

ORIGINAL ARTICLE

WILEY

Adverse health outcomes associated with fetal alcohol exposure: A systematic review focused on immune-related outcomes

Natasha Reid¹   | Karen M. Moritz^{1,2}  | Lisa K. Akison^{1,2} 

¹Child Health Research Centre, The University of Queensland, South Brisbane, Queensland, Australia

²School of Biomedical Sciences, The University of Queensland, St Lucia, Queensland, Australia

Correspondence

Natasha Reid, Child Health Research Centre, The University of Queensland, Room 408, 62 Graham St, South Brisbane, Qld 4101, Australia.
Email: n.reid1@uq.edu.au

Funding information

National Health and Medical Research Council, Grant/Award Number: APP1078164; Aboriginal and Torres Strait Islander Health Branch; Department of Health, Queensland

Edited by: Jon Genuneit

Abstract

Prenatal alcohol exposure (PAE) has well-known teratogenic effects on the developing fetus, potentially resulting in neurologic impairments. However, there is increasing interest regarding other potential adverse health outcomes related to prenatal alcohol exposure. The objective of this study was to undertake a systematic review to identify all the available clinical and preclinical literature investigating immune-related outcomes in offspring with PAE. A systematic review searching four electronic databases (PubMed, CINAHL, Web of Science, and Embase) was conducted. Potential articles were screened against strict inclusion/exclusion criteria. This review specifically focused on evaluating studies related to immune-related outcomes following PAE. Twelve clinical studies were included in the current review. Six included allergy outcomes, four included infection outcomes, and two studies included both. Thirty-nine preclinical studies were identified examining a wide range of immune outcomes. The current review provides some preliminary clinical evidence that PAE can influence immune function, including atopic allergy and infection outcomes. However, there was variability in results across studies, particularly in the atopy area. Preclinical studies demonstrated some changes in lymphocytes and cytokines in offspring following PAE. More research investigating the effects of PAE on immune responses is warranted.

KEYWORDS

allergy, fetal alcohol spectrum disorder, infection, prenatal alcohol exposure

1 | INTRODUCTION

Exposure to alcohol in utero can lead to a wide range of negative outcomes, including miscarriage, prematurity, growth restriction, and neurobehavioral impairments.¹ Some individuals who experience prenatal alcohol exposure (PAE) will go on to receive a diagnosis of fetal alcohol spectrum disorder (FASD), a neurodevelopmental condition with lifelong implications.² While the most widely known consequences of PAE are neurobehavioral in nature, recent research is drawing attention to other potential

health impacts. Given the cumulative interest regarding the effects of PAE on other body systems, we conducted a systematic review of all the available literature. Due to the large number of studies identified, we have grouped studies according to domains of impairment to allow for in-depth analysis of study quality and outcomes. The current systematic review focuses on studies reporting impacts on the immune system. In the clinical studies, the focus is specifically on allergy and infection outcomes for individuals who have experienced PAE, including those who have a FASD diagnosis.

There is increasing incidence of allergies and immune-related complications in the population. For instance, atopic disease, including dermatitis, allergic rhinitis, and asthma, has increased in frequency and now affects approximately 20% of the population worldwide.³ The concept of the “atopic march” has been used to describe the disease progression from atopic dermatitis in infants to asthma in children.³ The development of these diseases is complex and influenced by both genetic and environmental factors. For example, exposure to allergens including certain foods and dust mites may trigger atopic dermatitis in children,⁴ but there is also increasing understanding that prenatal events, such as maternal nutrition or exposure to stress, may lead to increased immune disorder susceptibility for some children.⁵ Consequently, alcohol exposure during the prenatal period, a critical window of development, may play a role in the increasing incidence of these conditions. Alcohol use during pregnancy is common in many countries. For example, a recent Australian study found that many women (60.6%) reported consuming alcohol between conception and pregnancy recognition and patterns of binge and heavy drinking were more common than low-level drinking.⁶ Research has started to provide insights regarding how PAE may disrupt the developing immune system. For example, a previous clinical study documented a positive association between PAE and immunoglobulin E in cord blood.⁷ In animal studies, PAE increased pro-inflammatory cytokines in the serum and spleen of young rat pups.⁸ There have been a number of previous narrative reviews on this topic, including the review by Gauthier et al,⁹ who reviewed the effects of PAE on the developing lung and immune defenses, highlighting that there is currently a lack of clinical awareness of this topic. Gauthier¹⁰ also contributed a review on PAE and the developing immune system, which emphasized that PAE results in an increased risk for infections and disease in newborns, which may continue throughout life. This review highlighted that PAE may have direct teratogenic effects on the developing immune system, particularly during the second trimester. However, the review also summarized evidence that heavy to binge levels of PAE can increase the risk for chorioamnionitis and/or placental abruption, conditions known to increase the risk for premature delivery. Thus, given the link between prematurity and the increased risk of infections and infection-related illnesses, PAE can indirectly affect immune-related outcomes in neonates. Giliberti et al¹¹ contributed a review regarding implications of PAE for lung development and disease, which provided a summary of the toxic effects of PAE on the cellular development of the neonatal lung. To our knowledge, this is the first systematic review on the effects of PAE on the developing immune system.

2 | METHODS

2.1 | Search strategy

Articles were identified through a systematic search. The aim was to identify the full range of non-neurologic/behavioral impacts (ie, impacts to all other organs and body systems besides the brain) arising from PAE. Four bibliographic databases were searched (PubMed,

Key Message

The current review identified all the available clinical and preclinical literature investigating immune-related outcomes in offspring with prenatal alcohol exposure. The review provides preliminary evidence that PAE can influence immune function, including atopic allergy and infection outcomes. However, there was variability in findings across studies, particularly in the atopy area.

CINAHL, Web of Science, and Embase) from inception to December 2017. An updated search was conducted closer to submission (October 2018). Search terms were as follows: maternal OR prenatal OR neonatal OR fetal OR foetal OR pregnancy OR pregnant OR fetal programming AND alcohol OR ethanol OR fetal alcohol OR foetal alcohol AND renal OR kidney OR cardiac OR cardio OR heart OR metabolic OR diabetes OR obesity OR respiratory OR lung OR immune OR reproductive OR endocrine. This review conformed to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement¹² and was registered with the international prospective register of systematic reviews (PROSPERO; CRD42017082627).

