

Review

Fetal Alcohol Spectrum Disorder (FASD) Accelerates Local Neuroinflammation in Hippocampus: A Preclinical Systematic Review and Meta-Analysis Study

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Abstract: Background — Prenatal alcohol exposure (PAE) causes fetal alcohol spectrum disorder (FASD). The hippocampus, an important part of the limbic system, is pathologically affected in FASD. The goal of this systematic review and meta-analysis was to evaluate biomarkers of neuroinflammation and neurogenesis in the hippocampus in FASD.

Methods — All studies reporting the effects of PAE on the hippocampus were collected (as of June 12, 2025) in valid WoS, PubMed, Scopus, and ScienceDirect databases using MeSH keywords. After primary and secondary screening (PRISMA 2020 guidelines), the selected articles underwent study quality assessment (ARRIVE checklist). Data were extracted in two main areas: neuroinflammation (IL-1 β , IL-6, and TNF- α cytokines) and neurogenesis (Ki67, DCX, and BrdU markers). Random and fixed effects models (comprehensive meta-analysis software: CMA version 2) were used for statistical analysis. Subgrouping and the I^2 index were used to assess heterogeneity. Publication bias and sensitivity were also assessed. P-value of 0.05 or less was considered statistically significant, and a 95% confidence interval (95% CI) was calculated.

Results — Of the 231 articles, 8 high-quality experiments were used for data extraction. The total effect of FASD on hippocampal neuroinflammation and neurogenesis was 1.323 ± 0.125 (95% CI=1.078–1.568, $p=0.000$) and 0.288 ± 0.482 (95% CI=-0.655–1.232, $p=0.549$), respectively. The results of subgroup analysis were as follows: IL-1 β (0.972 ± 0.222 , 95% CI=0.537~1.407, $p=0.000$), IL-6 (1.954 ± 0.204 , 95% CI=1.555~2.354, $p=0.000$), TNF- α (0.916 ± 0.224 , 95% CI=0.476~1.356, $p=0.000$), BrdU⁺ cells (-1.468 ± 1.109 , 95% CI=-3.641~0.705, $p=0.185$), DCX⁺ cells (-0.234 ± 1.191 , 95% CI=-2.569~2.101, $p=0.844$), and Ki67⁺ cells (0.932 ± 0.598 , 95% CI=-0.241~2.104, $p=0.119$).

Conclusion — FASD may lead to hippocampal neuroinflammation in neonates.

Keywords: maternal alcohol exposure, fetal alcohol spectrum disorder, FASD, neuroinflammation, neurodegeneration, hippocampus, meta-analysis.

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Introduction

Prenatal alcohol exposure (PAE) in the form of maternal alcohol consumption during pregnancy is an important risk factor for the development of fetal alcohol spectrum disorder (FASD). FASD includes a variety of neurodevelopmental and physical impairments [1]. The teratogenic effects of alcohol disrupt normal fetal development, specifically affecting brain structure and function which leads to substantial problems in learning, memory, and emotional regulation [2]. Ethanol exposure activates glial cells (particularly microglia) and astrocytes resulting in an inflammatory response characterized by the release of proinflammatory cytokines such as IL-1 β and TNF- α . Neuroinflammatory changes disrupt normal neurodevelopment, induce aberrant neuronal differentiation, lead to excessive apoptosis, and affect synaptic connectivity, particularly in areas such as the neocortex and hippocampus [3]. Dysregulated microglia not only contribute to

neuroinflammation but also exacerbate neuronal damage leading to long-term cognitive impairment and behavioral problems characteristic of FASD [4]. The hippocampus, a critical component of the limbic system located in the medial temporal lobe of the brain, consists of two major interconnected structures, including the Ammon's horn and the dentate gyrus [5].

Systematic reviews and meta-analyses are evidence-based solutions in clinical practice. Numerous studies were published on the role of FASD in the hippocampus. Since no overall effects have been found to date, the present systematic review and meta-analysis were conducted to evaluate the pathological role of FASD in hippocampal neuroinflammation (via assessing the IL-1 β , IL-6, and TNF- α cytokines) and neurogenesis (by assessing the Ki67, DCX, and BrdU markers).

Methods

Ethical considerations and research registration

All protocols for this study were approved by the Tabriz University of Medical Sciences, Iran (research ID: 74923), and the Tabriz University Ethics Committee (ethical approval number: IR.TBZMED.VCR.REC.1403.172).

Study objective

Regarding the research question, this systematic review and meta-analysis were designed to examine the neuroinflammatory (by assessing IL-1 β , IL-6, and TNF- α cytokines) and neurogenetic (by assessing Ki67, DCX, and BrdU markers) effects of PAE during pregnancy on the fetal hippocampus.

MeSH keywords and search strategy

To select relevant publications for our systematic review and meta-analysis, we used the following MeSH-based keywords;

'prenatal', 'alcohol', 'exposure', 'neonate', 'fetal alcohol spectrum disorder', 'FASD', 'maternal alcohol exposure', 'neuroinflammation', 'neurodegeneration', 'hippocampus', 'TNF- α ', 'IL-1 β ', 'IL-6', 'Ki67', 'DCX', 'BrdU', 'rodents', 'mice', and 'rat'. The search strategy included various English-language databases: WoS, PubMed, Scopus, and ScienceDirect. Finally, the Google Scholar search engine and citations of all revealed articles were manually screened to identify the maximum number of eligible studies. No publication time limit was applied, and all relevant articles published before June 12 of 2025 were included. The search strategy was as follows: ('prenatal') AND ('neonate') AND ('postnatal') AND ('alcohol') AND ('maternal alcohol exposure') AND ('Fetal alcohol spectrum disorder' OR 'FASD') AND ('neurodegeneration') AND ('neurogenesis') AND ('hippocampus') AND ('TNF- α ' OR 'IL-1 β ' OR 'IL-6') AND ('Ki67' OR 'DCH' OR 'BrdU') AND ('rodents' OR 'mice' OR 'rat').

