



**Transgenerational Abnormalities Induced by Paternal
Preconceptual Alcohol Drinking. Findings from Humans and
Animal Models**

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6 **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. Solid human and animal model data demonstrated that PPAC may affect sperm function eliciting oxidative stress. In newborns, PPAC may induce changes in the 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 disclosed also changes in brain function and structure by the disruption of 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.

Keywords: neurotrophins; brain; fetal alcohol spectrum disorders; fetal alcohol syndrome; epigenetics; paternal preconceptional alcohol consumption; transgenerational changes.

Running head: fathers and alcohol drinking

1. INTRODUCTION

Alcohol-use disorders (AUD) and alcoholism, are complex multifactorial conditions [1–10] being related also 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), 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] (Figure 1, point I).

Despite the acknowledgment of 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 in the world [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 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

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3 documentation that the biological mother consumed alcohol during the pregnancy; sentinel facial
4 features; neurodevelopmental assessment in search of evidence of pervasive brain dysfunction [35].
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6 The life expectancy of patients with FAS is estimated to be 34 years (95% confidence interval: 31 to
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8 37 years) even though the leading causes of death are "external causes" (44%), which include suicide
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10 (15%), accidents (14%) and poisoning by illegal drugs or alcohol (7%) as well as other causes [36].
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14 The observation, in twin and adoption studies, of heritability of about 50% of alcohol use disorder
15 associated with the demonstration that chronic exposure to ethanol causes chromatin remodeling in
16 the brain that promotes the transition from use to abuse and addiction, render even more challenging
17 the efficacy of prevention campaigns in many situations [37–40]. In the overall population, it has
18
19 been estimated that about 8.9% of women drink alcohol during pregnancy, while the global
20 prevalence of FASD is about 1 in 100 people and the prevalence of FAS in the same population is
21 about 14.6 per 10,000 people [27]. It has been calculated that one in every 67 women who consume
22 alcohol during pregnancy would carry a child with FAS, which translates to about 119,000 children
23 born with FAS every year in the world. Provided that FASD is a lifelong disease, it has been estimated
24 that more than 11 million individuals between 0 and 18 years of age, and 25 million individuals
25 between 0–40 years of age, have FASD worldwide [41].
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40 It is now widely established that the harmful effects of prenatal alcohol exposure on the embryo
41 and fetus development cause noticeable cognitive and neurobiological deficits in the newborns, the
42 most common clinical features are listed in Table 1 [42]. The deleterious role of oxidative stress
43 induced by alcohol drinking during pregnancy has been established suggesting how supplementing a
44 mother's diet with protective and antioxidant substances in addition to folic acid, choline, neuroactive
45 peptides, and supportive psychological therapies could protect newborns from being affected
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47 [16,18,43].
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56 It has been demonstrated how phenotypic heterogeneity in human FASD is strongly related to a
57 genetic vulnerability in affected brain regions [44]. Interestingly, it has been hypothesized that the
58 principles of non-Mendelian inheritance, or "exceptions" to Mendelian genetics, in particular when
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3 one parent has an exaggerated influence over the offspring's vulnerability to prenatal alcohol (e.g.
4 maternal alleles leading to an altered intrauterine hormonal environment or when epigenetic
5 regulation of allele-specific gene expression causes an imbalance between the maternal and paternal
6 genetic contributions) could be the main factor determining the severity of the FASD symptomology
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14 While the negative effects of gestational alcohol use have been well documented and known for
15 some time, the capacity of parental life history to influence the offspring phenotype has been highly
16 underestimated and not fully explored [16,26,43,45–47]. Knowing that about 75 % of children with
17 FAS have heavy drinkers or alcoholic biological fathers it seems logical that in recent times it has
18 been gaining a subtle role, in FASD manifestation, of alcohol consumption by fathers before mating
19 [48]. Differently from maternal alcohol exposure, paternal alcohol consumption does not directly
20 come into contact with developing embryos and the direct relationship between paternal alcohol
21 consumption and genetic or epigenetic mutations that result in developmental abnormalities has been
22 studied only recently, challenging the common misconception that sperm does not transmit heritable
23 information beyond the genetic code. In 2004, it was partially investigated the paternal influence on
24 offspring's low birth weight, congenital malformations, deficits of the immune system and, of course,
25 cognitive and behavioral abnormalities including learning and memory deficits, hyperactivity, and
26 poor stress tolerance, resulting in a renewed interest on this topic [48]. Recently many studies have
27 also suggested the existence of 'transgenerational' consequences of alcohol for the offspring, more
28 often related to male chronic alcohol abuse, demonstrating the tendency to epigenetic variations that
29 might be transmitted through multiple generations [37,49,50].
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51 This report aims to provide a summary and subsequent review of past researches, which highlights
52 the evidence on the role of paternal preconceptual alcohol consumption (PPAC) in FASD
53 etiopathogenesis.
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2. METHODS

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, preconceptual ethanol consumption, prenatal ethanol exposure. When judged relevant, other items listed in the references of the included articles were considered for eligibility.