2.2 | Inclusion and exclusion criteria

Screening of titles and abstracts was performed against the following criteria: (a) animal/in vivo study (any mammal) with fetal alcohol exposure or children diagnosed with FASD or PAE; (b) alcohol exposure occurred during pregnancy or during the third-trimester-equivalent period in the rat (up to postnatal day [PD] 9); (c) details of the alcohol exposure: for preclinical studies, alcohol concentration/dose and accurate timing of exposure must be provided (any route of administration); for clinical studies, children diagnosed with FASD using specified diagnostic criteria or suspected to have been exposed to moderate-to-heavy fetal alcohol exposure via a stated method of maternal alcohol consumption assessment; (d) a physical health impact of PAE was assessed in offspring (eg, renal, cardiac, metabolic, reproductive, immune); and (e) inclusion of an appropriate unexposed control group for comparison. Studies were excluded if they: (a) were not published in English; (b) only included assessments related to brain-based, neurologic or behavioral outcomes; (c) only included outcomes related to malformations, growth rates, or growth restriction; (d) only documented effects on embryos, fetuses, or neonates during the third-trimester-equivalent period in the rat; (e) only focused on maternal effects of alcohol exposure rather than offspring effects; or (f) were conference abstracts, PhD dissertations, or editorials.

2.3 | Study quality assessment

The methodological quality of the included clinical studies was assessed using the Downs and Black Checklist.¹³ The checklist

assesses quality of reporting, internal and external validity, and statistical power. The checklist was adapted by removing items relating to interventions, and item 27, instead of rating to an available range of study power, was rated as to whether the study performed a power calculation. Two reviewers (NR and LA) independently rated each of the included studies. Any disagreements were resolved by discussion between assessors. Initial review of the preclinical studies indicated most were published more than 20 years ago (28 of the 39 studies were published prior to 1999) and many methodologies used are no longer considered "gold standard."¹⁴ Thus, instead of undertaking a quality assessment as we have previously performed,^{15,16} studies were summarized with regard to the alcohol dosage, age of offspring studied, immune factor assessed, methodology used, and outcome (See Data S1).

2.4 | Data extraction and synthesis

Data were extracted from each study for the following pre-defined parameters: study type, participant details, assessment and definition of PAE, relevant outcome assessments, key results, and conclusions. Given the wide variability in the identified studies, meta-analysis was not possible and consequently a narrative synthesis method was used.

3 | RESULTS

3.1 | Search results and classification of included studies by health outcomes

The electronic database search located 3230 citations (after duplicates were removed), which following title and abstract screening resulted in 245 articles for the full-text review. Reasons for exclusion are provided in the study flow diagram (Figure 1). A total of 142 articles met the inclusion criteria. Given the large amount of studies identified, we decided to report detailed results separately. For further details on the search results, refer to Akison et al,¹⁵ Akison et al,¹⁶ or Reid et al (Revised, JSAD). The current review provides an overview of the 39 preclinical studies (Data S1) that were identified, but predominately focuses on the clinical immune studies, for which twelve studies were included.

3.2 | Quality of methodological reporting in the clinical studies

See Figure 2 and Data S2 for a summary of the quality assessment. The quality of the included studies was variable. Two older case-control studies^{17,18} obtained the lowest scores, both with 8 out of a possible 19. In comparison, the highest score was obtained by Wada et al¹⁹ with 16 out of a possible 19. Items commonly insufficiently addressed

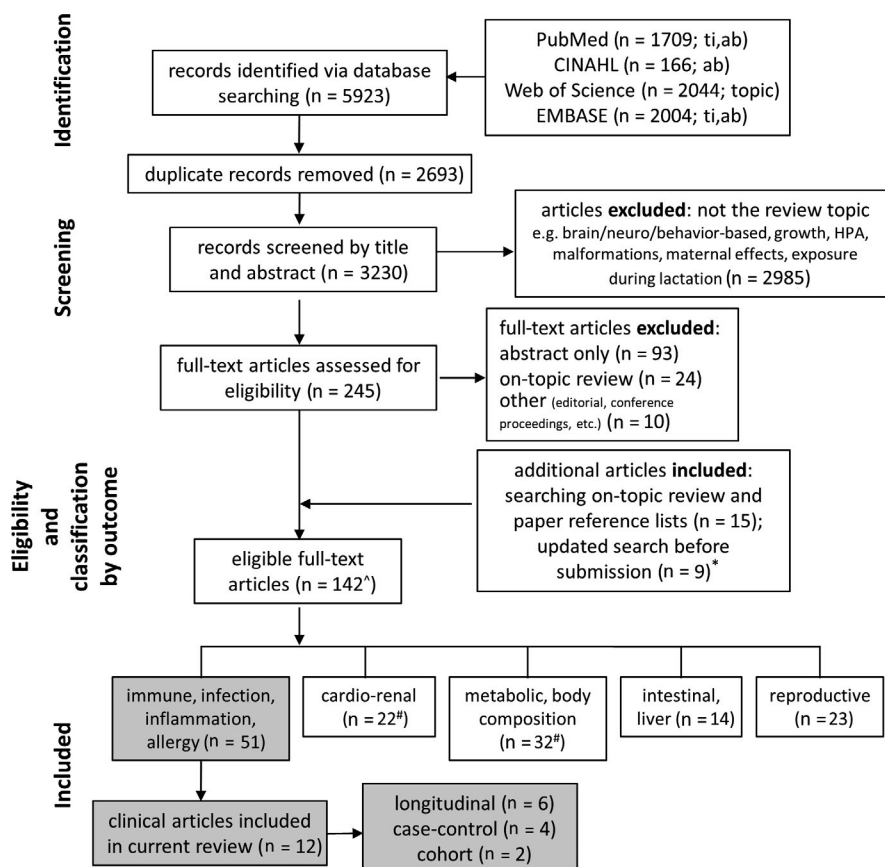


FIGURE 1 Flow diagram of the literature search strategy and study selection process based on the PRISMA statement.¹² Studies were grouped into specific health domains affected in offspring exposed to alcohol during development. Data extraction and reporting in the current review were specifically done only on clinical studies reporting on outcomes related to allergy, infection, inflammation, or immune function. *Search repeated October 2018. †Only one study reported on structural and functional deficits in the lung so is not classified. #One of the papers contains data on both metabolic and cardio-renal outcomes so is counted in both of these groups. ab, abstract; ti, title

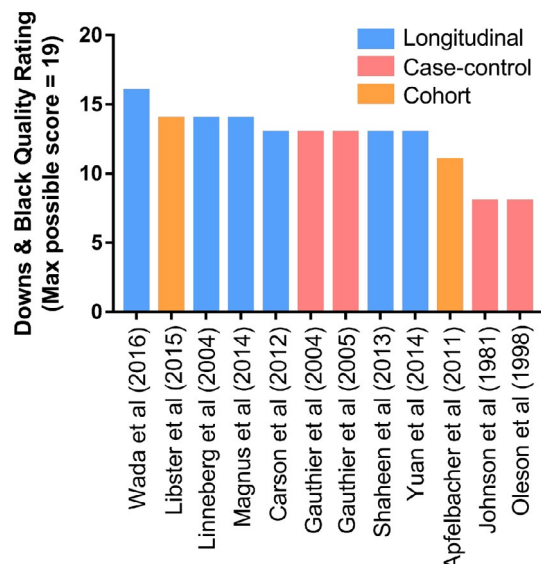


FIGURE 2 Quality assessment scores for all included studies based on the Downs and Black assessment criteria.¹³ Highest possible score is 19. See Data S2 for details regarding how the scores were calculated [Colour figure can be viewed at wileyonlinelibrary.com]

included the following: reporting on the representativeness of study participants (items 11 and 12), mentioning if an attempt was made to blind those measuring the main outcomes (item 15), and including a power calculation to assess sample size validity for detecting a significant difference between PAE and control group, if it occurs (item 27).

