

Transgenerational Abnormalities Induced by Paternal Preconceptional Alcohol Drinking: Findings from Humans and Animal Models

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Abstract: Alcohol consumption during pregnancy and lactation is a widespread preventable cause of neurodevelopmental impairment in newborns. While the harmful effects of gestational alcohol use have been well documented, only recently, the role of paternal preconceptional alcohol consumption (PPAC) prior to copulating has drawn specific epigenetic considerations. Data from human and animal models have demonstrated that PPAC may affect sperm function, eliciting oxidative stress. In newborns, PPAC may induce changes in behavior, cognitive functions, and emotional responses. Furthermore, PPAC may elicit neurobiological disruptions, visuospatial impairments, hyperactivity disorders, motor skill disruptions, hearing loss, endocrine, and immune alterations, reduced physical growth, placental disruptions, and metabolic alterations. Neurobiological studies on PPAC have also disclosed changes in brain function and structure by disrupting the growth factors pathways. In particular, as shown in animal model studies, PPAC alters brain nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) synthesis and release. This review shows that the crucial topic of lifelong disabilities induced by PPAC and/or gestational alcohol drinking is quite challenging at the individual, societal, and familial levels. Since a nontoxic drinking behavior before pregnancy (for both men and women), during pregnancy, and lactation cannot be established, the only suggestion for couples planning pregnancies is to completely avoid the consumption of alcoholic beverages.

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1. INTRODUCTION

Alcohol-use disorders (AUD) and alcoholism are complex multifactorial conditions [1-10] and are also related to epigenetic mechanisms [11-18]. Alcohol can modify gene expression through DNA methylation (it enhances the catabolism and loss of methyl groups, which in turn disrupts subsequent SAM-dependent transmethylation reactions of the folate pathway, which are lastly required for DNA methylation) and nucleosomal remodeling *via* histone modifications and microRNA expression. Meanwhile, it has also been observed, similarly to some psychiatric diseases, how persists in adulthood an oxidative stress response mainly at the cellular level (including reactive oxygen species generated as byproducts of cytochrome P450 2E1 (CYP2E1), decreased

endogenous antioxidant levels, and mitochondrial damage) and a global upregulation of neuroendocrine stress response, regulated by the hypothalamic-pituitary-adrenal axis [5] (Fig. 1, point I).

Despite acknowledging the disastrous impact of gestational alcohol abuse on the offspring's health, it is still very common for women and men to drink before and during pregnancy. Indeed, alcohol consumption before conception and during pregnancy and lactation is widely known among the leading preventable causes of neurodevelopmental impairment worldwide [19-22]. The umbrella term fetal alcohol spectrum disorders (FASD) is widely used to define such impairments, including distinctive craniofacial malformations, central nervous system defects, mental retardation, and both prenatal and postnatal growth restriction [23-28]. The terminology FASD is officially adopted to identify a broader spectrum of presentations and disabilities resulting

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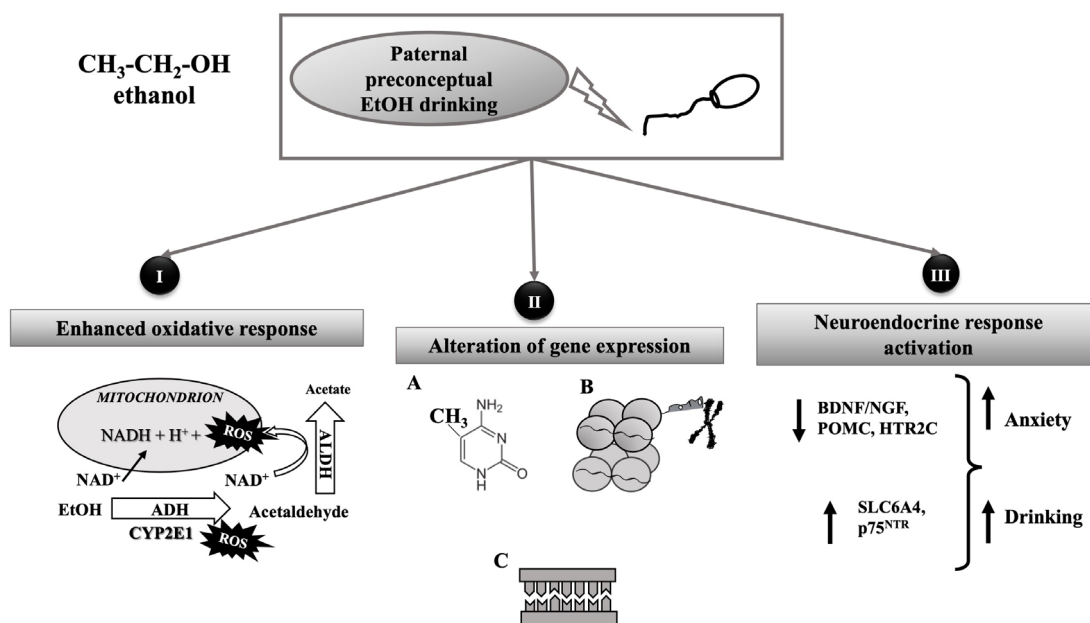