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.

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 when compared to an animal model.

3. TRANSGENERATIONAL EFFECTS OF PATERNAL PRECONCEPTUAL ALCOHOL CONSUMPTION

In 1968, Lemoine and colleagues have been the first to describe the situation in which children who presented clinical features of FAS were born from mothers who did not drink but alcoholics fathers [53]. This seems to be the first time that it was introduced 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

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3 male rats that had consumed alcohol for seven months did not differ significantly from controls in
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5 their immobility response in the forced swimming test but, on the other hand, it was the offspring of
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7 these males that exhibited a significant dose-related decrease in immobility [54]. These results
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9 showed for the first time that, while alcohol's effects may not be observable in males consuming it,
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11 this does not necessarily mean that it has no important biological effects on these males and that these
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13 effects may not be appreciated until their offspring are examined. This discovery brought new light
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15 on the potential role of PPAC in the alcohol disorders-related effects on the offspring. In the years it
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17 has been shown how not only the offspring's next generation may be influenced by PPAC, but also
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19 multiple generations may be affected, so that the term “transgenerational” may be used to identify the
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21 effects of prenatal alcohol exposure on the offspring [37,49,50]. Epimutations in the critical genes
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23 required for normal development can be transmitted through fertilization and, since only germ cells
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25 are in contact with alcohol in the case of PPAC, male sperm has been identified as the main mediator
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27 of the toxic effects. Therefore, the transgenerational toxic effects of PPAC are due to alcohol-induced
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29 epigenetic mutations in sperm DNA [55–57]. Interestingly, using bisulfite mutagenesis and second-
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31 generation deep sequencing, no evidence was found that suggested that the alcohol-associated
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33 phenotypes and the transcriptional changes are linked to alterations in the sperm-inherited DNA
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35 methylation profile emphasizing the importance of epigenetic mechanisms of paternal inheritance
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37 beyond DNA methylation [58].
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47 **4. PRECONCEPTUAL ALCOHOL CONSUMPTION IS ASSOCIATED WITH OXIDATIVE** 48 **STRESS**