Table 1. Static characteristics presenting the extracted data of the included articles

First author	Title	Year	Main findings	Sample characterization	Study groups	Adjuvant therapy	ARRIVE score (%)
Baker JA [9]	Choline supplementation alters hippocampal cytokine levels in adolescence and adulthood in an animal model of FASD	2023	Choline supplementation modulates hippocampal cytokines (IL-1 β , IL-6, TNF- α) in adolescent and adult rats with FASD	Adolescent and adult rats	Control, FASD + choline supplementation, FASD only	Choline supplementation	80
Baker JA [10]	Choline supplementation modifies the effects of developmental alcohol exposure on immune responses in adult rats	2022	Alcohol consumption during development alters immune markers; choline supplementation modifies the immune response and inflammation	Adult rats	Control, Alcohol-exposed, Alcohol + choline	Choline supplementation	75
Doremus-Fitzwater TL [11]	Lingering effects of prenatal alcohol exposure on basal and ethanol-evoked expression of inflammatory-related genes in the CNS of adolescent and adult rats	2020	Prenatal alcohol exposure increased the abundance of proinflammatory genes in the CNS during adolescence and adulthood; persistent inflammation was noted	Adolescent and adult rats	Prenatal alcohol exposed vs. control	None	75
Xu W [12]	Early ethanol exposure inhibits the differentiation of hippocampal dentate gyrus granule cells in a mouse model of FASD	2020	Ethanol decreased hippocampal granule cell differentiation; markers of neurogenesis decreased, but inflammation analysis was limited	Baby mice	Control, prenatal ethanol exposure	None	78
Gil-Mohapel J [13]	Prenatal ethanol exposure differentially affects hippocampal neurogenesis in the adolescent and aged brain	2014	Prenatal ethanol exposure decreased neurogenesis markers in adolescent and aged rats; neuroinflammation was assessed.	Adolescent and old rats	Control, prenatal ethanol exposure	None	75
Hamilton GF [14]	Housing in environmental complexity following wheel running augments survival of newly generated hippocampal neurons in a rat model of binge alcohol exposure during the third trimester equivalent	2012	Environmental enrichment improved neurogenesis after alcohol exposure; inflammation was reduced by exercise	Newborn rats	Control, alcohol-exposed, alcohol + exercise, alcohol + enriched environment	Exercise	80
Boehme F [15]	Voluntary exercise induces adult hippocampal neurogenesis and BDNF expression in a rodent model of FASD	2011	Exercise intensified neurogenesis and BDNF expression; they compensated for some of the alcohol-induced deficits, but there was no direct evidence of inflammation	Adult rodents	Control, ethanol-exposed, ethanol + exercise	Exercise	75
Gil-Mohapel J [16]	Altered adult hippocampal neuronal maturation in a rat model of fetal alcohol syndrome	2011	Ethanol exposure slowed neuronal maturation; inflammatory processes were demonstrated by an increase in the levels of cytokines and activation of NF- κ B	Adult rats	Control, prenatal ethanol exposure	None	78

FASD, fetal alcohol spectrum disorder; CNS, central nervous system; BDNF, brain-derived neurotrophic factor.

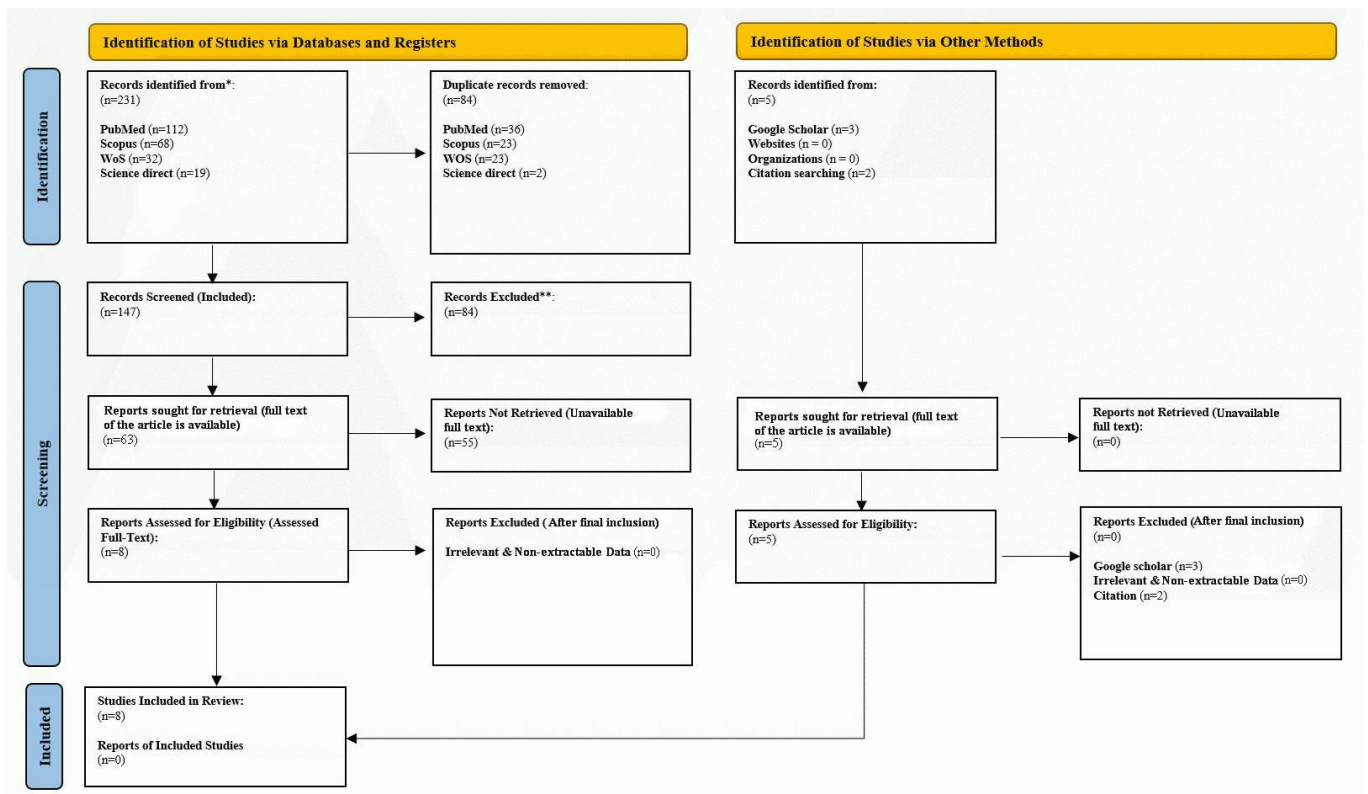


Figure 1. PRISMA 2020 flow diagram regarding the inclusion of relevant studies based on the inclusion and exclusion criteria.