Fig. (1). Epigenetics refers to biochemical changes in chromatin structure that can modify gene expression without altering the DNA sequence. Paternal preconceptual alcohol consumption (PPAC) causes epigenetic alterations in male sperm which have been related to paternal FASD: 1) PPAC alters gene expression with 3 major processes, **A**) enhancement of the catabolism and loss of methyl groups, which in turn disrupts subsequent SAM-dependent transmethylation reactions of the folate pathway, which are lastly required for DNA methylation; **B**) nucleosomal remodeling *via* histone modifications; **C**) abnormal microRNA expression. 2) PPAC also enhances oxidative stress response due to the metabolism of ethanol to acetaldehyde, catalyzed either by alcohol dehydrogenase, CYP2E1, or catalase, leading to the generation of ROS and modifying the intracellular redox state, ultimately causing neuronal cell death and altered gene expression as a result of DNA oxidation; 3) global upregulation of neuroendocrine stress response regulated by the HPA axis and the linked drinking behavior have been related to epigenetic modulation of neurotrophins and POMC genes and variations of the pathways regulating mood, emotion, serotonergic tone. ADH, alcohol dehydrogenase; ALDH, acetaldehyde dehydrogenase; BDNF, brain-derived neurotrophic factor; CYP2E1, cytochrome P450 2E1; EtOH, ethanol; FASD, fetal alcohol spectrum disorders; HPA, hypothalamic-pituitary-adrenal; HTR2C, 5-Hydroxytryptamine Receptor 2C; NGF, nerve growth factor; p75NTR, low-affinity pan-neurotrophins receptor; POMC, proopiomelanocortin; ROS, reactive oxygen species; SAM, S-adenosyl-L-methionine; SLC6A4, Solute Carrier Family 6 Member 4.

from prenatal alcohol exposure, including a range of categories referred to by the Institute of Medicine (IOM) as alcohol-related neurodevelopmental disorders (ARND), alcohol-related birth defects (ARBD), the partial fetal alcohol syndrome (pFAS) and finally, the FAS, which is the most serious form [29]. The diagnosis of this clinical condition remains difficult [30-34]; it requires a multidisciplinary team and is based on: screening in pregnant and postpartum women, referral of individuals for a possible FASD diagnosis whenever there is the suspect, support to promote abstinence from alcohol, medical assessment including compiling a social and medical history and complete physical examination and documentation of the biological mother consumed alcohol during the pregnancy, sentinel facial features, and neurodevelopmental assessment in search of evidence of pervasive brain dysfunction [35].

The life expectancy of patients with FAS is estimated to be 34 years (95% confidence interval: 31 to 37 years) even though the leading causes of death are "external causes" (44%), which include suicide (15%), accidents (14%) and poisoning by illegal drugs or alcohol (7%) as well as other causes [36].

The observation, in twin and adoption studies, of heritability of about 50% of alcohol use disorder associated with the demonstration that chronic exposure to ethanol causes

chromatin remodeling in the brain that promotes the transition from use to abuse and addiction, render even more challenging the efficacy of prevention campaigns in many situations [37-40]. In the overall population, it has been estimated that about 8.9% of women drink alcohol during pregnancy, while the global prevalence of FASD is about 1 in 100 people, and the prevalence of FAS in the same population is about 14.6 per 10,000 people [27]. It has been calculated that one in every 67 women who consume alcohol during pregnancy would carry a child with FAS, which translates to about 119,000 children born with FAS every year in the world. Provided that FASD is a lifelong disease, it has been estimated that more than 11 million individuals between 0 and 18 years of age, and 25 million individuals between 0-40 years of age, have FASD worldwide [41].

It is now widely established that the harmful effects of prenatal alcohol exposure on the embryo and fetus development cause noticeable cognitive and neurobiological deficits in the newborns; the most common clinical features are listed in Table 1 [42]. The deleterious role of oxidative stress induced by alcohol drinking during pregnancy has been established, suggesting how supplementing a mother's diet with protective and antioxidant substances in addition to folic acid, choline, neuroactive peptides, and supportive psychological therapies could protect newborns from being affected [16, 18, 43].

Table 1. Fetal alcohol spectrum disorders with common clinical features.

Congenital Malformations	Organs and Apparatus	Behavioral Functioning	Cognitive Functioning
Abnormal facial features: flat nasal bridge, smooth ridge between the nose and upper lip, thin upper lip, extra crease in the outer ears, an upturned nose	Hearing problems	Sleep problems	Learning disabilities
Curved pinky finger	Heart	Sucking problems	Problems following directions
Smaller head size	Kidneys	Hyperactive behavior	Difficulty understanding the consequences of their actions
Low body weight	Bones	Inattentiveness	Poor memory skills
Shorter than average height	Immune dysfunction	Poor social skills	Poor reasoning skills
-	Endocrine disruptions	-	Speech and language delays

It has been demonstrated how phenotypic heterogeneity in human FASD is strongly related to a genetic vulnerability in affected brain regions [44]. Interestingly, it has been hypothesized that the principles of non-Mendelian inheritance, or “exceptions” to Mendelian genetics, in particular when one parent has an exaggerated influence over the offspring's vulnerability to prenatal alcohol (*e.g.*, maternal alleles leading to an altered intrauterine hormonal environment or when epigenetic regulation of allele-specific gene expression causes an imbalance between the maternal and paternal genetic contributions) could be the main factor determining the severity of the FASD symptomatology [21].