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51 Oxidative pathways involve the metabolism of ethanol to acetaldehyde, catalyzed either by alcohol
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53 dehydrogenase (ADH), CYP2E1 or catalase [59]. It is currently known how ethanol exposure can
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55 lead to the generation of reactive oxygen species (ROS) modifying the intracellular redox state, and
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57 ultimately leading to an overall increase in oxidative stress and neuronal cell death by the oxidation
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59 of proteins, lipids and DNA [60,61]. In particular, DNA lesions can result in genetic mutations
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3 involving the DNA sequence of a single cell, resulting in cellular immortalization and clonal
4 expansion ultimately leading to postnatal cancer or, otherwise, it can result in direct or indirect
5 epigenetic modifications to DNA, histones or RNA that involves gene expression of many cells,
6 contributing to teratogenesis in the form of birth defects and postnatal neurodevelopmental
7 abnormalities [59] (Figure 1, point II). Alcohol toxicity is caused both directly by ethanol and
8 indirectly by its metabolic products, including the ROS produced during its biotransformation
9 involving CYP2E1 [62,63]. Interestingly, both maternal and paternal preconceptional alcohol exposure
10 have been related to mitochondrial dysfunction and a significant increase in oxidative stress in
11 developing organs, especially in the central nervous system [64,65]. The relationship between
12 oxidative stress and central nervous system abnormalities should not surprise given that, when
13 compared to other organs, the brain is more susceptible to ROS generation (that include superoxide
14 anions, hydrogen peroxide, and hydroxyl radicals) holding the highest oxygen metabolic rate (its cells
15 utilize 20% of the oxygen consumed by the entire organism), and brain tissues are rich in unsaturated
16 fatty acids that can be used as substrates for ROS production [66]. Furthermore, some brain regions
17 have a high content of iron and several neurotransmitters tend to spontaneously react with oxygen
18 (namely dopamine, levodopa, serotonin and norepinephrine), which may further promote the
19 generation of ROS [67]. Finally, it should be considered that the activities of the antioxidant enzymes
20 (superoxide dismutase, catalase, and glutathione peroxidase) are lower in the brain than in other
21 organs such as the liver or kidney, while fetal cells are more vulnerable than adult cells to the
22 neurotoxic effects of oxidative stress so that the role of ROS in FASD pathophysiology is clearly of
23 primary importance [68,69]. It should be noted that ROS participate in intracellular signaling
24 pathways and that, despite being unstable, are produced naturally in the embryo and fetus and are
25 essential for normal development, so that the problem resides in the imbalance due to excessive ROS
26 production related to the preconceptional alcohol consumption that ultimately causes oxidative stress
27 [70]. Increased activity of the enzyme NADPH oxidase (NOX), which may be the major source of
28 ROS production in the brain, has been related to mouse embryos preconceptional alcohol consumption,
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3 while co-treatment with the NOX inhibitor (diphenyleneiodonium) has shown to prevent ethanol-
4 induced increases in NOX activity, ROS generation and oxidative DNA damage [64,71–73]. In
5 rodents, antioxidant vitamin E (alpha-tocopherol) increases levels of anti-apoptotic proteins (e.g.,
6 Bcl-2, Bcl-xl, activated Akt kinase), downregulates pro-apoptotic proteins (e.g., Bcl-xs), improves
7 organs alterations and DNA damage, and adjusts the level of hyperhomocysteinemia showing
8 promising therapeutic applications in FASD [74–77]. Many other antioxidants have shown, mainly
9 in rodents, promising therapeutic effects: ascorbic acid (vitamin C), beta-carotene, black ginseng,
10 EUK-134 (synthetic superoxide dismutase plus catalase mimetic), folic acid, melatonin, N-
11 acetylcysteine, phenyl butyl nitron, pycnogenol, silymarin, superoxide dismutase [60,78–85]. Also,
12 the content of antioxidants (polyphenols, resveratrol) in alcoholic beverages (*i.e.* red wine) may
13 counteract the toxic effect of alcohol *per se* as shown in FASD/AUD animal models [86–98]. The
14 effects may vary depending on the dose, duration, exposure period and route of exposure for both the
15 ethanol and the antioxidant.
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35 **5. IMPACT OF CHRONIC ALCOHOL USE ON SPERM FUNCTION**

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37 The impact of chronic alcohol use on sperm function and male reproductive physiology has been
38 the center of many studies demonstrating how chronic alcohol consumption has a detrimental impact
39 on male reproductive hormones and semen quality significantly altering the sperm structure and its
40 heritage [99–103]. Furthermore, paternal heavy alcohol consumption, before conception, increases
41 the risk of early pregnancy loss and stillbirth [104,105]. These findings may be considered the pillars
42 that drew attention to the PPAC's role in heritable disorders. Since then, literature expanded and many
43 items employed animal and human models to analyze the relationship between ethanol exposures and
44 the long-term effects on the offspring, highlighting paternally inherited variations in epigenetic
45 coding and their role in numerous disease states of the offspring, including behavior and cognitive
46 alterations, growth deficit, organic and physiological abnormalities related to FASD.
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6. PATERNAL PRECONCEPTUAL ALCOHOL CONSUMPTION IMPACTS ON THE OFFSPRING BEHAVIOR AND COGNITIVE FUNCTIONS

In literature, most of the earlier studies about the PPAC role in offspring 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 tend to perform differently when administered with neuropsychological tests: sons of active alcoholics perform 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 show 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