3.3 | Description of included clinical studies

Details of the included studies are summarized in Table 1. Six studies were longitudinal designs, of which three were from Denmark.^{20–22} Each of the Danish studies examined a different population of participants. One²¹ used data from the National Birth Cohort, where women were recruited through their general practitioners at their first antenatal appointment. Yuan et al²² recruited pregnant women who attended midwife centers and then followed up child outcomes through the National Hospital Discharge Registry. Most recently, Carson et al²⁰ analyzed data from the Copenhagen Study on Asthma in Childhood, which recruited children born to women with a history of asthma. The three remaining longitudinal studies were from Norway,²³ the United Kingdom (UK),²⁴ and Japan.¹⁹ The Norwegian study²³ assessed outcomes from the Mother and Child Cohort Study, which recruited pregnant women and followed up children until 7 years of age. The Japanese study¹⁹ recruited women during pregnancy from a maternal clinic and followed up until children were 5 years old. The UK study²⁴ examined outcomes from the Avon Longitudinal Study of Parents and Children, predominately Caucasian women from Bristol, UK.

Four of the included studies were case-control designs, all of which were from the United States (US). Two studies^{25,26} recruited infants born at Atlanta hospitals. One study²⁶ focused on very

low-birthweight infants (ie, <1500 g). The other study included both small-for-gestational-age infants (SGA; birthweight <10th percentile) and infants who were appropriate for gestational age (AGA). Johnson et al¹⁷ was the only study to assess infants and children with fetal alcohol syndrome (FAS). Oleson et al¹⁸ tested adolescents (PAE vs controls) in Atlanta who had been followed up from a longitudinal cohort of women who were screened for alcohol use during pregnancy. The sample was predominately African American and of low socioeconomic status. The remaining two studies were cohort designs. One was conducted in Argentina,²⁷ which surveyed children under 2 years of age who received care from one of the 12 public hospitals in the southern region of Buenos Aires. The other study²⁸ was from Germany and analyzed data obtained from the German Interview and Examination Survey, which assessed children and adolescents from 167 communities, stratified by federal state and community size.

3.4 | Details of alcohol exposure

Details regarding assessment of PAE are summarized in Table 1. Notably, a number of studies included in the current review were large prospective studies with a different primary goal and therefore specific information regarding PAE tended to be lacking. Seven of the included studies assessed PAE during pregnancy, frequently referred to as “prospective” reporting, either by interview or by questionnaire. Four studies assessed PAE retrospectively, either by interview or by questionnaire from as early as 48 hours post-delivery²⁵ up to when children were 17 years old.²⁸ There was a wide variability regarding the classification of PAE. For example, some studies^{19,27} classified into low- and high-exposure groups, but definitions of the groups differed between studies. Two studies^{21,25} provided detailed information regarding PAE, broken down by number of drinks per week. Other studies provided a range and/or mean for the PAE group (eg, ranged from 1 oz of absolute alcohol per week to 75 oz per week).¹⁸ One study²⁸ did not provide information regarding the level, but indicated that there was a 13.4% prevalence of PAE. Only one study¹⁷ was identified that included children with FASD; however, no details were provided regarding the diagnostic criteria, level of PAE, or how this was assessed.

3.5 | Details of control groups

Given that the majority of the studies were longitudinal designs, most studies did not actively recruit control groups. All children were recruited at the same time and then classified based on PAE. Of the four studies that specifically included control groups, one²⁶ assessed very low-birthweight infants across both PAE and non-exposed groups, who were matched for race, sex, and birthweight. Subsequently, Gauthier et al²⁵ compared the effects of PAE across two groups of infants who were SGA or AGA. The AGA group was randomly selected for inclusion as the comparison group. Johnson et al¹⁷ compared children with FAS, most of whom were SGA, to two groups of typically developing age-matched control children (one SGA group and one AGA group). Oleson et al¹⁸ included a no-exposure control group of adolescents, whose mothers reported no alcohol use during pregnancy.

TABLE 1 Participant and study characteristics

Study	Study type: Country	Participants	Alcohol exposure
Focused on newborns only			
Gauthier et al ²⁶	Case-control: United States	VLBW newborns: alcohol exposed (n = 20); matched ^a control (n = 168); alcohol + cocaine (n = 64)	Prospective interview. Self-report of any alcohol/drug use in pregnancy. Further details not provided
Gauthier et al ²⁵	Case-control: United States	Of 872 newborns, 16%-34% PAE; 1.6%-5.2% to excessive alcohol	Retrospective interview within 48 h of delivery ^b
Included children			
Carson et al ²⁰	Longitudinal: Denmark	108 of 411 children born to mothers with a history of asthma had PAE. Followed up until 7 y of age	Retrospective online questionnaire completed when child was 1 mo old ^c
Johnson et al ¹⁷	Case-control: United States	13 FAS, 13 SGA, 13 AGA control ^d children. 12 mo to 10.5-y of age	"Excessive alcohol consumption" further details not provided
Libster et al ²⁷	Cohort: Argentina	3423 children <2 y hospitalized with severe acute respiratory infections. 398 with PAE (low = 210; high = 188) ^e	Retrospective questionnaire for PAE during 3rd trimester
Linneberg et al ²¹	Longitudinal: Denmark	24 341 mother-child pairs in the Danish Birth Cohort (30%-37% PAE). Assessed at 18 mo of age	Prospective interview at 12 ^f and 30 wk ^g gestation
Magnus et al ²³	Longitudinal: Norway	Assessed: 49 138 children for asthma at 36 mo; 39 791 recurrent LRTIs ^h at 36 mo; 13 253 asthma at 7 y of age. 32% PAE in 1st trimester	Prospective questionnaire at 18 and 30 wk gestation ⁱ
Shaheen et al ²⁴	Longitudinal: United Kingdom	13 988 children followed up at 7.5 y of age. 18% reported PAE	Prospective questionnaire at 8 and 18 wk gestation. Retrospective questionnaire 8 wk post-delivery ^j
Wada et al ¹⁹	Longitudinal: Japan	350 children participated in follow-up surveys at 3-5 y of age (180 males; 166 females) ^k . 12% reported PAE	Prospective questionnaire using a 5-d dietary record at 10 and 29 wk
Included adolescents			
Apfelbacher et al ²⁸	Cohort: Germany	16 761 reported on PAE. 13.4% reported PAE. Children 0-17 y of age randomly selected. 66.6% response rate	Retrospective questionnaire. Details regarding exposure not provided
Oleson et al ¹⁸	Case-control: United States	22 controls (M _{age} 14.4 y; 10 males, 12 females) and 35 PAE (M _{age} 14.9 y; 19 males, 16 females). 95% African American	Prospective (further details not provided) ^l
Yuan et al ²²	Longitudinal: Denmark	10 440 full-term infants followed up to 12 y of age through linked hospital records. 81% reported PAE	Prospective questionnaire at 36 wk gestation ^m

Abbreviations: AGA, appropriate for gestational age; d, day; FAS, fetal alcohol syndrome; h, hour; M, mean; mo, month; PAE, prenatal alcohol exposure; SGA, small for gestational age (ie, <10th percentile for gestational age); VLBW, very low birthweight (ie, <1500 g); wk, week; y, year.