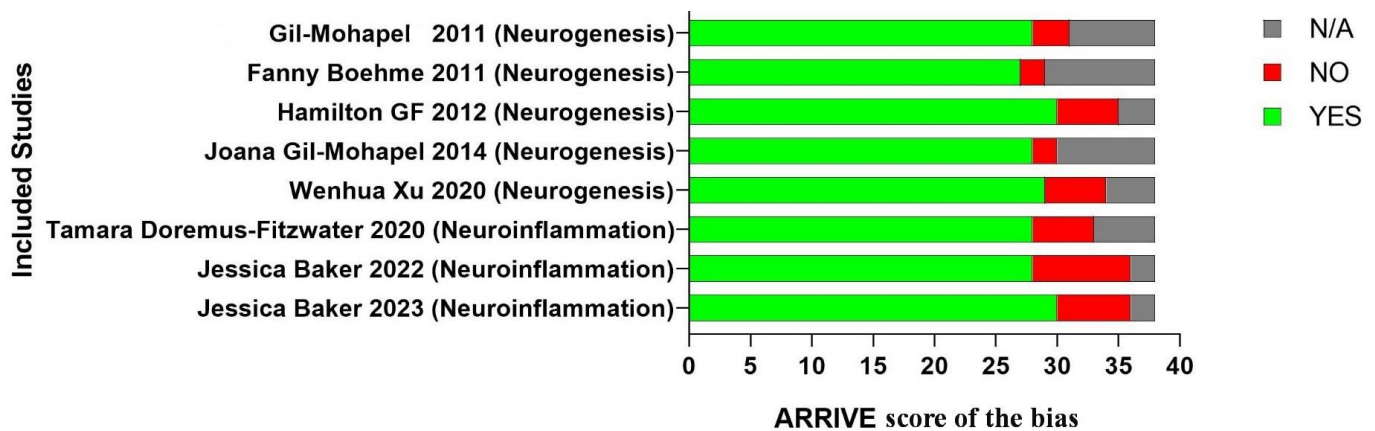


Figure 2. Article quality reports reflecting risk of bias according to the ARRIVE checklist. YES: items mentioned in the text according to the ARRIVE criteria; NO, items not mentioned in the text according to the ARRIVE criteria; N/A, not applicable items according to the ARRIVE criteria; ARRIVE, Animal Studies: Reporting of In Vivo Experiments.

Primary and secondary screening

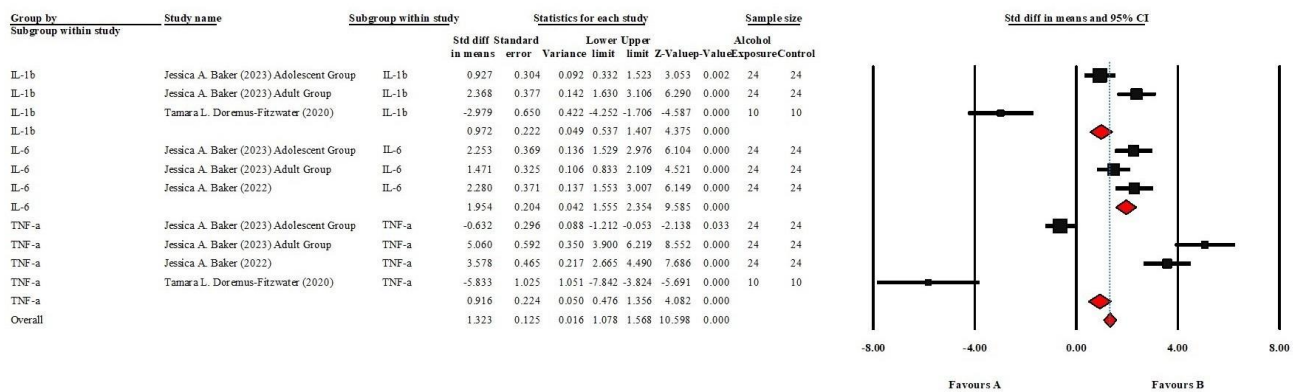
After collecting articles, all associated citations were imported into citation management software (EndNote x8, USA), duplicate articles were identified and merged. The primary screening of articles was based on the title and abstract. Then, the full texts of the included studies were subjected to a secondary assessment. In vivo experimental studies on rodents reporting neuroinflammatory cytokines (TNF- α , IL-1 β , and IL-6) and neurogenesis markers (Ki67, CDX, and BrdU) in the fetal hippocampus following prenatal maternal exposure were included in all stages of the primary and secondary screening. In addition, other irrelevant papers

(publications in languages other than English), papers with non-extractable data, reviews, letters to the editor, and non-animal studies) were excluded from our study. The entire article selection process was performed by independent authors (VSHA, HAS, and SHM) in a blinded manner. The corresponding authors (HHP and FKHH) resolved potential disagreements. The article screening process was conducted based on the PRISMA 2020 flowchart (Figure 1) [6]. The *Animal Research: Reporting of In Vivo Experiments* (ARRIVE) guidelines, which is a comprehensive guide to improving experimental design and reporting standard in animal research (developed by the National Centre for the Replacement, Refinement, and Reduction of Animals in Research),

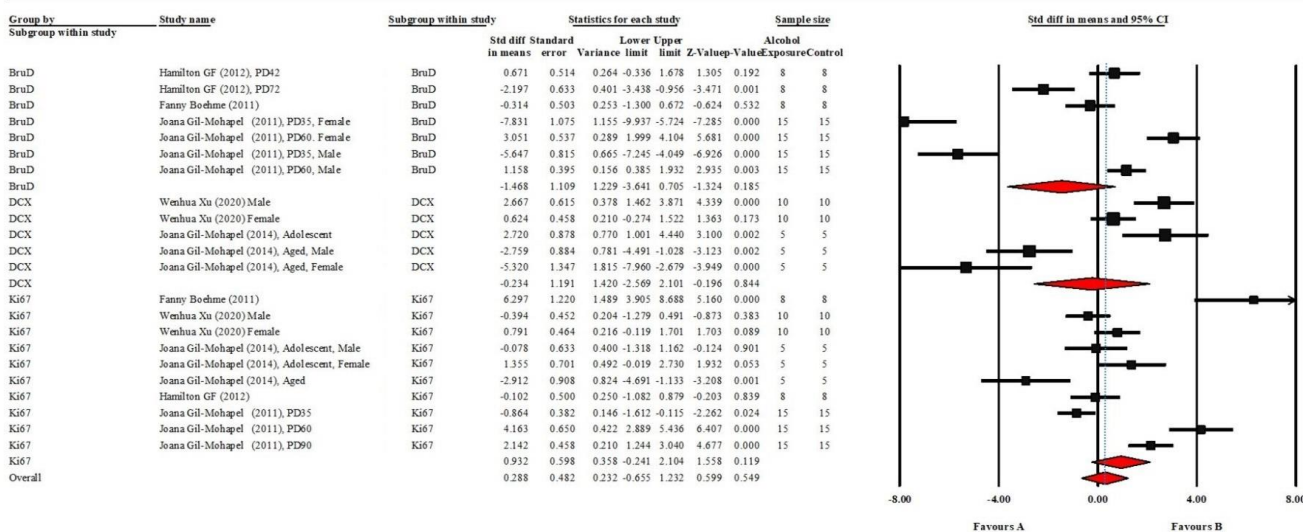
were used to assess the quality of reviewed studies (Figure 2) [7]. The ARRIVE guidelines contain 20 different items assessing the quality of articles for possible bias, including: Title, Abstract, Background, Objective, Ethics, Study design, Experimental procedure, Experimental animal, Animal care, Sample size, Animal allocation, Experimental results, Statistical methods, Baseline data, Data analyzed, Results and evaluation, Adverse events, Interpretation/Scientific implications, Generalizability/Translational nature of the data, and Funding. An ARRIVE score of <25, 25-50, 50-75, and >75 pts was interpreted as poor, low-quality, moderate-quality, and high-quality studies, respectively. In this systematic review, moderate-quality and high-quality studies were included for data extraction. (ARRIVE score ≥50).