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3 without familial alcoholism [121]. Furthermore, reduced resting-state synchrony between the left
4 amygdala and left superior frontal gyrus was related to poorer response inhibition.
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7 As ethanol exposure activates the hypothalamic-pituitary-adrenal axis acting as a physiological
8 stressor, it has been hypothesized that PPAC impacts stress responsivity of offspring [122,123]. This
9 hypothesis seems to be partially confirmed as the studies show ethanol-sired male offspring sex-
10 specific blunted plasma corticosterone levels in response to acute restraint stress, resistance to stress-
11 induced excessive fluid intake, enhanced sensitivity to the anxiolytic and motor-enhancing effects of
12 ethanol, reduced ethanol preference and consumption, altered BDNF (brain-derived neurotrophic
13 factor) and/or NGF (nerve growth factor) (well-known regulators of ethanol drinking behavior
14 [16,26,117,124–142]) expression in the ventral tegmental area compared to control-sired male
15 offspring. No differences among ethanol- and control-sired female offspring have been found on these
16 assays. Consistently with the hypothesis of epigenetic effects of PPAC, ethanol exposure also showed
17 to reduce DNA methylation at the BDNF/Promoter of sire's germ cells, while hypomethylation is
18 maintained in the ventral tegmental area of both male and female ethanol-sired offspring [123]. In
19 another study, a voluntary oral route of paternal preconception EtOH exposure, i.e., intermittent
20 every-other-day two-bottle choice drinking, altered offspring behavior [143]. The authors found that
21 paternal preconception every-other-day two-bottle choice drinking resulted in reduced EtOH
22 consumption selectively in male offspring in the drinking in the dark assay compared to control-sired
23 offspring. No differences were detected in either sex in the unlimited access two-bottle choice and
24 elevated plus maze assays. Open field analysis revealed complex changes in basal behavior and
25 EtOH-induced behaviors that were sex specific [143]. All together these studies show that PPAC has
26 persistent effects that impact the next generation.
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56 **9. PATERNAL PRECONCEPTUAL ALCOHOL CONSUMPTION AND NEUROTROPHINS**

57 Among neurotrophic factors (which regulate cell growth, proliferation, differentiation, and
58 maturation, but also cell migration, metabolism and apoptotic cell death), neurotrophins play a critical
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3 role in proper brain development, in mediating synaptic plasticity throughout adulthood and in
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5 maintaining a balance between neuroendocrine, immune and metabolic systems [26,144,145].
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8 Neurotrophins include NGF, BDNF, neurotrophin-3 (NT-3) and NT-4/5. In particular, NGF and
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10 BDNF and their receptors, high-affinity receptor tropomyosin receptor kinase A (TrkA for NGF) and
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12 B (TrkB for BDNF) and low affinity p75 neurotrophin receptor (p75^{NTR} for both NGF and BDNF),
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14 have been shown to play a crucial role within the pathogenesis of prenatal alcohol exposure since
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16 alcohol exposure has been related to neurotrophins alterations in the frontal cortex, striatum and
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18 olfactory lobes and disruption of neurotrophin pathways that in turn affects brain cell growth and
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20 development and newborns behavior [129,146–150]. More specifically, we found that PPAC induces
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22 a higher susceptibility to the ethanol rewarding effects, affecting BDNF in the frontal cortex, striatum
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24 and olfactory lobes, TrkA in the hippocampus and p75^{NTR} in the frontal cortex, ultimately inducing
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26 ethanol intake preference in the male offspring [129]. Furthermore, alcohol inhibits the expression of
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28 the endogenous extracellular signal-regulated kinase (ERK) and the phosphatidylinositol-3-kinase
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30 (PI3K) of the neurotrophins pathways [151,152]. Possible links between the epigenetic modulation
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32 of NGF and BDNF, the serum levels of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) and
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34 the symptomatology of alcohol dependence have been suggested indicating that changes in the
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36 methylation of neurotrophins genes may contribute to the development of alcohol dependence by
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38 affecting relevant downstream signaling cascades [151,152].
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45 Modifications in NGF and BDNF levels in the serum and plasma are shown during the onset and
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47 progression of many disorders, including neurological, psychiatric and immune diseases [128,153–
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49 164] and during physiopathological conditions as cardiometabolic disruptions [165–169], stressful
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51 events [126], aging [169–171] and the post-partum period [127,172]. Recent findings on murine
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53 models have analyzed the relationship between PPAC, FASD and neurotrophins alterations
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55 suggesting that these proteins could act in a compensatory manner as neuroprotective molecules that
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57 can oppose the harmful effects of prenatal alcohol exposure [173,174].
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3 More recently, we have demonstrated how PPAC may disrupt the epigenetic regulation of
4 postnatal alcohol sensitivity in the offspring by investigating pathways regulating mood, emotion,
5 serotonergic tone and neurotrophins [26,98,117]. In these studies, we demonstrated how PPAC alters
6 the sensitivity to the serotonergic/neurotrophic-associated effects of alcohol affecting the postnatal
7 alcohol preference in the offspring. The analysis of the brainstem gene expression showed serotonin
8 transporter Solute Carrier Family 6 Member 4 (SLC6A4) and low-affinity pan-neurotrophins receptor
9 (p75^{NTR}) elevation and decreased 5-Hydroxytryptamine Receptor 2C (HTR2C) in the offspring of
10 chronic alcohol-exposed sires [117]. In a different study, male offspring sired by adult male mice
11 exposed to chronic intermittent vapor ethanol before mating showed attenuated ethanol drinking
12 behavior, increased sensitivity to the anxiolytic effects of ethanol, and epigenetic alterations (DNA
13 hypomethylation) and BDNF gene increased expression that persist in the ventral tegmental area
14 [123].