^aMatched for race, sex, and birthweight.

^bReported alcohol use 3 mo prior to pregnancy and during 1st, 2nd, and 3rd trimester; separated into none, <3.5, 3.5-7, 7-14, and >14 drinks/wk. Excessive alcohol was defined as ≥7 drinks/wk.

^cMothers drinking minimum of 1 unit of alcohol per week in minimum one of three trimesters were defined as having PAE during pregnancy. One unit of alcohol was defined as 15 mL or 12 g of alcohol.

^dMatched for age and sex.

^eLow = < drinking once/wk; high = drinking ≥ once/wk.

^fAt 12 wk: 13 054 non-drinkers; 3909 reported 0.5 drinks/wk; 5651 reported 1-1.5 drinks/wk; 5993 reported 2-3.5 drinks/wk; 7191 reported ≥4 drinks/wk.

^gAt 30 wk: 11 551 non-drinkers; 3611 reported 0.5 drinks/wk; 4917 reported 1-1.5 drinks/wk; 3253 reported 2-3.5 drinks/wk; 861 reported ≥4 drinks/wk. PAE defined as ≥1 drink/wk.

^hLower respiratory tract infections (LRTIs) defined as three or more LRTIs during the 1st 36 mo of life. LRTIs included maternal report of pneumonia and/or bronchitis.

ⁱ31.8% reported PAE during 1st trimester (68.9% < once per month 80.8% ≤2 per occasion); 9.7% during 2nd trimester; and 15.6% during 3rd trimester. Only 7.7% consumed >11 drinks throughout pregnancy.

^jFor the last 2 mo of pregnancy, 3780 reported never consuming alcohol; 2947 reported < once/wk; 1475 reported ≥ once/wk (=PAE).

^k315 children followed up until 3 y old, 240 followed up until 5 y old.

^lPAE ranged from 1 oz of absolute alcohol per week to 75 oz/wk, with a mean of 12.4 oz/wk.

^m85% reported PAE during pregnancy, and 2.1% consumed 120 g/wk.

3.6 | Summary of atopy study outcomes

The study outcomes are summarized in Table 2. Eight studies included at least one atopic outcome (ie, eczema, dermatitis, and/or

asthma), with mixed findings evidenced across the included studies. Of the three studies that assessed for eczema, two^{24,28} reported no increased risk associated with PAE and the other study¹⁹ reported an increased risk. Differences in results may be due to the age of

TABLE 2 Relevant assessments and key results of included studies

Study	Relevant assessments	Key results ^a	Conclusions
Included atopy outcomes			
Apfelbacher et al ²⁸	Physician diagnosed eczema	Univariate analysis demonstrated significant effect of PAE; multivariate analysis not significant	PAE not associated with eczema prevalence once accounted for other social and environmental factors
Carson et al ²⁰	Age at 1st atopic dermatitis diagnosis	177 of 411 children developed atopic dermatitis before 7 y of age; significant association of PAE with atopic dermatitis development	PAE was associated with an increased risk of atopic dermatitis in children born to women with a history of asthma
Linneberg et al ²¹	Diagnosis of atopic dermatitis based on caregiver interview	PAE (at both 12 and 30 wk) associated with ↑ risk of atopic dermatitis during the 1st 2 mo of life	PAE increases the risk of early-onset atopic dermatitis, mainly in predisposed infants ^b
Shaheen et al ²⁴	Maternal report of atopic outcomes ^c or positive reaction to an allergy test	PAE negatively associated with asthma and hay fever	No increased risk of atopic outcomes in children
Wada et al ¹⁹	American Thoracic Society Division of Lung Diseases questionnaire; International Study of Asthma & Allergies in Childhood questionnaire	↑ Risk of atopic eczema before 3 and 5 y of age; non-significant trend for asthma before 3 y of age	PAE increases the risk of developing atopic eczema in young children
Yuan et al ²²	Discharge diagnoses of asthma from the Danish Hospital Discharge Registry	PAE was not associated with increased risk of hospitalization with asthma	No support for a causal link between PAE and hospitalization for asthma
Included infection outcomes			
Gauthier et al ²⁶	Early-onset ^d and multiple late-onset sepsis ^e	Early-onset sepsis 15-fold ↑; ↔ compared to alcohol/cocaine control; ↔ multiple late-onset sepsis	PAE increased the risk of early-onset sepsis in a group of VLBW newborns
Gauthier et al ²⁵	Documentation of newborn infections ^f from a review of medical records	Increasing PAE led to ↑ risk of infections; SGA infants exposed to any PAE with 2.5-fold ↑ risk; excessive alcohol ^g threefold to fourfold ↑ risk	PAE associated with increased risk of newborn infections
Johnson et al ¹⁷	Immune cell assays ^h and documented rates of infections	↓ T and B lymphocytes; eosinophilia; ↓ mitogen-induced lymphocytes; ↔ absolute lymphocyte or neutrophil numbers; all 13 children with FAS had major and/or minor infections ⁱ	Children with FAS had impairments in cell-mediated and humoral immune function and high rates of major and minor infections
Libster et al ²⁷	Nasopharyngeal aspirates at the time of hospital admission evaluated for viruses ^j	↑ Risk life-threatening infections, particularly in males (OR rose from 3.67 to 13.52 with PAE)	PAE associated with life-threatening ^k respiratory infections in males
Included both atopy and infection outcomes			
Magnus et al ²³	Maternal report of asthma and recurrent LRTIs ^l	No association between higher PAE during 1st trimester and outcomes; PAE showed inverse association with asthma at 36 mo of age	Comparatively low levels of PAE not associated with increased risk of asthma or LRTIs
Oleson et al ¹⁸	Cumulative prevalence of infectious disease and allergy; leukocyte counts from whole blood smears and flow cytometry	Non-significant trend for ↑ rates of asthma, allergic rhinitis, and persistent skin rashes; ↑ number of circulating lymphocytes in PAE group (dysmorphic and non-dysmorphic combined), possibly due to an acute stressor ^m ; ↔ eosinophil, neutrophil, monocyte numbers	Adolescents with PAE may be hyper-responsive to stress ⁿ

Abbreviations: E, erythrocyte; EAC, erythrocyte antibody complement; FAS, fetal alcohol syndrome; mo, month; OR, odds ratio; PAE, prenatal alcohol exposure; SGA, small for gestational age (ie, <10th percentile for gestational age); wk, week; y, year.

