of those cells (78). The brain growth retardation associated with FAS is a result of the diminished fetal brain concentration of IGF-I and decreased gene expression of IGFs (79).

Epidermal growth factor (EGF) is a polypeptide which stimulates maxillofacial development and DNA synthesis (80). Besides cell division, EGF affects other cellular functions such as synthesis of various macromolecules, bone remodelation and differentiation of both epithelial and mesenchymal cells derived from tooth germ (81,82). Furthermore, EGF plays an important role in the control of tooth eruption (83,84). This growth factor is of particular interest because of the reported retardation of maxillofacial growth (85), including small teeth, enamel anomalies and retardation in the tooth eruption of humans (23) and rats (47) characteristic of FAS.

Ethanol intake during gestation reduces EGF in the odontogenesis of the offspring of alcoholic mice (86). The effect of maternal alcoholism on EGF was studied in the development of rat mandibular molar using immunohistochemistry. The result showed that immunoexpression of EGF was reduced in dentinogenesis and amelogenesis, therefore causing a reduction of dentin and enamel formation (87). Jiménez-Farfán *et al.* (88) stated that epidermal growth factor receptors (EGF-Rs) participate in dental proliferation and differentiation, and changes in these receptors were considered to be a likely mechanism associated to the dental anomalies observed in the FAS. This study revealed an enhanced immunoreactivity to EGF-R and erbB-2 during the morphogenesis of the lower first molar in 16.5- and 18.5-day prenatal mice of mothers who consumed ethanol during pregnancy; additionally, ethanol delayed differentiation, caused degenerative changes in dental epithelial tissues and reduced dental

size. Retardation of the growth of tooth germs may be due to the inhibition of EGF by alcohol (47,87,88). One of the key findings is that the effects of ethanol on tooth development are not homogeneous; some cells are significantly more resistant to ethanol toxicity than others. As reported by Luo and Miller (89), the differential cell sensitivity to ethanol is directly proportional to their response to growth factors, that is, cells that are actively regulated by EGF are much more susceptible to ethanol than cells that are less or unresponsive to EGF. These findings provide additional insight into the mechanism(s) of ethanol teratogenicity in formation of dental hard tissues and should stimulate further study.

Future directions and treatment strategies

The past 30 years research has focused on describing the adverse outcomes of ethanol exposure during development. The search for additional mechanisms and systematic studies of prevention and intervention are the critical questions that will drive FAS/FAE research in the future (90). There are some basic science studies that focus on possible interventions. These include studies on pre-treatment with numerous agents: neuroprotective proteins (91), growth factor (92), and antioxidants (93). These studies are based on the assumption that restoring neuroanatomical integrity would result in subsequent functional recovery (94).

Advances in molecular and cellular biology have opened multiple avenues for exploring alcohol's action mechanisms on pre-natal development. Many of alcohol's target tissues, including face, heart, brain, limb and urogenital tract tissues employ a common set of genes that are responsible for coordinating their growth and development (11,95). Therefore, it is not surprising that certain toxicants and/or genetic mutations will disrupt all of these tissues to some degree. Several alcohol-related birth defects are consistent with altered embryo development, and examining the role that genes play in regulating early development in animal models, including craniofacial and tooth development, may yield insights into how susceptible tissues respond to maternal alcohol exposure. Studies with transgenic and knockout mice also will assist investigators to better evaluate the alcohol's action mechanisms on tissues development.

Although the cranial neural crest contributes to many tissues, alcohol-related research on these cells predominantly focuses on facial bone and cartilage. Examination of other tissues arising from the cranial neural crest could uncover less recognized targets of prenatal alcohol exposure. The tooth is a good representative organ model for studying developmental toxicity of ethanol and other teratogenic drugs (96) and the role of genetic factors in determining individual sensitivity. Unlike in the case of bone, dentin and enamel matrices are not remodeled. Thus, any disturbance in the function of odontoblasts and ameloblasts leads to permanent defects in dentin and enamel, respectively. Under appropriate culture conditions, tooth's development can be monitored *in vitro* up to the stage of dentin and enamel mineralization, which allows experimental interference under controlled conditions (97). Approaches similar to those undertaken by researches studying facial and tooth defects also will benefit studies of brain, heart, urogenital tract, and limb development (11).