10. ATTENTION DEFICITS AND HYPERACTIVITY DISORDERS

15 Simultaneous preconceptual paternal or maternal exposure to alcohol and environmental tobacco
16 smoke increase the risk of attention deficit hyperactivity disorder (ADHD) diagnosed using the
17 DuPaul Rating Scale [175]. In rats, the presence of a differential effect of amphetamine suggests that
18 the paternal effect on hyperactivity may be mediated by catecholaminergic action [176]. Based on
19 previous experiments which demonstrated how the offspring of male mice chronically exposed to
20 ethanol before mating present attention deficit hyperactivity, altered expression and epigenetic
21 regulation of the dopamine transporter in the frontal cortex, but also altered neurobehavioral
22 outcomes (reflex acquisition, activity, gait) and increased cerebral cortical layer thickness, a recent
23 study demonstrated, in male mice who self-administered 25% ethanol for an extended period before
24 conception, how PPAC impacts the development of the neocortex and behavior in offspring similarly
25 as previously demonstrated in maternal consumption models of FASD [177–179]. Furthermore,
26 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 insemination 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 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 preconceptual 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 preconceptual 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 behavioral effects of PPAC on mice offspring are 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 PRECONCEPTUAL ALCOHOL CONSUMPTION IS RELATED TO ORGANIC AND PHYSIOLOGICAL ABNORMALITIES:

12.1 Organic Abnormalities

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3 Higher odds of human microcephaly at birth are associated with higher paternal, but not maternal,
4 alcohol consumption before pregnancy, and similar but weaker effects are estimated for the first
5 drinking trimester [185]. Meanwhile, in mice, it has been demonstrated how PPAC is related to
6 agenesi s and skull malformation (exencephaly), as well as the reduced weight of heart, kidney and
7 ventral prostate, causing teratogenic and developmental defects in the next generation at fetal,
8 prenatal and postnatal stages [56].
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12 Male rats consuming alcohol for 8–9 weeks, before conception, produce rat offspring with
13 increased adrenal weights, decreased spleen weights and hypoactivity [186].
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15 16 17 **12.2 Hearing Loss**

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19 Data show how chronic PPAC in mice may have a dose-dependent role in offspring's increased
20 hearing loss [187]. In the same study, the authors, also assessed the methylation of H19, Peg3, Ndn
21 and Snrpn in paternal spermatozoa and the cerebral cortices of deaf mice confirming the epigenetic
22 bases of the FASD pathophysiology that have been suggested in previous studies: PPAC alters the
23 methylation of imprinted genes in sire spermatozoa and these genes could be passed on to the
24 offspring ultimately giving rise to developmental disorders [187,188].
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37 38 **12.3 Neurogenesis, Obesity and Reproductive System Effects**

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40 While most of the experiments have evaluated the effects of prenatal alcohol exposure in the
41 gestational and short term periconceptional period, an interesting study evaluates the effects of
42 adolescent binge alcohol exposure on hypothalamic gene expression patterns in the F1 generation
43 offspring [189]. In this study the authors exposed adolescent male and female rats to a repeated binge
44 EtOH exposure paradigm and then mated them in adulthood. Data showed that the offspring of
45 alcohol-exposed parents had significant differences compared to offspring from alcohol-naïve parents
46 [189]. Specifically, major differences were observed in the expression of genes that mediate
47 neurogenesis and synaptic plasticity during neurodevelopment, genes important for directing
48 chromatin remodeling, post-translational modifications or transcription regulation, as well as genes
49 involved in regulation of obesity and reproductive function [189].
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3 There is limited literature on the effects of PPAC on the reproductive development in humans
4 offspring: a recent study that included 1,292 pregnant women shows how paternal alcohol
5 consumption within 3 months before conception may be associated with shorter anogenital distance
6 (the distance from the center of the anus to the genitals) in the newborns, especially in boys [190].
7
8 Anogenital distance, which usually persists throughout life, is associated with sperm quality and
9
10 serum reproductive hormones, it can also predict other androgen-responsive outcomes, such as
11
12 hypospadias, cryptorchidism and prostate cancer, and so it has an essential role as a sensitive
13
14 biomarker of reproductive hormone abnormalities during the critical developmental window. These
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16 results suggest that paternal alcohol drinking may harm newborns' reproductive development.
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24 **12.4 Endocrine and Immune Alterations**