While the negative effects of gestational alcohol use have been well documented and known for some time, the capacity of parental life history to influence the offspring phenotype has been highly underestimated and not fully explored [16, 26, 43, 45-47]. Knowing that about 75 % of children with FAS have heavy drinkers or alcoholic biological fathers, it seems logical that in recent times it has been gaining a subtle role, in FASD manifestation, of alcohol consumption by fathers before mating [48]. Differently from maternal alcohol exposure, paternal alcohol consumption does not directly come into contact with developing embryos, and the direct relationship between paternal alcohol consumption and genetic or epigenetic mutations that result in developmental abnormalities has been studied only recently, challenging the common misconception that sperm does not transmit heritable information beyond the genetic code. In 2004, the paternal influence on offspring's low birth weight, congenital malformations, deficits of the immune system, and, of course, cognitive and behavioral abnormalities, including learning and memory deficits, hyperactivity, and poor stress tolerance, was partially investigated, resulting in a renewed interest on this topic [48]. Recently many studies have also suggested the existence of 'transgenerational' consequences of alcohol for the offspring, more often related to male chronic alcohol abuse, demonstrating the tendency to epigenetic variations that might be transmitted through multiple generations [37, 49, 50].

This report aims to provide a summary and subsequent review of past research works, highlighting the evidence on the role of paternal preconceptional alcohol consumption (PPAC) in FASD etiopathogenesis.

2. METHODS

2.1. Search Strategy

All the studies indexed in PubMed and relevant to identify the paternal alcohol consumption role in FASD were sought using a combination of keywords selected, with authors' agreement, using the PICOS approach: participants, interventions, comparisons, outcomes, and study design [51, 52]. The following keywords were used: alcohol-related birth defects, fetal alcohol syndrome, fetal alcohol spectrum disorders, paternal alcohol consumption, preconceptional ethanol consumption, prenatal ethanol exposure. When judged relevant, other items listed in the references of the included articles were considered for eligibility.

2.2. Study Selection and Inclusion Criteria

Only full-text articles published in English analyzing the role of paternal alcohol consumption in FASD in humans or animal models were considered eligible.

2.3. Data Extraction and Data Analysis

Investigators independently screened and assessed titles and abstracts before retrieval of the full manuscripts. The selected full papers were reviewed for eligibility according to the inclusion criteria. References in the selected papers were scrutinized for additional articles in a further effort to ensure that relevant publications were not missed. All controversies concerning study selection or data extraction were resolved by consensus with a third group of reviewers. Evidence from available literature is presented in this review in a narrative way to offer a clear overview of the many findings regarding the effects of PPAC and its role in FASD etiopathogenesis. Results are presented, distinguishing many organized subchapters and prioritizing findings from human clinical studies compared to an animal model.

3. TRANSGENERATIONAL EFFECTS OF PATER- NAL PRECONCEPTIONAL ALCOHOL CONSUMPTION

In 1968, Lemoine and colleagues were the first to describe the situation in which children who presented clinical

features of FAS were born from mothers who did not drink but had alcoholic fathers [53]. This seems to be the first time introducing the idea that PPAC may have a role in FASD pathogenesis. More than 20 years later, in 1991, Professor Abel found that the response of male rats that had consumed alcohol for seven months did not differ significantly from controls in their immobility response in the forced swimming test but, on the other hand, it was the offspring of these males that exhibited a significant dose-related decrease in immobility [54]. These results showed for the first time that, while alcohol's effects may not be observable in males consuming it, this does not necessarily mean that it has no important biological effects on these males and that these effects may not be appreciated until their offspring are examined. This discovery brought new light on the potential role of PPAC in the alcohol disorders-related effects on the offspring. In the years, it has been shown how not only the offspring's next generation may be influenced by PPAC, but also multiple generations may be affected, so that the term "transgenerational" may be used to identify the effects of prenatal alcohol exposure on the offspring [37, 49, 50]. Epimutations in the critical genes required for normal development can be transmitted through fertilization and, since only germ cells are in contact with alcohol in the case of PPAC, male sperm has been identified as the main mediator of the toxic effects. Therefore, the transgenerational toxic effects of PPAC are due to alcohol-induced epigenetic mutations in sperm DNA [55-57]. Interestingly, using bisulfite mutagenesis and second-generation deep sequencing, no evidence was found that suggests that the alcohol-associated phenotypes and the transcriptional changes are linked to alterations in the sperm-inherited DNA methylation profile, emphasizing the importance of epigenetic mechanisms of paternal inheritance beyond DNA methylation [58].

4. PRECONCEPTUAL ALCOHOL CONSUMPTION IS ASSOCIATED WITH OXIDATIVE STRESS

Oxidative pathways involve the metabolism of ethanol to acetaldehyde, catalyzed either by alcohol dehydrogenase (ADH), CYP2E1, or catalase [59]. It is currently known how ethanol exposure can lead to the generation of reactive oxygen species (ROS), modifying the intracellular redox state, and ultimately leading to an overall increase in oxidative stress and neuronal cell death by the oxidation of proteins, lipids, and DNA [60, 61]. In particular, DNA lesions can result in genetic mutations involving the DNA sequence of a single cell, resulting in cellular immortalization and clonal expansion, ultimately leading to postnatal cancer or, otherwise, it can result in direct or indirect epigenetic modifications to DNA, histones, or RNA that involve gene expression of many cells, contributing to teratogenesis in the form of birth defects and postnatal neurodevelopmental abnormalities [59] (Fig. 1, point II). Alcohol toxicity is caused both directly by ethanol and indirectly by its metabolic products, including the ROS produced during its biotransformation involving CYP2E1 [62, 63]. Interestingly, both maternal and paternal preconceptual alcohol exposures have been related to mitochondrial dysfunction and a significant increase in oxidative stress in developing organs, especially in the central nervous system [64, 65]. The relationship between oxidative stress and central nervous system abnormalities is not