Data collection and meta-analysis

Relevant data collected from eligible articles are summarized in Table 1, including the first author's name, article title, article publication year, main results, and all methods relevant to the study. After data extraction, the results were divided into two main subgroups: neuroinflammatory cytokines (IL-1β, IL-6, and TNF-α) and neurogenesis markers (Ki67+, DCX+, and BrdU+ cells). Subgroup analysis was used to assess heterogeneity, and the I² index was reported. Publication bias was assessed, and a corresponding funnel plot was constructed. Finally, the leave-one-out meta-analysis was employed to report the sensitivity. Statistical significance was assumed at p<0.05, and a 95% confidence interval (95% CI) was determined using the Cochran's sample size formula. All statistical analyses were performed using comprehensive meta-analysis software (CMA version 2) [8].



Meta Analysis



Meta Analysis

Figure 3. Forest plot displaying mean differences and 95% CI for included studies that met the inclusion criteria for markers of neuroinflammation (A) and neurogenesis (B) in animals with FASD (random effects model). CI, confidence interval; FASD, fetal alcohol spectrum disorder.

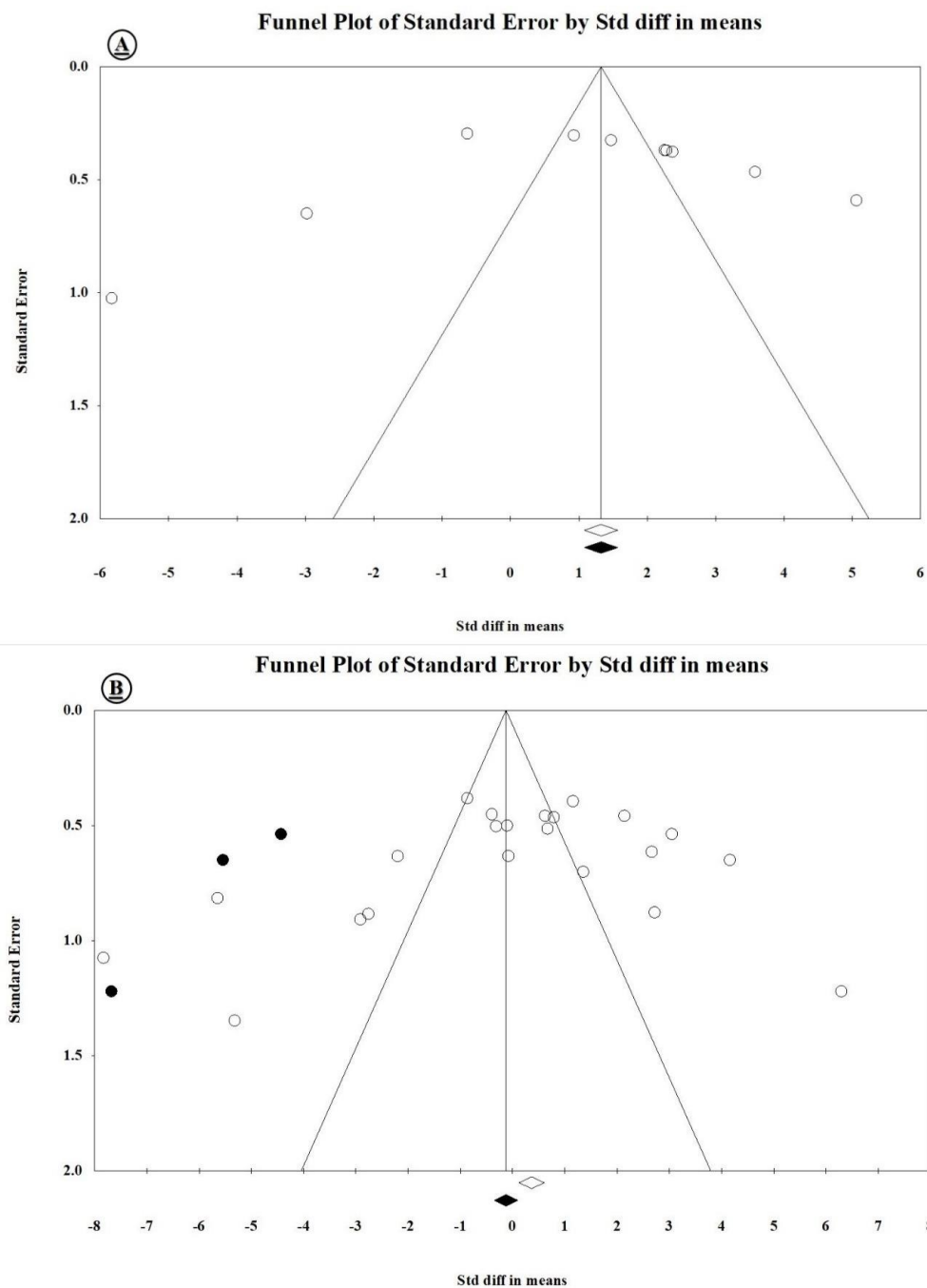


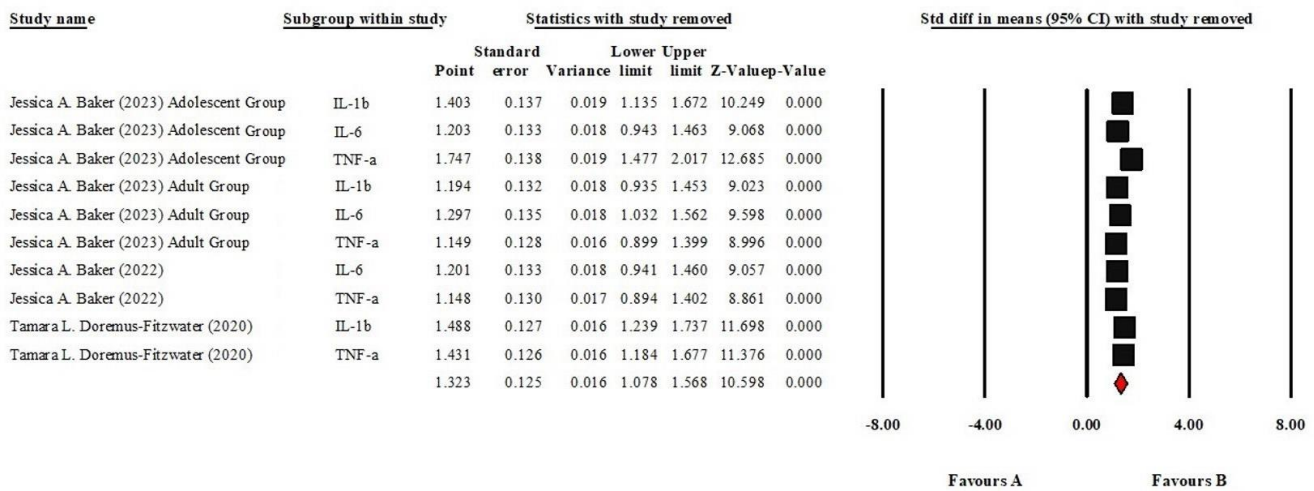
Figure 4. Funnel plot depicting publication bias for markers of neuroinflammation (A) and neurogenesis (B) in animals with FASD (random effects model). FASD, fetal alcohol syndrome.

Results