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26 The proopiomelanocortin (Pomc) gene, that controls neuroendocrine-immune functions has been
27
28 proven to be imprinted by fetal alcohol exposure. Interestingly, the Pomc gene is hypermethylated
29
30 through three generations, while the alcohol epigenetic marks on the Pomc gene are maintained in
31
32 the male but not in the female germline during this transgenerational transmission confirming how
33
34 the male-specific chromosome is involved in transmitting alcohol epigenetic marks through multiple
35
36 generations [49].
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40 Another study showed that children with FASD are more susceptible to infections than normal
41
42 children, and this is also true for children born with PPAC, especially in the case of diseases caused
43
44 by *Pseudomonas aeruginosa* [191]. An old, but still actual, article centered on the neurochemical
45
46 effects of PPAC showed how PPAC causes sex-specific changes in levels of norepinephrine in male
47
48 offspring, as well as reduced 5-Hydroxytryptamine levels in the cerebrum and increased Met-
49
50 enkephalin levels in all brain regions of offspring from both maternally and paternally exposed rats
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52 [192].
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56 **12.5 Reduced Physical Growth, Placental Disruptions and Metabolic Alterations**

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58 Although the first report of an association, in humans, between father's drinking before conception
59
60 and decreased infant birth weight dates back to 1987, the subsequent findings have been controversial

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3 for a while, finally demonstrating the importance of the timing, quality and quantity of alcohol
4 exposure in the manifestation of this phenotype [56,193,194]. Moreover, only recently the epigenetic
5 mechanisms at the base of this condition are starting to be unraveled.
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10 In literature, mostly using rodents as models, it has been linked chronic PPAC to fetal growth
11 restriction and metabolic alterations [47,195–199].
12
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14 Knowing that chronic ethanol exposure can affect small non-coding RNA (ncRNAs) abundance
15 and nucleoside modifications in mice sperm and that ncRNAs in sperm have been shown to causally
16 induce heritable phenotypes in the offspring, recently the potential effects of ethanol-responsive
17 sperm ncRNAs on offspring health and development have been investigated [47,200]. The findings
18 associated with these studies have been partially related to the growth deficit of the offspring.
19 However, the mechanisms through which the effects of sperm-inherited ncRNAs persist into later life
20 and how the memory of chronic alcohol use transmits to the offspring needs to be further studied.
21 Chronic alcohol consumption alters the sperm constitution at a profound level shifting the
22 proportional ratio of transfer RNA-derived small RNAs to Piwi-interacting RNAs, as well as altered
23 enrichment of microRNAs miR21, miR30, and miR142; interestingly these changes cannot be linked
24 to alcohol-induced changes in the profile of circulating corticosterone but instead may primarily be
25 mediated by effects on epididymal trafficking [47].
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42 Interestingly, as preconceptual alcohol consumption has been demonstrated to reduce global DNA
43 methylation in the developing mouse fetus, in literature it has been specifically examined the effect
44 of PPAC on DNA methylation at H19 imprinting control region (ICR), a paternally methylated ICR
45 in the sperm of exposed males and somatic DNA of sired offspring [55,201,202]. Interestingly, H19
46 has a role in placental formation and works in combination with Igf2, a potent mitogen, to regulate
47 fetal growth and development. Significant reductions at the H19 CCCTC-binding factors 1 and 2
48 binding sites were observed in the offspring of ethanol-treated sires and were significantly correlated
49 with reduced weight at postnatal days 35-42 but without affecting birth weight [55]. Furthermore,
50 growth was only delayed during the postnatal weaning period, with subsequent reconvergence,
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3 suggesting that this transient growth reduction may be the result of a mental deficit that causes the
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5 delayed establishment of independent feeding following weaning.
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8 In literature, it has been also emphasized how both the male and female offspring growth
9
10 restriction associated with PPAC correlates with a reduction in placental efficiency and manifesting
11
12 mainly during the later phases of pregnancy [47,58,195].
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15 While FASD growth phenotypes can be linked to alcohol-induced intrauterine growth restriction
16
17 mediated by impaired placentation, it is also true that impaired placentation, in turn, is associated
18
19 with poor cognitive development and long-term alterations in metabolic programming within the
20
21 offspring. Additionally, ethanol-impaired placentation has been associated with inhibition of aspartyl-
22
23 (asparaginy) beta-hydroxylase (AAH) expression, a gene that is regulated by insulin-like growth
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25 factors (IGF) and has a critical role in cell motility and invasion, in trophoblasts [203]. Interestingly,
26
27 it has been suggested a specific pattern of increased placental iodothyronine deiodinase III (Dio3)
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29 and decreased thyroid hormone receptor $\alpha 1$ (TR $\alpha 1$) and glucocorticoid receptor (GR) protein levels
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31 in the placenta as a potential biomarker for intrauterine alcohol exposure [204].
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36 Alcohol-induced growth phenotypes are accompanied in a mouse model by a prolonged period of
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38 fetal gestation and sex-specific patterns of postnatal growth restriction characterized by reductions in
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40 height, weight, and body mass index that manifest at birth and continue to persist through young
41
42 adulthood [195].
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45 In rodents, the male offspring of alcohol-exposed fathers continue to exhibit growth deficits in
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47 postnatal life, persisting into adolescence, which may be linked to sex-specifically insulin
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49 hypersensitivity in the male offspring as well as increased markers of hepatic fibrosis (up-regulation
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51 of genes within the pro-fibrotic TGF- β signaling pathway), suppressed cytokine profiles within the
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53 liver and altered cholesterol trafficking and up-regulation of genes in the
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55 LiverX/RetinoidX/FarnesoidX receptor (LRX) pathways (within the liver and pancreas of both the
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57 male and female offspring) [195,196]. In particular, among liver X receptors (LXR), that regulate
58
59 many aspects of cholesterol, fatty acid, and glucose homeostasis, and have important roles in
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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-kB 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 Preconceptual 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 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 a 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,