surprising, considering that, when compared to other organs, the brain is more susceptible to ROS generation (that include superoxide anions, hydrogen peroxide, and hydroxyl radicals), holding the highest oxygen metabolic rate (its cells utilize 20% of the oxygen consumed by the entire organism), and brain tissues are rich in unsaturated fatty acids that can be used as substrates for ROS production [66]. Furthermore, some brain regions have a high content of iron, and several neurotransmitters tend to spontaneously react with oxygen (namely dopamine, levodopa, serotonin, and norepinephrine), which may further promote the generation of ROS [67]. Finally, it should be considered that the activities of the antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) are lower in the brain than in other organs such as the liver or kidney, while fetal cells are more vulnerable than adult cells to the neurotoxic effects of oxidative stress so that the role of ROS in FASD pathophysiology is clearly of primary importance [68, 69]. It should be noted that ROS participate in intracellular signaling pathways and that, despite being unstable, they are produced naturally in the embryo and fetus and are essential for normal development, so that the problem resides in the imbalance due to excessive ROS production related to the preconceptual alcohol consumption that ultimately causes oxidative stress [70]. Increased activity of the enzyme NADPH oxidase (NOX), which may be the major source of ROS production in the brain, has been related to mouse embryos preconceptual alcohol consumption, while co-treatment with the NOX inhibitor (diphenyleneiodonium) has been shown to prevent an ethanol-induced increase in NOX activity, ROS generation, and oxidative DNA damage [64, 71-73]. In rodents, antioxidant vitamin E (alpha-tocopherol) increased the levels of anti-apoptotic proteins (*e.g.*, Bcl-2, Bcl-x1, activated Akt kinase), downregulated pro-apoptotic proteins (*e.g.*, Bcl-xs), improved organs alterations and DNA damage, and adjusted the level of hyperhomocysteinemia, showing promising therapeutic applications in FASD [74-77]. Many other antioxidants have shown, mainly in rodents, promising therapeutic effects, such as ascorbic acid (vitamin C), beta-carotene, black ginseng, EUK-134 (synthetic superoxide dismutase plus catalase mimetic), folic acid, melatonin, N-acetylcysteine, phenyl butyl nitron, pycnogenol, silymarin, and superoxide dismutase [60, 78-85]. Also, the content of antioxidants (polyphenols, resveratrol) in alcoholic beverages (*i.e.*, red wine) may counteract the toxic effect of alcohol *per se*, as shown in FASD/AUD animal models [86-98]. The effects may vary depending on the dose, duration, exposure period, and route of exposure for both the ethanol and the antioxidant.

5. IMPACT OF CHRONIC ALCOHOL USE ON SPERM FUNCTION

The impact of chronic alcohol use on sperm function and male reproductive physiology has been the center of many studies demonstrating how chronic alcohol consumption has a detrimental impact on male reproductive hormones and semen quality, significantly altering the sperm structure and its heritage [99-103]. Furthermore, before conception, paternal heavy alcohol consumption increases the risk of early pregnancy loss and stillbirth [104, 105]. These findings may be considered the pillars that drew attention to the PPAC's

role in heritable disorders. Since then, literature expanded, and many studies employed animal and human models to analyze the relationship between ethanol exposures and the long-term effects on the offspring, highlighting paternally inherited variations in epigenetic coding and their role in numerous disease states of the offspring, including behavior and cognitive alterations, growth deficit, organic and physiological abnormalities related to FASD.

6. PATERNAL PRECONCEPTUAL ALCOHOL CONSUMPTION IMPACTS ON THE OFFSPRING BEHAVIOR AND COGNITIVE FUNCTIONS

In literature, most of the earlier studies on the PPAC role in offspring's disorders focused on the behavioral, cognitive, and psychological consequences imputable to the impact of the harmful conduct of the fathers [106-109]. It is now factually known that sons of alcoholics, when compared to sons of non-alcoholics, may show delayed cognitive performance, increased anxiety and depression, certain neuropsychological deficits in perceptual-motor ability, memory, and language processing as well as auditory and visual attentional impairments, and a lower level of achievement in reading comprehension [110-113]. Furthermore, sons of male alcoholics are at markedly increased genetic risk to develop alcohol abuse and neurotic personality profiles [114-117].

7. VISUOSPATIAL INFORMATION IMPAIRMENT

When compared to non-alcoholics, chronic alcoholics and their children show significant deficits in the processing of visuospatial information [118, 119]. Sons of active alcoholic, recovering alcoholic, and social drinking fathers tended to perform differently when administered with neuropsychological tests: sons of active alcoholics performed significantly worse on visuospatial, memory, and attentional tasks as well as general intellectual functioning than sons of social drinking fathers and sons of recovering alcoholic fathers (who showed no significant difference in their cognitive functioning) [120].

8. EMOTIONAL RESPONSE

More recently, the altered emotional processing and brain activity in adolescents with familial alcoholism has been deeply analyzed using functional magnetic resonance imaging, showing reduced activity during emotional processing in the superior temporal cortex, as well as during cognitive control within emotional contexts in frontal and striatal regions when compared to adolescents without familial alcoholism [121]. Furthermore, reduced resting-state synchrony between the left amygdala and left superior frontal gyrus was related to poorer response inhibition.