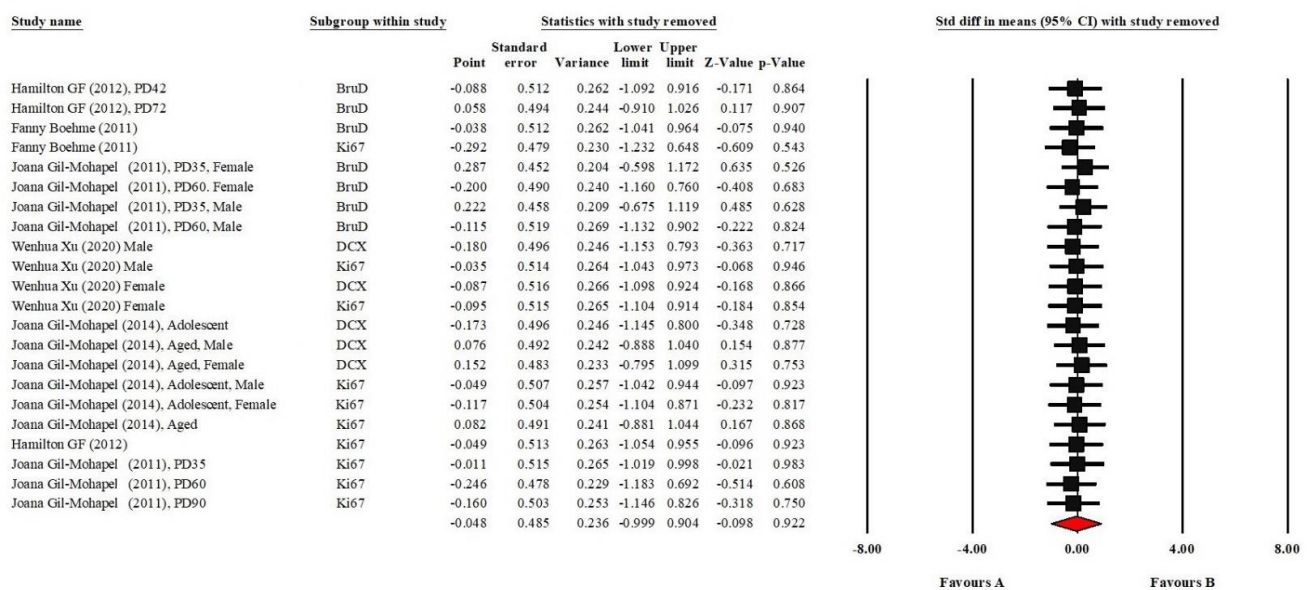
Selecting eligible articles and considering PRISMA guidelines

The primary search of the following databases retrieved 231 studies: PubMed (n=112), Scopus (n=68), WoS (n=32), and ScienceDirect (n=19). Besides that, 5 studies (n=5) were found after additional search in Google Scholar (n=3) and manual reference search (n=2), but were later excluded due to non-meeting the eligibility criteria. Thus, 231 studies were imported into EndNote software, and 84 duplicate studies were found and merged together (n=36, 23, 23, and 2 for PubMed, Scopus, WoS,

and ScienceDirect databases, respectively). Among the 147 remaining articles, 84 irrelevant studies were also excluded during the primary screening. In the secondary screening, 63 articles were excluded due to the impossibility of data extraction. Ultimately, 8 eligible studies were identified for data analysis (Figure 1), including Jessica Baker et al. (2023) [9], Jessica Baker et al. (2022) [10], Tamara Doremus-Fitzwater et al. (2020) [11], Wenhua Xu et al. (2020) [12], Joana Gil-Mohapel et al. (2014) [13], Hamilton GF et al. (2012) [14], Fanny Boehme et al. (2011) [15], and Joana Gil-Mohapel et al. (2011) [16] (Table 1). All included articles were rated as high quality (ARRIVE score ≥ 75 pts.) (Figure 2).



Meta Analysis



Meta Analysis

Figure 5. Sensitivity assessment using the leave-one-out random-effects model for neuroinflammation (A) and neurogenesis (B) in animals with FASD. FASD, fetal alcohol spectrum disorder.