^aCompared to unexposed/low exposure controls unless stated otherwise.

^bPredisposed infants were defined as those with two parents with an allergic disease.

^cAtopic outcomes included wheezing, asthma, eczema, or hay fever.

^dEarly-onset sepsis defined as blood culture positive for microorganisms within first 72 h of life.

^eMultiple late-onset sepsis defined as two or more blood cultures positive for microorganisms in the neonate after 7 d of life.

^fNewborn infections included Group B streptococcus and sepsis.

^gExcessive alcohol was defined as ≥7 drinks/wk.

^hIncluded erythrocyte rosetting (E-rosetting) to specifically measure T lymphocytes; erythrocyte antibody complement rosetting (EAC-rosetting) to specifically measure B lymphocytes; total leukocyte cell counts using Hypaque-Ficoll gradient; and mitogen-induced lymphocyte production. Note these assays are no longer used and have been replaced with immunohistochemistry and flow cytometry.

ⁱMajor infections included sepsis, pneumonia, and meningitis; minor infections included recurrent otitis media and urinary tract infections.

Documentation of infection rates in the SGA or typical control groups were not reported so no comparison could be made.

^jViruses included respiratory syncytial virus (RSV), human rhinoviruses (HRV), and influenza viruses.

^kLife-threatening infection was defined as patient presenting with an oxygen saturation of ≤87% on admission.

^lRecurrent lower respiratory tract infections (LRTIs) defined as three or more LRTIs during the 1st 36 mo of life. LRTIs included maternal report of pneumonia and/or bronchitis.

^mBefore blood collection, participants underwent neuropsychologic testing, which may have been differentially stressful to the PAE group.

ⁿAuthors suggest that adolescents with PAE found the neuropsychologic testing more difficult and was therefore a more stressful experience compared to the control group.

the participants assessed. Apfelbacher et al²⁸ and Shaheen et al²⁴ included older children (ie, up to 7.5 years²⁴ and 17 years²⁸), whereas Wada et al¹⁹ only assessed children at 3 and 5 years of age. Wada et al¹⁹ reported that although there was still a risk of eczema before age 5, this risk was reduced compared to before age 3 (hazard ratio of 1.90 compared to 1.74).

Two studies^{20,21} assessed dermatitis, with both finding that PAE was associated with increased risk. Linneberg et al²¹ reported a dose-dependent effect from PAE and the risk of developing dermatitis during the first 2 months of life, but not beyond this period. This effect was mainly seen in high-risk infants (ie, two parents with allergic disease). Carson et al²⁰ found that in a sample of mothers with a history of asthma, PAE was associated with increased risk of dermatitis during the first 7 years of life. Five studies assessed asthma as an outcome, with none reporting significant results. Oleson et al¹⁸ found a non-significant trend for adolescents with PAE to have increased rates of asthma. Wada et al¹⁹ also found a non-significant trend for children with PAE to develop asthma before age 3. Yuan et al²² assessed rates of hospitalization due to asthma and found no link with PAE. Finally, two studies^{23,24} found no association between PAE and asthma in two large longitudinal studies with comparatively low levels of PAE.

3.7 | Summary of infection study outcomes

Six studies included at least one infection-based outcome of interest. Two studies^{25,26} focused on newborns, with both finding that PAE was associated with increased risk for infections, which included sepsis and Group B streptococcus. Gauthier et al²⁵ also found that tobacco exposure increased the risk of infections. However, when controlling for smoking, race, and low maternal income, high PAE (ie, ≥ 7 drinks/wk) during the 2nd trimester still increased the risk of newborn infections (odds ratio 3.7). Of note, this study also performed a subgroup analysis of the SGA sample, which demonstrated an even stronger association between PAE and newborn infections. The authors concluded that these results support the hypothesis that alcohol and not poor nutrition increased the risk of infection in these newborns. Libster et al²⁷ evaluated children under 2 years of age who were hospitalized for acute respiratory infections. A greater effect on life-threatening infections, defined as oxygen saturation of up to 87% upon admission, was observed with higher PAE during the third trimester. This was more pronounced for males, whose adjusted odds ratio increased from 3.67 to 13.52 when they had experienced PAE.

Johnson et al¹⁷ reported major bacterial (eg, pneumonia and meningitis) and/or minor (eg, otitis media) infections in all 13 children with FASD. They did not report on the incidence of infections in the corresponding non-PAE SGA group or normal age-matched control group. However, they did do a comprehensive immunologic comparison between the FASD and control groups, indicating significantly reduced T and B lymphocytes and mitogen-stimulated lymphocytes. They also reported eosinophilia, but no change in absolute neutrophil numbers. The immunologic assays used in this study were

appropriate at the time, but are now outdated. Oleson et al¹⁸ also assessed leukocytes from blood smears and specific lymphocyte cell types in whole blood using flow cytometry in a small group of adolescents with PAE compared to controls. They found a significant increase in circulating lymphocytes (specifically T cells, T-helper cells, and natural killer [NK] cells), regardless of gender or presence of dysmorphic features, but no change in neutrophils, eosinophils, or monocytes. However, the authors suggested that these increases may be due to adolescents with PAE being hyper-responsive to acute mental stress associated with neuropsychologic testing, which occurred just prior to blood collection. Adolescents with PAE may also have found the testing more challenging, due to their impairments, and therefore, their stress response may be considered appropriate given the circumstances. However, no direct assessments of stress responses (eg, cortisol, heart rate) were included. Magnus et al²³ found that comparatively low levels of PAE were not associated with increased risks of recurrent lower respiratory tract infections (LRTIs), which was categorized as three or more LRTIs during the first 36 months of life.

3.8 | Description of identified preclinical studies

A summary of the main methods and results within the preclinical studies is provided in Data S1. These studies were not analyzed in detail in the current review as only five out of the 39 studies were published in the last 10 years and the majority (72%) were >20 years old. They also included very different outcomes to the clinical studies. Studies were predominantly conducted in rats (82%), but there were also five studies in mouse, one in sheep, and one in non-human primates (macaque), with PAE-induced alterations to the immune response reported across all species. A range of offspring ages were assessed, although the majority of studies examined adults (67%). Many of the studies examined the effect of PAE on cell-mediated immune responses, particularly splenic or thymic lymphocyte proliferation to a mitogen such as lipopolysaccharide (LPS) endotoxin or concanavalin-A (Con-A).