As ethanol exposure activates the hypothalamic-pituitary-adrenal axis acting as a physiological stressor, it has been hypothesized that PPAC impacts stress responsivity of offspring [122, 123]. This hypothesis seems to be partially confirmed as the studies showed sex-specific blunted plasma corticosterone levels of ethanol-sired male offspring in response to acute restraint stress, resistance to stress-induced excessive fluid intake, enhanced sensitivity to the anxiolytic and motor-enhancing effects of ethanol, reduced ethanol

preference and consumption, altered BDNF (brain-derived neurotrophic factor) and/or NGF (nerve growth factor) (well-known regulators of ethanol drinking behavior [16, 26, 117, 124-142]) expression in the ventral tegmental area compared to control-sired male offspring. No differences among ethanol- and control-sired female offspring have been found on these assays. Consistent with the hypothesis of epigenetic effects of PPAC, ethanol exposure was shown to reduce DNA methylation at the BDNF/Promoter of sire's germ cells, while hypomethylation was maintained in the ventral tegmental area of both male and female ethanol-sired offspring [123]. In another study, a voluntary oral route of paternal preconception EtOH exposure, *i.e.*, intermittent every-other-day two-bottle choice drinking, altered offspring behavior [143]. The authors found that paternal preconception of two-bottle choice drinking every other day resulted in reduced EtOH consumption selectively in male offspring in the dark assay compared to control-sired offspring. No differences were detected in either sex in the unlimited access two-bottle choice and elevated plus-maze assays. Open field analysis revealed complex changes in basal behavior and EtOH-induced behaviors that were sex-specific [143]. These studies showed that PPAC has persistent effects that impact the next generation.

9. PATERNAL PRECONCEPTUAL ALCOHOL CONSUMPTION AND NEUROTROPHINS

Among neurotrophic factors (which regulate cell growth, proliferation, differentiation, and maturation, and also cell migration, metabolism, and apoptotic cell death), neurotrophins play a critical role in proper brain development, in mediating synaptic plasticity throughout adulthood, and in maintaining a balance between neuroendocrine, immune and metabolic systems [26, 144, 145].

Neurotrophins include NGF, BDNF, neurotrophin-3 (NT-3), and NT-4/5. In particular, NGF and BDNF and their receptors, high-affinity receptor tropomyosin receptor kinase A (TrkA for NGF) and B (TrkB for BDNF) and low affinity p75 neurotrophin receptor (p75^{NTR} for both NGF and BDNF), have been shown to play a crucial role within the pathogenesis of prenatal alcohol exposure since alcohol exposure has been related to neurotrophins alterations in the frontal cortex, striatum, and olfactory lobes and disruption of neurotrophin pathways that in turn affects brain cell growth and development and newborns behavior [129, 146-150]. More specifically, we found that PPAC induces a higher susceptibility to the rewarding effects of ethanol, affecting BDNF in the frontal cortex, striatum and olfactory lobes, TrkA in the hippocampus, and p75^{NTR} in the frontal cortex, ultimately inducing ethanol intake preference in the male offspring [129]. Furthermore, alcohol inhibits the expression of the endogenous extracellular signal-regulated kinase (ERK) and the phosphatidylinositol-3-kinase (PI3K) of the neurotrophins pathways [151, 152]. Possible links between the epigenetic modulation of NGF and BDNF, the serum levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), and the symptomatology of alcohol dependence have been suggested, indicating that changes in the methylation of neurotrophins genes may contribute to the development of alcohol dependence by affecting relevant downstream signaling cascades [151, 152].

Modifications in NGF and BDNF levels in the serum and plasma are observed during the onset and progression of many disorders, including neurological, psychiatric, and immune diseases [128, 153-164] and during physiopathological conditions, such as cardiometabolic disruptions [165-169], stressful events [126], aging [169-171], and the post-partum period [127, 172]. Recent findings on murine models have analyzed the relationship between PPAC, FASD, and neurotrophins alterations, suggesting that these proteins could act in a compensatory manner as neuroprotective molecules that can oppose the harmful effects of prenatal alcohol exposure [173, 174].

More recently, we have demonstrated how PPAC may disrupt the epigenetic regulation of postnatal alcohol sensitivity in the offspring by investigating pathways regulating mood, emotion, serotonergic tone, and neurotrophins [26, 98, 117]. In these studies, we demonstrated how PPAC alters the sensitivity to the serotonergic/neurotrophic-associated effects of alcohol affecting the postnatal alcohol preference in the offspring. The analysis of the brainstem gene expression showed serotonin transporter Solute Carrier Family 6 Member 4 (SLC6A4) and low-affinity pan-neurotrophins receptor (p75^{NTR}) elevation and decreased 5-Hydroxytryptamine Receptor 2C (HTR2C) in the offspring of chronic alcohol-exposed sires [117]. In a different study, male offspring sired by adult male mice exposed to chronic intermittent vapor ethanol before mating showed attenuated ethanol drinking behavior, increased sensitivity to the anxiolytic effects of ethanol, and epigenetic alterations (DNA hypomethylation) and BDNF gene increased expression that persists in the ventral tegmental area [123].

10. ATTENTION DEFICITS AND HYPERACTIVITY DISORDERS

Simultaneous preconceptional paternal or maternal exposure to alcohol and environmental tobacco smoke increases the risk of attention deficit hyperactivity disorder (ADHD) diagnosed using the DuPaul Rating Scale [175]. In rats, the presence of a differential effect of amphetamine suggests that the paternal effect on hyperactivity may be mediated by catecholaminergic action [176]. Based on previous experiments which demonstrated how the offspring of male mice chronically exposed to ethanol before mating present attention deficit hyperactivity, altered expression, and epigenetic regulation of the dopamine transporter in the frontal cortex, but also altered neurobehavioral outcomes (reflex acquisition, activity, gait) and increased cerebral cortical layer thickness, a recent study demonstrated, in male mice who self-administered 25% ethanol for an extended period before conception, how PPAC impacts the development of the neocortex and behavior in offspring similarly as previously demonstrated in maternal consumption models of FASD [177-179]. Furthermore, PPAC effects on the offspring may vary with the timing of alcohol exposure relative to conception [178]. In fact, it is sufficient 15 to 23 days of PPAC to produce altered behavior at 20 days after birth in a sex-specific manner, including increased activity in males and perturbed sensorimotor integration in both males and females, suggesting possible long-lasting, sex-specific effects of PPAC on offspring behavior. In animal models, it has also been shown that one acute dose of alcohol before insemina-

tion may cause early developmental delays and behavioral abnormalities such as being less fearful and more aggressive as adults when compared to a control group [180].