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3 no differences were observed in female offspring [116]. In PPAC self-administration mouse model
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5 authors discovered that PPAC elicited a sex-specific increase in activity and sensorimotor integration
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7 deficits at P20, and decreased balance, coordination, and short-term motor learning at P30 [177]
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9 suggesting that PPAC may generate long-lasting, sex-specific effects on offspring behavior. [177].
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12 As for human PPAC studies anecdotal evidence suggests that paternal alcoholism may play an
13
14 unidentified role in the aetiology of Turner syndrome, a sex chromosome aneuploidy that occurs as a
15
16 result of a non-disjunctional error in meiosis I or anaphase lag, however, a study suggested there is
17
18 no association between paternal or maternal alcohol consumption and Turner syndrome [206]. In a
19
20 study investigating the association between PPAC and anogenital distance [190], authors found that
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22 boys had shorter anogenital distance during growth but in girls shorter anogenital distance was
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24 observed only at birth [190].
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31 13. DISCUSSION

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33 In this narrative review, we have shown how PPAC plays a substantial but underestimated role in
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35 FASD pathogenesis. Increasing evidence from a variety of model organisms (mostly mouse) suggests
36
37 that the environment can affect epigenetic inheritance and that related non-genetically determined
38
39 phenotypes can be passed between generations with complex and mostly unclear means [207,208].
40
41 We have recently exposed how mechanisms controlling the alcohol drinking behavior and the
42
43 neurobiological sensitivity to alcohol may be due to disrupted epigenetic regulation of pathways
44
45 regulating mood, emotion and serotonergic states potentially involved in depression and sickness
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47 behavior throughout also changes in the expression of neurotrophins as NGF and BDNF [117] (Figure
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49 1, point III). This important link between alcohol and neurotrophins may imply a neuroprotective
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51 effect against alcohol of neuropharmacological approaches based on the increase in neurotrophins
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53 availability.
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58 As previously observed, the effects of PPAC involve not only the offspring's next generation but,
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60 as a result of epimutations in the critical sperm genes required for normal development, multiple