Meta-analysis

According to Figure 3A, the total effect of FASD on hippocampal neuroinflammation was 1.323±0.125 (95% CI =1.078–1.568, p=0.000), indicating a significant neuroinflammatory effect of FASD on the hippocampus in the early postnatal period. During this period, subgroup analysis showed that the levels of all proinflammatory cytokines were statistically significantly elevated (p<0.05) in the hippocampus after the onset of FASD, including IL-1β (0.972±0.222, 95% CI =0.537~1.407, p=0.000), IL-6 (1.954±0.204, 95% CI =1.555~2.354, p=0.000), and TNF-α

(0.916±0.224, 95% CI =0.476~1.356, p=0.000). The heterogeneity assessment of inflammatory cytokines yielded I²=96.06% for IL-1β (Cochran's Q=50.77, p=0.000), I²=44.94% for IL-6 (Q=3.63, p=0.16) and I²=98.03% for TNF-α (Q=152.51, p=0.000). The overall heterogeneity index for inflammatory cytokines was I²=95.95% (Q=222.28, p=0.000). According to the two-tailed asymmetry test, the p-values were 0.69 and 0.17 for the intercept of the Egger's regression test and Begg & Mazumdar rank correlation test, respectively. Thus, no significant publication bias was detected (p>0.05) (Figure 4A). According to the classical fail-safe N test, the number of missing studies (α=0.05) was 224. The sensitivity index

was also estimated using the leave-one-out meta-analysis with the corresponding p-value of 0.000 (Figure 5A), indicating a high sensitivity index among the eligible article collections. According to Figure 3B, showing the total effect of FASD on hippocampal neurogenesis, this value was 0.288 ± 0.482 (95% CI = $-0.655 \sim 1.232$, $p = 0.549$), indicating that the effect of FASD on hippocampal neurogenesis in the late postnatal period is not statistically significant. In this regard, subgroup analysis showed that all neurogenesis markers demonstrated statistically insignificant changes after the onset of FASD, including BrdU⁺ cells (-1.468 ± 1.109 , 95% CI = $-3.641 \sim 0.705$, $p = 0.185$), DCX⁺ cells (-0.234 ± 1.191 , 95% CI = $-2.569 \sim 2.101$, $p = 0.844$), and Ki67⁺ cells

(0.932 ± 0.598 , 95% CI = $-0.241 \sim 2.104$, $p = 0.119$). The heterogeneity assessment of neurogenesis markers showed $I^2 = 96.15\%$ ($Q = 155.97$, $p = 0.000$), $I^2 = 92.13\%$ ($Q = 50.85$, $p = 0.000$) and $I^2 = 91.18\%$ ($Q = 102.07$, $p = 0.000$) for BrdU⁺, CDX⁺ and Ki67⁺ cells, respectively. The overall heterogeneity index for neurogenesis, $I^2 = 93.33\%$ ($Q = 315.23$, $p = 0.000$). According to the two-tailed asymmetry test, p-values were 0.23 and 0.35 for the intercept of the Egger's regression test and Begg & Mazumdar rank correlation test, respectively. Thus, no statistically significant publication bias was detected ($p > 0.05$) (Figure 4B). The sensitivity index was also assessed by leave-one-out meta-analysis yielding $p = 0.922$ (Figure 5B), which implied no sensitivity among eligible paper collections.

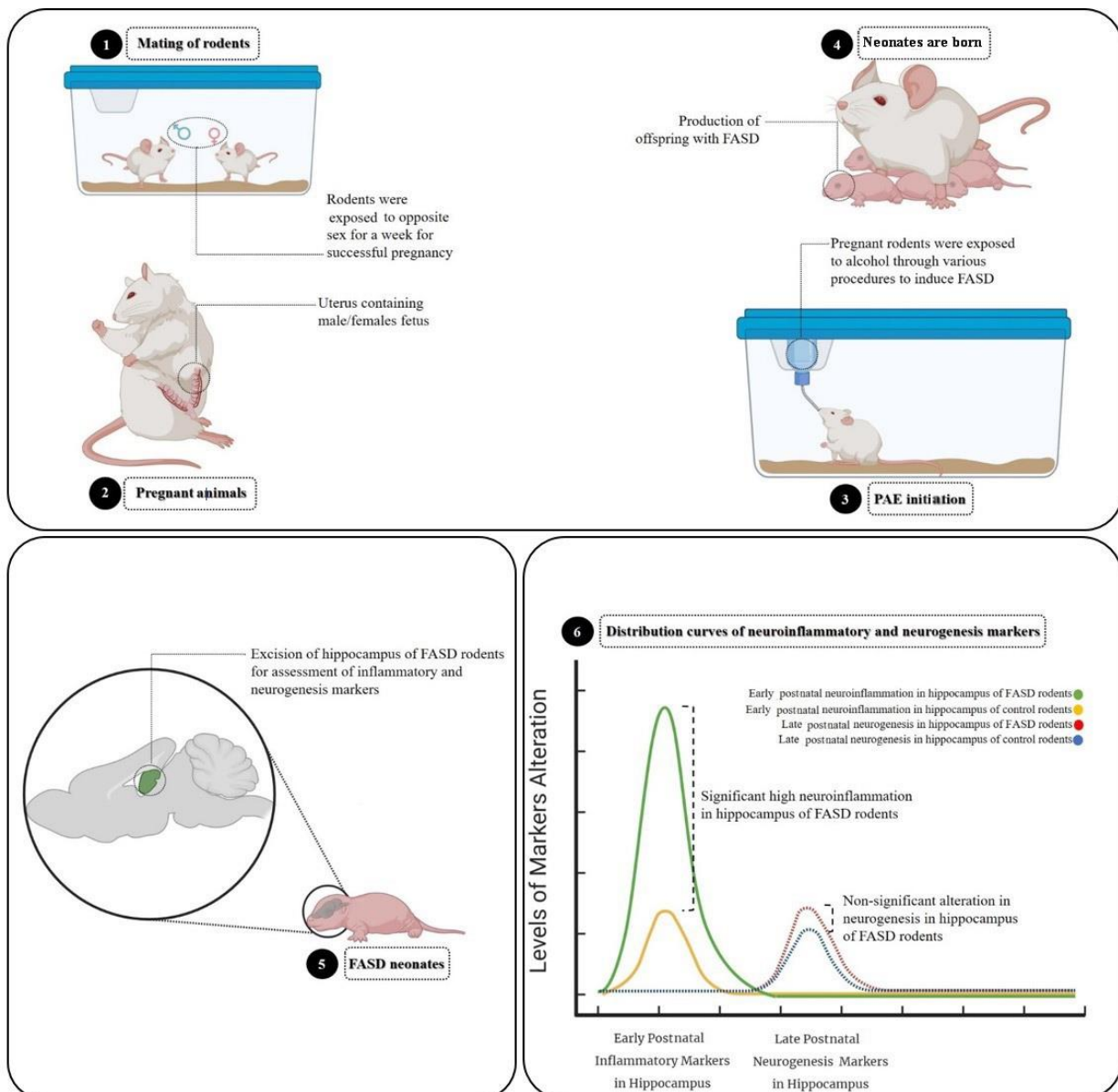


Figure 6. The development of FASD and associated neuroinflammation in the hippocampus is modeled here using a visual framework. 1) Rodents were mated to achieve successful pregnancy. 2) Pregnancy was confirmed for subsequent initiation of prenatal alcohol exposure (PAE). 3) Pregnant rodents were exposed to alcohol in various procedures inducing FASD in the fetus. 4) After birth, newborn rodents were whole-mounted for hippocampal excision and molecular analysis. 5) The hippocampus was dissected for molecular analysis including early postnatal neuroinflammation and late postnatal neurogenesis. 6) Changes in biomarkers of neuroinflammation and neurogenesis in the hippocampus were assessed based on published articles at two stages: early postnatal neuroinflammation and late postnatal neurogenesis.