11. COGNITIVE DEFICIT AND MOTOR SKILLS IMPAIRMENT

Knowing that high levels of paternal and maternal prenatal alcohol exposure are associated with impaired offspring motor development, two recent studies have assessed, using the Bayley Scales of Infant Development (BSID-III), the impact of low alcohol consumption before conception on gross motor skills (*i.e.*, coordination of movement using the large muscles of the body) and on cognitive development at 12-months of age [181, 182]. The experiments found no evidence that associates low preconceptional alcohol consumption with measurable impairment in infant gross motor skills or cognitive development at 12-months so that further research is needed to examine potential preconceptional alcohol consumption impacts on gross motor skills (and fine motor skills too) and cognitive development in heavier exposure groups especially through the childhood years, when subtle gross motor skills and cognitive deficits may be more easily detectable.

Other findings reported that behavioral effects of PPAC on mice offspring increased immobility in forced swimming tests, decreased grooming, hypoactivity (in contrast to the more often recorded hyperactivity), and failure to initiate and complete the T-maze performance tests (associated with reduced cognitive functioning) [54, 115, 183, 184].

12. PATERNAL PRECONCEPTIONAL ALCOHOL CONSUMPTION IS RELATED TO ORGANIC AND PHYSIOLOGICAL ABNORMALITIES:

12.1. Organic Abnormalities

Higher odds of human microcephaly at birth are associated with higher paternal, but not maternal, alcohol consumption before pregnancy, and similar but weaker effects are estimated for the first drinking trimester [185]. Meanwhile, in mice, it has been demonstrated how PPAC is related to agenesis and skull malformation (exencephaly), as well as the reduced weight of heart, kidney, and ventral prostate, causing teratogenic and developmental defects in the next generation at fetal, prenatal, and postnatal stages [56].

Male rats consuming alcohol for 8-9 weeks before conception produce rat offspring with increased adrenal weights, decreased spleen weights, and hypoactivity [186].

12.2. Hearing Loss

Data have shown how chronic PPAC in mice may have a dose-dependent role in offspring's increased hearing loss [187]. In the same study, the authors also assessed the methylation of H19, Peg3, Ndn, and Snrpn in paternal spermatozoa and the cerebral cortices of deaf mice, confirming the epigenetic bases of the FASD pathophysiology that have been suggested in previous studies; PPAC alters the methylation of imprinted genes in sire spermatozoa, and these genes could be passed on to the offspring ultimately giving rise to developmental disorders [187, 188].

12.3. Neurogenesis, Obesity, and Reproductive System Effects

While most of the experiments have evaluated the effects of prenatal alcohol exposure in the gestational and short term periconceptional period, an interesting study evaluated the effects of adolescent binge alcohol exposure on hypothalamic gene expression patterns in the F1 generation offspring [189]. In this study, the authors exposed adolescent male and female rats to a repeated binge EtOH exposure paradigm and then mated them in adulthood. Data showed that the offspring of alcohol-exposed parents had significant differences compared to offspring from alcohol-naïve parents [189]. Specifically, major differences were observed in the expression of genes that mediate neurogenesis and synaptic plasticity during neurodevelopment, genes important for directing chromatin remodeling, post-translational modifications or transcription regulation, as well as genes involved in the regulation of obesity and reproductive function [189].

There is limited literature on the effects of PPAC on the reproductive development in human offspring; a recent study that included 1, 292 pregnant women has shown how paternal alcohol consumption within 3 months before conception may be associated with shorter anogenital distance (the distance from the center of the anus to the genitals) in the newborns, especially in boys [190]. Anogenital distance, which usually persists throughout life, is associated with sperm quality and serum reproductive hormones. It can also predict other androgen-responsive outcomes, such as hypospadias, cryptorchidism, and prostate cancer. Furthermore, it has an essential role as a sensitive biomarker of reproductive hormone abnormalities during the critical developmental window. These results suggest that paternal alcohol drinking may harm newborns' reproductive development.

12.4. Endocrine and Immune Alterations

The proopiomelanocortin (Pomc) gene that controls neuroendocrine-immune functions has been proven to be imprinted by fetal alcohol exposure. Interestingly, the Pomc gene is hypermethylated through three generations, while the alcohol epigenetic marks on the Pomc gene are maintained in the male but not in the female germline during this transgenerational transmission, confirming how the male-specific chromosome is involved in transmitting alcohol epigenetic marks through multiple generations [49].

Another study showed that children with FASD are more susceptible to infections than normal children, and this is also true for children born with PPAC, especially in the case of diseases caused by *Pseudomonas aeruginosa* [191]. An old but authentic article centered on the neurochemical effects of PPAC showed how PPAC causes sex-specific changes in levels of norepinephrine in male offspring, as well as reduced 5-Hydroxytryptamine levels in the cerebrum and increased Met-enkephalin levels in all brain regions of offspring from both maternally and paternally exposed rats [192].