A further direction to research is the understanding of the precise relationship between maternal alcoholism and the molecules involved in tooth eruption. Eruption is a complex and highly regulated process that involves cells of the tooth organ and the surrounding alveolus. Molecules, such as, EGF, transforming growth factor-β1 (TGF-β1), colony-stimulating factor-one (CSF-1), interleukin-1α (IL-1α), monocyte chemotactic protein-1 (MCP-1), c-Fos and Nuclear factor kappa B (NFkB) interact to initiate the known cellular events of tooth eruption. EGF, for example, was reduced in the dental follicle of the mandibular first molar in the offspring of alcoholic mice on postnatal day 1.5 (86). Besides the dental follicle, the immunoexpression of EGF was also reduced in the stelatte reticulum and the alveolar bone (87). Considering that maternal ethanol exposure reduces tooth eruption it could be possible that other molecules involved in the signaling cascades of eruption have been affected by alcohol exposure. Such knowledge will provide us with a better understanding of the tooth eruption process itself, and it will contribute to the development of effective therapeutic interventions in the eruption defects, as seen in children with FAS/FAE. As research advances are made, it is now foreseeable that future treatment modalities could include therapeutics that re-creates eruption events. When the 'molecular orthodontic' procedures

are available for delivering the appropriate molecules or factors necessary to 'erupt' individual teeth, the clinician can select the appropriate treatment for the patient based on the underlying cause of the eruption problem (98).

Genetic technology can be used to achieve a better understanding of the influence of maternal genetic factors on determining fetal susceptibility to alcohol-induced damage and it could also be helpful for an early intervention in pregnancy (90). The work of Debelak and Smith (55) and more recently, Chambers and Jones (99) has been valuable as a first step toward this goal. In this revision, the authors associated the polymorphisms of the alcohol metabolizing enzyme genes with susceptibility to alcohol's teratogenicity.

More realistic for the immediate future is to find ways to identify heavy drinking women so that intervention can be made as early as possible in pregnancy or, preferably, before conception. Approaches to this issue include developing effective screening tools for pregnant women, such as the TWEAK (Tolerance, Worry, Eye Opener, Amnesia, Cut-Down) (100) and/or T-ACE (Tolerance, Annoyed, Cut-Down, Eye Opener) (101). In addition, the development and testing of reliable biomarkers for identifying drinking in pregnant women and to diagnose, and possibly to treat FAS early, in fetal life, would be desirable. Recent innovations have led to the development of laser surface scanning, a non-invasive method for acquiring three-dimensional images (102). This technique is promising in the analysis of facial features associated with prenatal alcohol exposure, but, at present, is a research tool only (5).

There are also research opportunities in the clinical realm specifically in the prevention of alcohol abuse by pregnant women (90). There is no known safe level of alcohol consumption during pregnancy. Hence, there is a need for wide spread education of the general public regarding FAS/FAE (1). In November 1989, alcoholic beverage container warning labels were introduced. A significant reduction in periconceptional alcohol intake occurred beginning in 1990. However, the decrease in drinking was limited to women who were already light drinkers, with no significant change among those drinking at risk levels. The lack of success to reduce fetal alcohol exposure was related to differences between who are risk drinkers and those who are not. Such differences could range from economic and educational status to genetic determinants of alcohol metabolic efficiency (103). Research has suggested that pregnant women identified as heavy drinkers do respond to treatment with a series of brief interventions (with booster sessions) more effectively than a single suggestion to stop drinking. For women who are not at risk, selective prevention (general information about drinking during pregnancy) can be provided (104).

In view of the serious sequelae of FAS/FAE, it is important that dentists are aware of the general and oral effects. There are two areas of particular interest to the dentist: the craniofacial anomalies and the medical problems that may affect the dental management of these patients. They are likely to have maxillary hypoplasia and micrognathia. Any of these may necessitate follow-up for malocclusion resulting from skeletal disharmonies. Among the medical problems are deficiencies in growth and intelligence. Because of the various congenital cardiac defects (especially atrial and ventricular valvular defects), the patient's medical practitioner needs to be consulted on the patient's susceptibility to subacute bacterial endocarditis and the need for prophylaxis prior to dental treatment. Since the children are mentally challenged, there may be behavior management (28). General dental practitioners, who see patients regularly for routine examination, are in an ideal position to identify drinking in pregnant women (105), for example, using the alcohol screening tests (i.e. T-ACE, TWEAK). These tests consist of questions that may be asked as part of the anamnesis. The awareness and recognition of patients with FAS is important so they can be correctly diagnosed and referred appropriately.

In conclusion, it can be said the FAS/FAE is a 100% preventable tragedy. Extensive efforts need to be on social, experimental, health, and legislature fronts to empower alcoholic mothers to make lifestyle changes, not allowing that the unprotected child will continue to suffer through out its life for no fault of its own.

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