Discussion

PAE can cause serious detrimental effects on the fetus, particularly on the nervous system. Furthermore, the hippocampus is a critical brain structure, and there are several reports of hippocampal damage associated with alcohol consumption [17]. This meta-analysis was designed to examine the effects of PAE on the induction of hippocampal inflammation and neurodegeneration. The findings suggest that maternal alcohol exposure can induce neuroinflammation in the fetal hippocampus by accelerating the production of proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α . Furthermore, although markers of neurogenesis (including DCX⁺, Ki67⁺, and BrdU⁺) exhibited reduced levels in the hippocampus, no statistically significant changes were observed in the late postnatal period following alcohol exposure. In this regard, the overall effects of FASD on neuroinflammation and neurogenesis in the hippocampus were 1.323 \pm 0.125 (95% CI=1.078–1.568, p =0.000) and 0.288 \pm 0.482 (95% CI=-0.655-1.232, p =0.549), respectively.

The hippocampus is an important structure in the medial temporal lobe of the brain, primarily known for its role in memory formation and spatial navigation [18]. Neuroinflammation plays a significant role during embryogenesis, particularly in the development of the central nervous system (CNS). Microglia, the brain's resident immune cells, play a crucial role in this process. They originate from yolk sac progenitors, migrate to the developing brain, and differentiate into various brain regions [19]. During embryogenesis, microglia are actively involved in the regulation of neurogenesis and gliogenesis through cytokine signaling and interactions with neural progenitor cells. For instance, microglia can influence the proliferation and differentiation of neural progenitor cells by secreting proinflammatory cytokines such as CCL2 and CXCL10, which can promote neural differentiation while inhibiting astrocyte differentiation [20]. Moreover, neuroinflammation can also be induced by maternal environmental factors such as infections or immune activation. These factors can lead to changes in microglial function and phenotype, potentially leading to abnormal brain development [21]. For example, maternal inflammation is associated with increased IL-6 levels in fetal microglia, which may disrupt normal neurodevelopmental processes [22]. Dysregulation of microglial activity during embryogenesis is linked to various neurodevelopmental disorders, such as fetal alcohol syndrome [23]. Furthermore, although microglia are essential for normal CNS development, excessive or prolonged neuroinflammation can have detrimental consequences. For example, microglial hyperactivation can lead to phagocytosis of neural progenitors or disruption of synaptic organization, ultimately impairing cognitive function [24]. Thus, the balance between beneficial and detrimental neuroinflammatory responses during embryogenesis is crucial for elucidating the mechanisms underlying normal brain development and the etiology of neurodevelopmental disorders (Figure 6).

PAE, particularly during critical periods of fetal development [25], can significantly influence neuroinflammation in the hippocampus through modulation of proinflammatory cytokines such as IL-1 β , IL-6, and TNF- α . These cytokines are key mediators of neuroinflammatory responses and play a crucial role in various molecular pathways that influence brain development and function [26]. During embryogenesis and early postnatal development, ethanol exposure can activate microglia, the main

immune cells in the brain. For instance, some studies confirmed that maternal alcohol exposure increases IL-1 β and TNF- α levels in the hippocampus, thereby contributing to a neuroinflammatory environment that disrupts normal neurodevelopmental processes [27].

Cytokines IL-1 β , IL-6, and TNF- α are integral components of several neuroinflammatory pathways [28], primarily associated with the activation of immune responses in the CNS [29]. These cytokines are known to interact within the NF- κ B signaling pathway [30], which is a key regulator of inflammatory responses [31]. When activated by proinflammatory stimuli such as IL-1 β and TNF- α , the NF- κ B pathway promotes the transcription of various inflammatory genes, including those encoding IL-6. This pathway is critical in mediating the effects of these cytokines on neuronal survival and inflammation. For example, TNF- α can activate NF- κ B through its receptor, resulting in the expression of genes that promote inflammation and cell survival mechanisms in response to stressors [32]. The NF- κ B pathway appears to play a crucial role in mediating neuroinflammation following alcohol exposure. Alcohol consumption activates NF- κ B, particularly the p65 subunit [33], which translocates to the nucleus and binds to DNA to transcribe proinflammatory cytokines such as TNF- α and IL-1 β [34]. Moreover, chronic alcohol consumption appears to enhance the NF- κ B-mediated inflammatory response through various mechanisms [35]. Chronic NF- κ B activation in response to alcohol not only contributes to neuronal damage but also maintains an inflammatory state in the brain leading to long-term neuroimmune dysfunction associated with alcohol use disorders [36].

Regarding the role of significant neuroinflammation and insignificant neurogenesis in the fetal hippocampus following PAE, several critical hypotheses exist. Neuroinflammation is activated rapidly and early in response to injury, whereas neurogenesis is a late process requiring stable environmental conditions [37]. In studies of FASD, neuroinflammation is typically observed in the early postnatal period, as evidenced by the data. In contrast, neurogenesis was assessed in the late postnatal period, when the cellular environment is no longer conducive to the proliferation and differentiation of neural stem cells due to chronic inflammation and oxidative stress [38]. Furthermore, the present study showed that proinflammatory cytokines (including IL-1 β , IL-6, and TNF- α) were significantly increased, which could potentially activate the NF- κ B pathway (stimulating inflammatory gene expression) [39], microglia activation (ROS and nitric oxide release leading to oxidative stress) [40], and astrocyte reactivity (disruption of glutamate homeostasis and impaired metabolic support of neurons) [41]. These mechanisms are rapidly activated in response to ethanol toxicity resulting in an increase in inflammatory markers, as would be expected. Also, unlike inflammation, which is an immediate reaction, neurogenesis requires several supporting factors, including the presence of neurotrophic factors such as BDNF and FGF-2, reduction of oxidative stress and peripheral inflammation, as well as the integrity of the extracellular matrix and a healthy neuronal microenvironment [42]. Thus, although inflammation (as an immune response) occurs early, it also creates a toxic and unfavorable environment for neurogenesis. Microglial dynamics and their inhibitory role are another important cellular change. In FASD, microglia transition from a ramified (resting) state to an amoeboid (activated) state. These cells secrete IL-1 β and TNF- α and inhibit the differentiation of neural progenitor cells into mature neurons. Consequently, even if progenitor cells are