12.5. Reduced Physical Growth, Placental Disruptions, and Metabolic Alterations

Although the first report of an association, in humans, between father's drinking before conception and decreased in-

fant birth weight dated back to 1987, the subsequent findings have been controversial for a while, finally demonstrating the importance of the timing, quality, and quantity of alcohol exposure in the manifestation of this phenotype [56, 193, 194]. Moreover, only recently, the epigenetic mechanisms at the base of this condition have been started to be unraveled.

In literature, mostly using rodents as models, chronic PPAC has been linked to fetal growth restriction and metabolic alterations [47, 195-199].

Knowing that chronic ethanol exposure can affect small non-coding RNA (ncRNAs) abundance and nucleoside modifications in mice sperm and that ncRNAs in sperm have been shown to causally induce heritable phenotypes in the offspring, recently, the potential effects of ethanol-responsive sperm ncRNAs on offspring health and development have been investigated [47, 200]. The findings associated with these studies have been partially related to the growth deficit of the offspring. However, the mechanisms through which the effects of sperm-inherited ncRNAs persist into later life and how the memory of chronic alcohol use transmits to the offspring need to be further studied. Chronic alcohol consumption alters the sperm constitution at a profound level, shifting the proportional ratio of transfer RNA-derived small RNAs to Piwi-interacting RNAs, as well as alters the enrichment of microRNAs miR21, miR30, and miR142; interestingly, these changes cannot be linked to alcohol-induced changes in the profile of circulating corticosterone but instead may primarily be mediated by effects on epididymal trafficking [47].

Interestingly, as preconceptual alcohol consumption has been demonstrated to reduce global DNA methylation in the developing mouse fetus, in literature, the effect of PPAC on DNA methylation at H19 imprinting control region (ICR), a paternally methylated ICR in the sperm of exposed males and somatic DNA of sired offspring, has been specifically examined [55, 201, 202]. Interestingly, H19 has a role in the placental formation and works in combination with Igf2, a potent mitogen, to regulate fetal growth and development. Significant reductions at the H19 CCCTC-binding factors 1 and 2 binding sites were observed in the offspring of ethanol-treated sires and were significantly correlated with reduced weight at postnatal days 35-42 but without affecting birth weight [55]. Furthermore, growth was only delayed during the postnatal weaning period, with subsequent reconvergence, suggesting that this transient growth reduction may be the result of a mental deficit that causes the delayed establishment of independent feeding following weaning.

In literature, it has also been emphasized how both the male and female offspring growth restriction associated with PPAC correlates with a reduction in placental efficiency, manifesting mainly during the later phases of pregnancy [47, 58, 195].

While FASD growth phenotypes can be linked to alcohol-induced intrauterine growth restriction mediated by impaired placentation, it is also true that impaired placentation, in turn, is associated with poor cognitive development and long-term alterations in metabolic programming within the offspring. Additionally, ethanol-impaired placentation has been associated with inhibition of aspartyl-(asparaginyl) be-

ta-hydroxylase (AAH) expression, a gene that is regulated by insulin-like growth factors (IGF) and has a critical role in cell motility and invasion in trophoblasts [203]. Interestingly, it has been suggested a specific pattern of increased placental iodothyronine deiodinase III (Dio3) and decreased thyroid hormone receptor $\alpha 1$ (TR $\alpha 1$) and glucocorticoid receptor (GR) protein levels in the placenta as a potential biomarker for intrauterine alcohol exposure [204].

Alcohol-induced growth phenotypes are accompanied in a mouse model by a prolonged period of fetal gestation and sex-specific patterns of postnatal growth restriction characterized by reductions in height, weight, and body mass index that manifest at birth and continue to persist through young adulthood [195].

In rodents, the male offspring of alcohol-exposed fathers continue to exhibit growth deficits in postnatal life, persisting into adolescence, which may be linked to sex-specific insulin hypersensitivity in the male offspring as well as increased markers of hepatic fibrosis (up-regulation of genes within the pro-fibrotic TGF- β signaling pathway), suppressed cytokine profiles within the liver, and altered cholesterol trafficking and up-regulation of genes in the LiverX/RetinoidX/FarnesoidX receptor (LRX) pathways (within the liver and pancreas of both the male and female offspring) [195, 196]. In particular, among liver X receptors (LXR), that regulate many aspects of cholesterol, fatty acid, and glucose homeostasis and have important roles in controlling immune function and neurodevelopment, LXR α has been identified as a key mediator of male offspring long-term metabolic alterations induced by PPAC being related to resistance to diet-induced obesity and improved glucose homeostasis when challenged with a high-fat diet, as an adaptation mediated by LXR α trans-repression of inflammatory cytokines, following the release of the inhibitor of nuclear factor- κ B Kinase β (IKK β), related to the insulin signaling pathway. Curiously, paternally programmed increases in LXR α expression seem to be liver-specific and do not manifest in the pancreas or visceral fat.

12.6. Preconceptional Paternal Alcohol Exposure and Sex-specific Differences

Gender differences due to PPAC have been disclosed in animal models. Indeed, PPAC induced a prolonged period of fetal gestation and an increased incidence of intrauterine growth restriction, which affected the male offspring to a greater extent than the females [195]. While the female offspring of ethanol-exposed males were able to match the body weights of the controls within the first 2 weeks of postnatal life, male offspring continued to display an 11% reduction in weight at 5 weeks of age and a 6% reduction at 8 weeks of age [195]. The observed growth deficits were associated with insulin hypersensitivity in the male offspring, while in contrast, females displayed a modest lag in their glucose tolerance test [195]. These metabolic defects were associated with an up-regulation of genes within the pro-fibrotic TGF- β signaling pathway and increased levels of cellular hydroxyproline within the livers of the male offspring [195]. Suppressed cytokine profiles within the liver and pancreas of both the male and female offspring were also observed, which correlated with the up-regulation of genes in the LiverX/RetinoidX/FarnesoidX receptor pathways [195].