Most studies (~68%) reported that PAE attenuated immune responses to infection/challenge and therefore had an immunosuppressive effect, suggesting that this underlies the increased rates of infection seen in individuals with PAE. However, some studies found that PAE actually augmented the immune response,^{29,30} often in response to a postnatal stress or insult,^{31,32} while a few studies reported no change in immunocompetency.³³ There was also evidence that altered immune responses were transient, with pre-weanling, juvenile or adolescent PAE offspring exhibiting alterations that were normalized by adulthood.^{29,34-37} There was also some evidence for alterations to the humoral immune response, with changes in peripheral or central cytokine production,³⁸⁻⁴⁰ although other studies reported no change.⁴¹ Finally, studies examining LPS- and cytokine-induced fever in adult rats^{42,43} found an attenuated febrile response in PAE offspring, with the authors suggesting that this may also contribute to the decreased resistance to infection observed in animals and humans following PAE.

4 | DISCUSSION

Despite increasing interest in the wide range of possible health outcomes that can arise from PAE, this systematic review highlights the relative paucity of clinical studies that have investigated the impacts on immune functioning.

4.1 | Studies assessing atopy outcomes

Studies in this area demonstrated mixed findings. The two studies that assessed dermatitis^{20,21} both suggested that a genetic predisposition, in terms of parent history of atopic disease, modified the effect of PAE. The five studies that assessed asthma as an outcome found no significant effects. However, two of these studies reported comparatively low levels of PAE^{23,24} and one study only assessed hospitalization due to asthma.²² Future research with larger and more varied populations is therefore required to further elucidate the interactions of genetic predispositions and long-term immune function impacts following varying levels of PAE. Importantly, the age of the participants was highlighted in a number of the atopy studies as potentially influencing the findings. For example, Linneberg et al²¹ found that the effect of PAE on dermatitis was only present in the first 60 days of life and Wada et al¹⁹ reported that the risk of eczema reduced from age 3 to age 5. This has led some authors to suggest that the effects of alcohol regarding atopy outcomes may be transient and attenuate after ceased exposure to alcohol at birth.²¹ This finding was also supported by a number of the preclinical studies that found immune dysfunction was attenuated by adulthood.^{29,34-37}

4.2 | Studies assessing infection outcomes

The studies that included infection outcomes were more consistent in their findings, although there were slightly less studies identified in this area. All studies except one²³ reported changes related to infection outcomes. However, the two studies that specifically assessed lymphocyte populations reported conflicting results, with one study finding reduced levels,¹⁷ while the other found increased levels.¹⁸ These studies were conducted in children of different ethnicities, and consequently, this is an area that requires further research in multiple populations. Magnus et al suggested that their non-significant findings may be due to the low levels of PAE observed in their study. This may be possible, as a dose-response effect of PAE is common and other infection studies reported dose-response effects. For example, Gauthier et al²⁵ reported that high PAE (ie, ≥ 7 drinks/wk) was associated with threefold to fourfold increased risk of infections relative to unexposed pregnancies, compared to more moderate levels of alcohol consumption that resulted in a ~ 2.5 -fold increased risk.

However, it is also important to consider that although infection outcomes were not documented, previous research has suggested that low levels of PAE may underlie susceptibility to the development of autoimmune diseases through dysregulated cytokine

production.⁴⁴ Notably, preclinical research has demonstrated that both peripheral and central cytokines⁸ as well as C-reactive protein (CRP),³⁹ a marker of inflammation, were altered in female rat offspring with PAE. Specifically, animals showed increased pro-inflammatory cytokines in serum, hippocampus, and prefrontal cortex and decreased levels in the spleen and hypothalamus⁸ and increased serum CRP.³⁹ The authors suggested that the lower cytokine levels in the spleen, an important immune organ, and the hypothalamus, an important integrator of immune and endocrine responses, may underlie the increased susceptibility to infection.

Furthermore, Raine et al³⁹ found that exposure to early-life adversity differentially impacted the immune system of offspring with PAE compared to controls, such that offspring with PAE showed a lack of responsivity to early-life adversity. The authors suggested that PAE alters the development of the immune system, leading to inappropriate immune system responses that may be displayed when the system is challenged later in life. These immune markers are yet to be examined in clinical populations with PAE, and there are very few clinical studies that have investigated the concomitant effects of PAE and early-life adversity. This is a vital area for future research, as children with PAE are at increased risk of being exposed to adverse postnatal experiences, such as trauma and neglect.⁴⁵ Further research is needed across all levels of PAE to clarify findings and specifically, to include individuals with a diagnosis of FASD. Only one study was identified in the current review that included children with a FASD diagnosis.¹⁷

4.3 | Limitations

The present review provides a comprehensive synthesis of the available clinical evidence regarding the impact of PAE on immune functioning. However, a number of limitations need to be considered, including variability in the quality of reporting of the included studies, particularly two of the studies that included adolescents. Therefore, given the lack of high-quality evidence in older participants, this limits the conclusions that can be drawn for this age group. Future research that includes adolescents and adults with a history of PAE is needed to address this current gap in the literature. Furthermore, the current review was limited by the lack of detail that was reported for the PAE. A number of the included studies were large prospective studies with a different primary goal, and therefore, specific information regarding the timing and dose of alcohol consumed during pregnancy was lacking.

5 | CONCLUSIONS

The current review provides some initial evidence that PAE can influence immune function, including atopic allergy and infection outcomes in infants, children, and possibly adolescents with PAE or FASD. However, there was inconsistency in results across studies, particularly regarding atopy outcomes and lymphocyte levels. Compared to the comprehensive body of previous research

documenting the brain and behavioral outcomes associated with PAE, very few studies have investigated the potential influence of PAE on the developing immune system. Further research regarding the effects of PAE on immune responses is warranted, particularly including individuals with FASD to inform future clinical practice for this vulnerable population.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Lars Eriksson (Herston Health Sciences Library, the University of Queensland) for advice on the systematic search strategy. This work was supported by the Aboriginal and Torres Strait Islander Health Branch, Department of Health, Queensland; and the National Health and Medical Research Council [APP1078164 to KMM].