present, they lose the ability to differentiate and survive in an inflammatory environment [43]. Overall, neuroinflammation is an early, rapid, and visible response to FASD-induced damage, which is fully manifested in the early postnatal stages with an increase in inflammatory cytokines. However, neurogenesis is a process dependent on a healthy neural environment and is suppressed in the presence of inflammation, oxidative stress, and ethanol-induced metabolic changes; therefore, it does not significantly affect the statistical data.

Similar to the effects of many environmental toxins, such as exogenous melatonin, on the body, neuroinflammatory responses induced by FASD are well-documented features of this disorder [44]. However, increasing evidence suggests that the degree of neuroinflammation does not always directly correlate with the severity of neurological or functional consequences observed later in development. This phenomenon, referred to as the functional disconnect between neuroimmune activation and neurological consequences, can be explained by several biological, temporal, and spatial mechanisms [45]. First, there is often a time gap between the onset of inflammation and the manifestation of neurological impairment. The inflammatory response typically occurs in the acute phase following ethanol exposure, whereas neurological consequences (such as memory impairment, reduced hippocampal volume, or learning disability) emerge later in development. Consequently, by the time these consequences are assessed, the inflammation may have already subsided, although its molecular and structural effects on neural tissue persist. Thus, inflammation may act as an early initiator rather than a stable correlate of neuronal dysfunction [46]. Furthermore, the spatial heterogeneity of inflammatory responses contributes to this apparent discrepancy. In FASD, the intensity and distribution of neuroinflammation varies across brain regions; inflammation is often prominent in the hippocampus and prefrontal cortex, whereas structural and neurodegenerative changes may occur in other regions, such as the cerebellum or corpus callosum. Consequently, measuring inflammatory markers in one region (e.g., the hippocampus) may not spatially correspond to the site of functional impairment, resulting in a false lack of statistical correlation [47]. Another important factor is represented by neuroplasticity and compensatory mechanisms inherent to the developing brain. Even under inflammatory conditions, neuronal and glial cells can partially restore function through increased expression of neurotrophic factors such as BDNF, IGF-1, and VEGF, as well as through synaptic remodeling. These adaptive responses may temporarily mask the detrimental effects of inflammation on neuronal activity. However, in the long term, such compensatory adjustments may alter network organization and increase vulnerability to future cognitive decline. Moreover, it should be noted that neuroimmune responses exhibit nonlinear and adaptive dynamics. Low-grade or transient inflammation may play a beneficial role in debris removal and tissue repair, whereas persistent or chronic inflammation is typically neurotoxic [48]. Therefore, in some experimental settings, the observed inflammatory activity may represent a reactive, but not destructive, process, insufficient to cause measurable neurological impairment.

The heterogeneity of results related to neurogenesis after FASD exposure may be explained by a combination of biological and methodological factors influencing the results. First, differences in sampling timing across studies play a critical role, as each marker of neurogenesis represents different stages of

neuronal proliferation and differentiation; hence, their expression levels can vary significantly depending on the interval after alcohol exposure. Furthermore, differences in the nature and intensity of ethanol exposure (acute vs. chronic) have differential effects on the survival and maturation of neural stem cells, thereby contributing to the discrepancy in results. Also, regional differences in the brain explain some of this variability, as the neurogenic response to FASD is not uniform across brain regions such as the hippocampus, lateral ventricles, and cerebral cortex. Moreover, the degree of neuroinflammatory activation varies across studies; fluctuations in cytokine levels such as IL-1 β , TNF- α , and IL-6 may differentially suppress key regulatory pathways of neurogenesis, including the CREB/BDNF and Wnt/ β -catenin signaling cascades. Finally, methodological inconsistencies, including the use of different antibodies, cell counting protocols, and approaches to normalization relative to tissue volume, may further exacerbate statistical variability. Taken together, these temporal, spatial, molecular, and technical factors interact synergistically to create the high degree of heterogeneity reported in neurogenesis rates after FASD [49].

Key points

This study demonstrated that maternal alcohol exposure causes pathological consequences on the fetal central nervous system, leading to FASD. The latter induces neuroinflammation in the hippocampus through the proinflammatory secretion of cytokines such as IL-1 β , IL-6, and TNF- α . Furthermore, FASD slightly reduces neurogenesis in hippocampal neuronal cells (as confirmed by the assessment of neurogenesis markers, Ki67⁺, BrdU⁺, and DCX⁺).

Limitations and future prospects

After reviewing numerous publications on the role of PAE on the CNS, we concluded that additional experimental studies are needed to identify the actual molecular mechanism of alcohol-induced neuroinflammation. Furthermore, we propose to evaluate some important aspects of hippocampal neuroinflammation, such as memory. Many studies were omitted due to the language of publication other than English. Since markers of neurogenesis in the fetal hippocampus were only slightly reduced after PAE exposure, experimental studies are recommended to assess the precise role of alcohol in neurogenesis.

Conclusion

PAE induces FASD neuropathology with significant effects on the hippocampus. Although the results of the present study demonstrated that PAE triggers early postnatal neuroinflammation in the hippocampus through excessive secretion of IL-1 β , IL-6, and TNF- α , no statistically significant inhibition of hippocampal neurogenesis was observed in patients with FASD during the late postnatal period.

Abbreviations

PAE, prenatal alcohol exposure; FASD, random-effects; ARRIVE, Animal Research: Reporting of In Vivo Experiments; CI, confidence interval.

Author contributions

VSHA: review and editing of text, initial draft, methodology, investigation, software, funding acquisition, formal analysis, project

administration, data curation. HAS: validation, software, methodology, conceptualization, data curation. SHM: methodology, data curation, validation. HH: review and editing of text, initial draft, visualization, validation, scientific supervision, project administration, methodology, conceptualization. FKHH: methodology, conceptualization, data curation, validation, review and editing of text, initial draft.

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Conflict of interest

The authors declare no conflicts of interest.

Availability of data and materials

The datasets used and analyzed in this publication are available from the corresponding author upon reasonable request.

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