In a PPAC chronic intermittent vapor ethanol mouse model [205], authors found that male offspring showed stress hyporesponsivity in a stress-induced hyperthermia assay, but female offspring had reduced binge-like ethanol consumption in drinking in the dark assay compared to control offspring [205]. Another PPAC chronic intermittent vapor ethanol mouse model showed that male offspring exhibited decreased two-bottle free-choice ethanol-drinking preference, increased sensitivity to the anxiolytic effects of ethanol, and increased VTA BDNF expression [116]; however, no differences were observed in female offspring [116]. In the PPAC self-administration mouse model, authors discovered that PPAC elicited a sex-specific increase in activity and sensorimotor integration deficits at P20 and decreased balance, coordination, and short-term motor learning at P30 [177], suggesting that PPAC may generate long-lasting, sex-specific effects on offspring behavior [177].

As for human PPAC studies, anecdotal evidence suggests that paternal alcoholism may play an unidentified role in the aetiology of Turner syndrome, a sex chromosome aneuploidy that occurs as a result of a non-disjunctional error in meiosis I or anaphase lag; however, a study suggested that there is no association between paternal or maternal alcohol consumption and Turner syndrome [206]. In a study investigating the association between PPAC and anogenital distance [190], the authors found that boys had shorter anogenital distance during growth, but in girls, shorter anogenital distance was observed only at birth [190].

13. DISCUSSION

In this narrative review, we have shown how PPAC plays a substantial but underestimated role in FASD pathogenesis. Increasing evidence from a variety of model organisms (mostly mouse) suggests that the environment can affect epigenetic inheritance and that related non-genetically determined phenotypes can be passed between generations with complex and mostly unclear means [207, 208]. We have recently exposed how mechanisms controlling the alcohol drinking behavior and the neurobiological sensitivity to alcohol may be disrupted due to altered epigenetic regulation of pathways regulating mood, emotion, and serotonergic states potentially involved in depression and sickness behavior and changes in the expression of neurotrophins, such as NGF and BDNF [117] (Fig. 1, point III). This important link between alcohol and neurotrophins may imply a neuroprotective effect against alcohol in the neuropharmacological approaches based on the increase in neurotrophins availability.

As previously observed, the effects of PPAC involve not only the offspring's next generation but, as a result of epimutations in the critical sperm genes required for normal development, multiple generations may be affected [37, 49, 50]. Nevertheless, further studies are needed to uncover the mechanisms through which the effects of sperm-inherited ncRNAs persist into later life and how the memory of chronic alcohol use could be transmitted to the offspring [47, 181, 200].

Most of the clinical studies involving human subjects concern the PPAC role in offspring's behavioral, cognitive, and psychological consequences; therefore, our data provide

evidence that sons of alcoholics, when compared to sons of non-alcoholics, may show delayed cognitive performance, abnormal emotional responses, increased genetic risk to develop alcohol abuse and neurotic personality profiles, impairments in perceptual-motor ability, memory, auditory and visuospatial information, and language processing [106, 110, 112].

The PPAC has also been related to a series of organic and physiological abnormalities: fetal growth restriction, higher odds of human microcephaly, agenesis and skull malformation (exencephaly), the reduced weight of heart, kidney, spleen, and ventral prostate, increased hearing loss, shorter anogenital distance, increased adrenal weights, altered neuroendocrine-immune functions, metabolic variations, teratogenic and developmental defects in the next generation at fetal, prenatal, and postnatal stages [47, 185-190, 196, 203].

Despite the sizable amount of data presented in this review, it is worthy to note that, in contrast to the data on the role of maternal alcohol consumption in FASD, it should be still considered scarce; therefore, other studies are needed to further investigate most of the relationships already related and analyzed for maternal alcoholics and to generate multiple and new lines of evidence.

13.1. Limitations

The first limitation of this narrative review is the prevalence of studies based on animal models compared to human clinical studies. This condition suggests that most of the evidence actually available is transposed from animals to humans. Furthermore, most of the included human studies are observational, instead of experimental, due to the severity of the outcome of FASD in the involved subjects. Despite the extensive research, because of the vast literature on this topic, we cannot rule out the possibility that some pieces of evidence maybe not be included in this review.

CONCLUSION

Alcohol abuse is a dangerous condition that involves both females and males, causing significant dangerous effects not only to their health but also to their offspring's wellbeing. Despite the abundance of evidence on the role of paternal preconceptional alcohol consumption in fetal alcohol spectrum disorders pathogenesis, this topic is still scarcely known among the general population; therefore, it is of primary importance to raise awareness among citizens on the impact of paternal alcohol use disorders on their and their newborn's health. As oxidative stress caused by preconceptional alcohol use has been clearly related to fetal alcohol spectrum disorders, the evidence suggests how antioxidants could be potential candidates for the development of novel therapeutic strategies for the treatment of such neurodevelopmental disorders. The crucial topic of lifelong disabilities induced by paternal preconceptional alcohol consumption and/or gestational alcohol drinking is quite challenging at the individual, societal, and familial levels. Since a nontoxic drinking behavior before pregnancy (for both men and women), during pregnancy, and lactation cannot be established, the only suggestion for couples planning pregnancies is to completely avoid the consumption of alcoholic beverages.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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