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ORCID

Natasha Reid  <https://orcid.org/0000-0001-9813-5613>

Karen M. Moritz  <https://orcid.org/0000-0002-8085-0034>

Lisa K. Akison  <https://orcid.org/0000-0002-2667-3743>

REFERENCES

- Bailey BA, Sokol RJ. Prenatal alcohol exposure and miscarriage, stillbirth, preterm delivery, and sudden infant death syndrome. *Alcohol Res Health*. 2011;34(1):86-91.
- Lange S, Rovet J, Rehm J, Popova S. Neurodevelopmental profile of Fetal Alcohol Spectrum Disorder: a systematic review. *BMC Psychol*. 2017;5(1):22.
- Bantz SK, Zhu Z, Zheng T. The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *J Clin Cell Immunol*. 2014;5(2):202.
- Fuiano N, Incorvaia C. Dissecting the causes of atopic dermatitis in children: less foods, more mites. *Allergol Int*. 2012;61(2):231-243.
- Prescott SL. Early nutrition as a major determinant of 'Immune health': implications for allergy, obesity and other noncommunicable diseases. *Nestle Nutr Inst Workshop Ser*. 2016;85:1-17.
- McCormack C, Hutchinson D, Burns L, et al. Prenatal alcohol consumption between conception and recognition of pregnancy. *Alcohol Clin Exp Res*. 2017;41(2):369-378.
- Bjerke T, Hedegaard M, Henriksen TB, Nielsen BW, Schiøtz PO. Several genetic and environmental factors influence cord blood IgE concentration. *Pediatr Allergy Immunol*. 1994;5(2):88-94.
- Bodnar TS, Hill LA, Weinberg J. Evidence for an immune signature of prenatal alcohol exposure in female rats. *Brain Behav Immun*. 2016;58:130-141.
- Gauthier TW, Brown L. In utero alcohol effects on foetal, neonatal and childhood lung disease. *Paediatr Respir Rev*. 2017;21:34-37.
- Gauthier TW. Prenatal alcohol exposure and the developing immune system. *Alcohol Res*. 2015;37(2):279-285.
- Giliberti D, Mohan SS, Brown L, Gauthier TW. Perinatal exposure to alcohol: implications for lung development and disease. *Paediatr Respir Rev*. 2013;14(1):17-21.
- Moher D, Liberati A, Tetzlaff J, Altman DG, Altman DG, The PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Medicine*. 2009;6(7):e1000097.
- Downs SH, Black N. The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *J Epidemiol Community Health*. 1998;52(6):377-384.
- Abraham RS. Assessment of functional immune responses in lymphocytes. In: Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM, eds. *Clinical Immunology: Principles and Practice*, 5th edn. London, UK: Elsevier Science Health Science; 2019:1253-1271.e1251.
- Akison LK, Reid N, Wyllie M, Moritz KM. Adverse health outcomes in offspring associated with fetal alcohol exposure: a systematic review of clinical and preclinical studies with a focus on metabolic and body composition outcomes. *Alcohol Clin Exp Res*. 2019. <https://doi.org/10.1111/acer.14078> [Epub ahead of print].
- Akison L, Moritz K, Reid N. Adverse reproductive outcomes associated with fetal alcohol exposure: a systematic review. *Reproduction*. 2019;157(4):329-343.
- Johnson S, Knight R, Marmer DJ, Steele RW. Immune deficiency in fetal alcohol syndrome. *Pediatr Res*. 1981;15(6):908-911.
- Oleson DR, Magee RM, Donahoe RM, Falek A, Coles CD. Immunity and prenatal alcohol exposure. In: Friedman H, Madden JJ, Klein TW, eds. *Drugs of Abuse, Immunomodulation, and Aids. Advances in Experimental Medicine and Biology*. Vol 437. Boston, MA: Springer; 1998:255-264.
- Wada K, Konishi K, Tamura T, Shiraki M, Iwasa S, Nagata C. Alcohol intake during pregnancy and offspring's atopic eczema risk. *Alcohol Clin Exp Res*. 2016;40(5):1037-1043.
- Carson CG, Halkjaer LB, Jensen SM, Bisgaard H. Alcohol intake in pregnancy increases the child's risk of atopic dermatitis. The COPSAC prospective birth cohort study of a high risk population. *PLoS ONE*. 2012;7(8):e42710.
- Linneberg A, Petersen J, Grønbaek M, Benn C. Alcohol during pregnancy and atopic dermatitis in the offspring. *Clin Exp Allergy*. 2004;34(11):1678-1683.
- Yuan W, Sørensen HT, Basso O, Olsen J. Prenatal maternal alcohol consumption and hospitalization with asthma in childhood: a population-based follow-up study. *Alcohol Clin Exp Res*. 2004;28(5):765-768.
- Magnus MC, DeRoo LA, Håberg SE, et al. Prospective study of maternal alcohol intake during pregnancy or lactation and risk of childhood asthma: the norwegian mother and child cohort Study. *Alcohol Clin Exp Res*. 2014;38(4):1002-1011.
- Shaheen SO, Rutterford C, Zuccolo L, et al. Prenatal alcohol exposure and childhood atopic disease: a Mendelian randomization approach. *J Allergy Clin Immunol*. 2014;133(1):225-232.e225.
- Gauthier TW, Drews-Botsch C, Falek A, Coles C, Brown L. Maternal alcohol abuse and neonatal infection. *Alcohol Clin Exp Res*. 2005;29(6):1035-1043.
- Gauthier TW, Manar MH, Brown L. Is maternal alcohol use a risk factor for early-onset sepsis in premature newborns? *Alcohol*. 2004;33(2):139-145.
- Libster R, Ferolla FM, Hijano DR, et al. Alcohol during pregnancy worsens acute respiratory infections in children. *Acta Paediatr*. 2015;104(11):e494-e499.
- Apfelbacher C, Diepgen T, Schmitt J. Determinants of eczema: population-based cross-sectional study in Germany. *Allergy*. 2011;66(2):206-213.
- Wong C, Chiappelli F, Chang MP, et al. Prenatal exposure to alcohol enhances thymocyte mitogenic responses postnatally. *Int J Immunopharmacol*. 1992;14(2):303-309.
- Chiappelli F, Tio D, Tritt SH, Pilati ML, Taylor AN. Selective effects of fetal alcohol exposure on rat thymocyte development. *Alcohol*. 1992;9(6):481-487.