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3 generations may be affected [37,49,50]. Nevertheless, further studies are needed to uncover the
4 mechanisms through which the effects of sperm-inherited ncRNAs persist into later life and how the
5 memory of chronic alcohol use could be transmitted to the offspring [47,181,200].
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10 Most of the clinical studies involving human subjects concern the PPAC role in offspring's
11 behavioral, cognitive and psychological consequences, so that our data provide evidence that sons of
12 alcoholics, when compared to sons of non-alcoholics, may show delayed cognitive performance,
13 abnormal emotional responses, increased genetic risk to develop alcohol abuse and neurotic
14 personality profiles, impairments in perceptual-motor ability, memory, auditory and visuospatial
15 information, and language processing [106,110,112].
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24 The PPAC has also been related to a series of organic and physiological abnormalities: fetal growth
25 restriction; higher odds of human microcephaly, agenesis and skull malformation (exencephaly); the
26 reduced weight of heart, kidney, spleen and ventral prostate; increased hearing loss; shorter anogenital
27 distance; increased adrenal weights; altered neuroendocrine-immune functions; metabolic variations;
28 teratogenic and developmental defects in the next generation at fetal, prenatal and postnatal stages
29 [47,185–190,196,203].
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38 Despite the sizable amount of data presented in this review please note that, in contrast to the data
39 on the role of maternal alcohol consumption in FASD, it should be considered as still scarce, so that
40 other studies are needed to further investigate most of the relationships already related and analyzed
41 for maternal alcoholics and to generate multiple and new lines of evidence.
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47 Limitations: the first limitation of this narrative review is due to the prevalence of studies based
48 on animal models when compared to human clinical studies. This condition suggests that most of the
49 evidence actually available is transposed from animals to humans. Furthermore, most of the included
50 human studies are observational, instead of experimental, due to the severity of the outcome of FASD
51 in the involved subjects. Despite the extensive research, because of the vast literature on this topic,
52 we can't rule out the possibility that some pieces of evidence maybe not included in this review.
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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 preconceptual alcohol consumption in fetal alcohol spectrum disorders pathogenesis, this topic is still scarcely known among the general population so that it is of primary importance to raise awareness of citizens on the impact of paternal alcohol use disorders on their and their newborn's health. As oxidative stress caused by preconceptual 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 preconceptual 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.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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| Congenital malformations | Organs and apparats | Behavioral | 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 |

Table 1. Fetal alcohol spectrum disorders common clinical features.

Figure 1 caption

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.

CH₃-CH₂-OH
ethanol

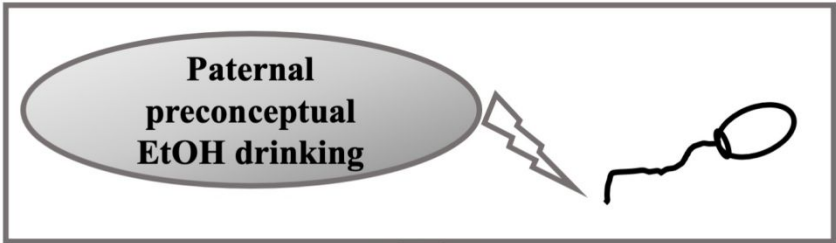
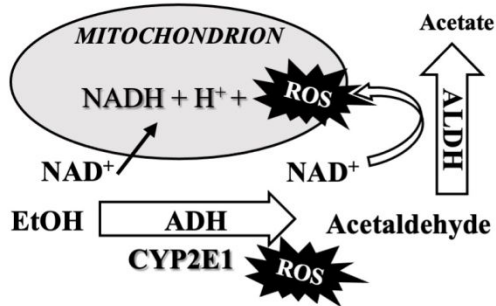


Figure 1

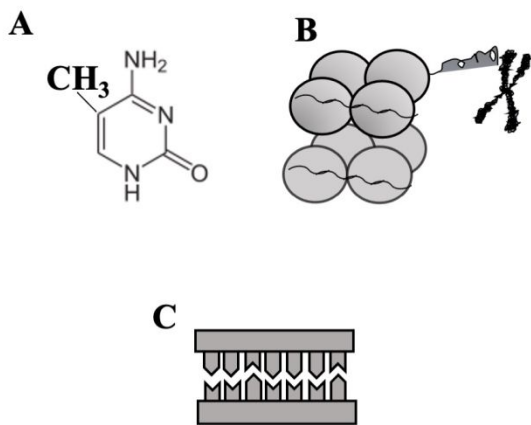
I

Enhanced oxidative response



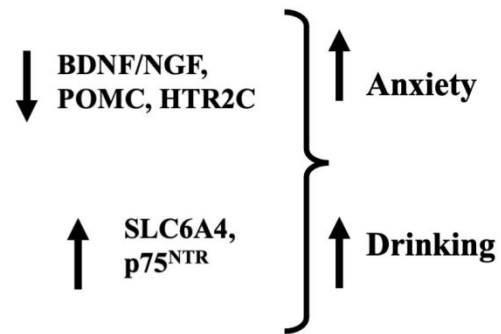
II

Alteration of gene expression



III

Neuroendocrine response activation



Transgenerational Abnormalities Induced by Paternal Preconceptual Alcohol Drinking. Findings from Humans and Animal Models

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Conflict of interest

The authors declare that they have no conflict of interest.

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