31. Giberson PK, Kim CK, Hutchison S, Yu W, Junker A, Weinberg J. The effect of cold stress on lymphocyte proliferation in fetal ethanol-exposed rats. *Alcohol Clin Exp Res*. 1997;21(8):1440-1447.
32. Halasz I, Aird F, Li L, Prystowsky MB, Redei E. Sexually dimorphic effects of alcohol exposure in utero on neuroendocrine and immune functions in chronic alcohol-exposed adult rats. *Mol Cell Neurosci*. 1993;4(4):343-353.
33. Zidell RH, Hatoum NS, Thomas PT. Fetal alcohol effects: evidence of developmental impairment in the absence of immunotoxicity. *Fundam Appl Toxicol*. 1988;10(2):189-198.
34. Wolcott RM, Jennings SR, Chervenak R. In utero exposure to ethanol affects postnatal development of T- and B-lymphocytes, but not natural killer cells. *Alcohol Clin Exp Res*. 1995;19(1):170-176.
35. Taylor AN, Tio DL, Chiappelli F. Thymocyte development in male fetal alcohol-exposed rats. *Alcohol Clin Exp Res*. 1999;23(3):465-470.
36. Norman DC, Chang MP, Wong C, Branch BJ, Castle S, Taylor AN. Changes with age in the proliferative response of splenic T cells from rats exposed to ethanol in utero. *Alcohol Clin Exp Res*. 1991;15(3):428-432.
37. Gottesfeld Z, Ullrich SE. Prenatal alcohol exposure selectively suppresses cell-mediated but not humoral immune responsiveness. *Int J Immunopharmacol*. 1995;17(3):247-254.
38. Grossmann A, Astley SJ, Liggitt HD, et al. Immune function in offspring of nonhuman primates (*Macaca nemestrina*) exposed weekly to 1.8 g/kg ethanol during pregnancy: preliminary observations. *Alcohol Clin Exp Res*. 1993;17(4):822-827.
39. Rainecki C, Bodnar TS, Holman PJ, Baglot SL, Lan N, Weinberg J. Effects of early-life adversity on immune function are mediated by prenatal environment: role of prenatal alcohol exposure. *Brain Behav Immun*. 2017;66:210-220.
40. Terasaki LS, Schwarz JM. Effects of moderate prenatal alcohol exposure during early gestation in rats on inflammation across the maternal-fetal-immune interface and later-life immune function in the offspring. *J Neuroimmune Pharmacol*. 2016;11(4):680-692.
41. Weinberg J, Jerrells TR. Suppression of immune responsiveness: sex differences in prenatal ethanol effects. *Alcohol Clin Exp Res*. 1991;15(3):525-531.
42. Yirmiya R, Tio DL, Taylor AN. Effects of fetal alcohol exposure on fever, sickness behavior, and pituitary-adrenal activation induced by interleukin-1 beta in young adult rats. *Brain Behav Immun*. 1996;10(3):205-220.
43. Yirmiya R, Pilati ML, Chiappelli F, Taylor AN. Fetal alcohol exposure attenuates lipopolysaccharide-induced fever in rats. *Alcohol Clin Exp Res*. 1993;17(4):906-910.
44. Noor S, Milligan ED. Lifelong impacts of moderate prenatal alcohol exposure on neuroimmune function. *Front Immunol*. 2018;9:1107.
45. Price A, Cook PA, Norgate S, Mukherjee R. Prenatal alcohol exposure and traumatic childhood experiences: a systematic review. *Neurosci Biobehav Rev*. 2017;80:89-98.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Reid N, Moritz KM, Akison LK. Adverse health outcomes associated with fetal alcohol exposure: A systematic review focused on immune-related outcomes. *Pediatr Allergy Immunol*. 2019;30:698-707. <https://doi.org/10.1111/pai.13099>

Supplemental Data File S2: Quality assessment of included studies.

A) Quality assessment of included clinical studies using the Downs and Black Checklist. The published checklist was adapted to remove items relating to an intervention. Maximum possible score = 19.

Study	Items*																		Total
	1	2	3	5	6	7	10	11	12	15^	16	17	18	20	21	22	25	27 ⁺	
Apfelbacher et al (2011)	1	1	1	0	0	0	1	0	0	0	1	1	1	1	1	1	1	0	11
Carson et al (2012)	1	1	1	2	1	1	1	0	0	0	1	1	1	1	0	1	0	0	13
Gauthier et al (2004)	1	1	1	2	1	1	0	0	0	0	1	1	1	1	1	1	0	0	13
Gauthier et al (2005)	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	13
Johnson et al (1981)	1	1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	8
Libster et al (2015)	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	14
Linneberg et al (2004)	1	1	1	2	1	1	0	1	0	0	1	1	1	1	0	1	1	0	14
Magnus et al (2014)	1	1	1	2	1	1	0	0	0	0	1	1	1	1	1	1	1	0	14
Oleson et al (1998)	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	8
Shaheen et al (2013)	1	1	1	2	0	0	1	0	0	0	1	1	1	1	1	1	1	0	13
Wada et al (2016)	1	1	1	2	1	1	1	1	0	0	1	1	1	1	1	1	1	0	16
Yuan et al (2004)	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	13

* Item numbers are as per Downs and Black (1998). The following items were removed as they refer to reporting of an intervention: 4, 8, 9, 13, 14, 19, 23, 24, and 26. Scale of item score: for items 1 to 3 and 6 to 10: 1 = Yes, 0 = No; for item 5 – 2 = Yes, 1 = Partially, 0 = No; for items 11 to 27 - 1 = Yes, 0 = No or unable to determine. The Downs and Black Checklist covers the following areas: *Reporting* (items 1 -10; includes reporting of hypothesis/aim/objective, outcomes, patient characteristics, confounders/covariates, main findings, estimates of variability, confidence intervals and probabilities); *external validity* (items 11, 12; assessment of how representative the study participants are of the recruitable population); *internal validity* (bias, items 15-20; confounding, items 21-25); and *statistical power* (item 27). See Downs and Black (1998) for full details of the criteria for each item.

^ For item 15, the ‘intervention’ was alcohol exposure.

Supplemental Data File 2: Quality assessment of included studies.

A) Quality assessment of included clinical studies using the Downs and Black Checklist. The published checklist was adapted to remove items relating to an intervention. Maximum possible score = 19.

Study	Items*																		Total
	1	2	3	5	6	7	10	11	12	15^	16	17	18	20	21	22	25	27 ⁺	
Apfelbacher et al (2011)	1	1	1	0	0	0	1	0	0	0	1	1	1	1	1	1	1	0	11
Carson et al (2012)	1	1	1	2	1	1	1	0	0	0	1	1	1	1	0	1	0	0	13
Gauthier et al (2004)	1	1	1	2	1	1	0	0	0	0	1	1	1	1	1	1	0	0	13
Gauthier et al (2005)	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	13
Johnson et al (1981)	1	1	1	0	1	1	0	0	0	0	1	0	0	1	0	0	1	0	8
Libster et al (2015)	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	0	14
Linneberg et al (2004)	1	1	1	2	1	1	0	1	0	0	1	1	1	1	0	1	1	0	14
Magnus et al (2014)	1	1	1	2	1	1	0	0	0	0	1	1	1	1	1	1	1	0	14
Oleson et al (1998)	0	1	1	0	1	1	1	0	0	0	0	0	1	1	1	0	0	0	8
Shaheen et al (2013)	1	1	1	2	0	0	1	0	0	0	1	1	1	1	1	1	1	0	13
Wada et al (2016)	1	1	1	2	1	1	1	1	0	0	1	1	1	1	1	1	1	0	16
Yuan et al (2004)	1	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1	1	0	13

* Item numbers are as per Downs and Black (1998). The following items were removed as they refer to reporting of an intervention: 4, 8, 9, 13, 14, 19, 23, 24, and 26. Scale of item score: for items 1 to 3 and 6 to 10: 1 = Yes, 0 = No; for item 5 – 2 = Yes, 1 = Partially, 0 = No; for items 11 to 27 - 1 = Yes, 0 = No or unable to determine. The Downs and Black Checklist covers the following areas: *Reporting* (items 1 -10; includes reporting of hypothesis/aim/objective, outcomes, patient characteristics, confounders/covariates, main findings, estimates of variability, confidence intervals and probabilities); *external validity* (items 11, 12; assessment of how representative the study participants are of the recruitable population); *internal validity* (bias, items 15-20; confounding, items 21-25); and *statistical power* (item 27). See Downs and Black (1998) for full details of the criteria for each item.

^ For item 15, the 'intervention' was alcohol exposure.