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Prenatal alcohol exposure and the early neurodevelopmental outcomes of children in a South African birth cohort study

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(HNDGAI003)

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DOCTOR OF PHILOSOPHY

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Declaration

I, Gaironeesa Hendricks, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions from others as outlined in the acknowledgements. The assistance I received with study management, data collection, analysis and manuscript review from the co-authors of the publications that form part of this thesis is described for each relevant chapter.

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1. Effects of prenatal alcohol exposure on language, speech and communication outcomes: a review longitudinal study. Hendricks G, Malcolm-Smith S, Adnams C, Stein DJ, Donald KA. Acta Neuropsychiatrica 2019, 74-83. [Published].

Contribution of co-authors and student:

I wrote the full draft and final version of this paper, collected the data, analysed and summarised the findings. My co-authors, KAD, SMS, DJS and CA reviewed the draft, made conceptual and intellectual contributions in specific areas of their expertise.

2. Prenatal alcohol exposure is associated with early motor, but not language development in a South African cohort. Hendricks G, Malcolm-Smith S, Stein DJ, Zar H, Wedderburn CJ, Nhapi RT, Chivese T, Adnams CM, Donald KA. 2020. Acta Neuropsychiatrica. [Published].

Contribution of co-authors and student:

I wrote the full draft and final version of this paper and integrated contributions from the other authors. I analysed the data, summarised and interpreted the findings into the final discussion. KAD was involved in the conceptual design, data collection, data management and quality control of the data, data analysis strategy and provided final approval of this manuscript. SMS was involved in the analysis strategy and provided a critical review of earlier drafts as well as final approval of this manuscript. All authors read the manuscript prior to submission and commented/contributed within their area of expertise. HZ is the PI of the umbrella DCHS and is responsible for the overall design of the parent project. DJS is the PI of the psychosocial component, and KAD is involved in the design and roll-out of the neurodevelopment aspects of the umbrella DCHS, from whom the mothers, infants or toddlers were recruited.

3. Prenatal alcohol exposure is associated with early developmental trajectory in a South African birth cohort: the first 4 years. Hendricks G, Malcolm-Smith S, Stein DJ, Zar HJ, Wedderburn CJ, Lewis R, Donald KA. [Draft].

Contribution of student and co-authors:

I wrote the full draft of this manuscript. I was involved in data collection, data management and quality control, coding, data analysis and final write-up of this study. KAD provided expert advice, was involved in the conceptual design and provided a critical review of earlier drafts. SMS was involved in the analysis strategy, provided a review of earlier drafts and final approval of this manuscript. Together with SMS, KAD and RL, we devised a data analysis plan. All authors were involved in review of the manuscript, provided feedback and approved the final version. HZ is the PI and is in charge of the overall design of the DCHS, while DJS is the PI of the psychosocial component. KAD is involved in the design and roll-out of the neurodevelopmental components of the DCHS.

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I wrote the full draft and final version of this manuscript. I was involved in data collection, data management and quality control, data transcribing and coding, data analysis and final write-up. Both SMS and KAD provided expert advice and were involved in the conceptual design of this study. The data analysis plan was conceptualised by myself with the statistical support of SMS and TC. All authors contributed to the writing of the manuscript and approved the submitted manuscript. As mentioned before, HZ is the PI and is responsible for the overall design of the DCHS. DJS is the PI of the psychosocial component, and KAD is involved in the design and roll-out of the neurodevelopmental aspects of the DCHS. SMS was involved in the roll-out of the video-audio recordings, from whom the mother-child dyads were recruited. All authors provided their input into the manuscript.

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Acknowledgments

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Abbreviations

ADHD	Attention deficit hyperactivity disorder
ARND	Alcohol related neurodevelopment disorder
ARBD	Alcohol related birth defect
ASSIST	Alcohol, Smoking and Substance Involvement Screening Test
BDI	Beck Depression Inventory
BMI	Body Mass Index
BSID-III	Bayley-III Scales of Infant Development
CLAN	Computerized language analysis
DCHS	Drakenstein Child Health Study
FASD	Foetal alcohol spectrum disorder
FAS	Foetal alcohol syndrome
HICs	High-income countries
LMICs	Low-and-middle income countries
KABC-II	Kaufman Assessment Battery for Children
MPSS	Modified Posttraumatic Stress Disorder Symptom Scale
PAE	Prenatal alcohol exposure
PI	Principal investigator
pFAS	Partial foetal alcohol syndrome
PPVT	Peabody Picture Vocabulary Test
PTSD	Post-traumatic stress disorder
QATSO	Quality Assessment Tool for Systematic Reviews of Observational Studies
SD	Standard deviation
SES	Socioeconomic status

Abstract

Introduction: Over the last few decades, prenatal alcohol exposure (PAE) has been a major public health problem both globally and in low- to-middle-income countries (LMICs) such as South Africa. Pregnant women and new mothers are particularly vulnerable; and PAE may be associated with adverse child neurodevelopmental outcomes. However, few studies have explored the association of PAE, including risk factors, and subsequent neurodevelopmental trajectories over multiple timepoints in the early years. Given the high burden of PAE and associated risk factors, and the relative paucity of empirical data, further work in South African populations is warranted. This thesis aimed to investigate the association between PAE and early neurodevelopmental outcomes in the Drakenstein Child Health Study (DCHS), a South African birth cohort. The specific objectives included: 1. a systematic review on the available longitudinal studies exploring the impact of PAE on language, speech and communication development (Chapter 3 Manuscript 1); 2. an exploration of the association between PAE and motor, language and cognitive outcomes in infancy (Chapter 4- Manuscript 2); 3. an investigation of the association between PAE, including interactions of tobacco smoking exposure, and the neurodevelopmental trajectories (motor, language and cognitive outcomes) of children across the first 4 years of life (Chapter 5 Manuscript 3); 4. a comparison of the conversational turn-taking between mothers and their alcohol exposed children compared to those between mothers and their unexposed children (Chapter 6 Manuscript 4).

Methods: This thesis included four publications, three of which present data from the DCHS. Pregnant women were enrolled from two public primary healthcare clinics, Mbekweni (a predominantly black African population) and TC Newman (a mixed-ancestry population), and more than 1000 mother-child dyads were followed longitudinally from birth through the first 5 years of life. For this study, both antenatal and postnatal maternal measures were used to assess moderate-to-severe levels of PAE. These measures included the (i) Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) antenatally, (ii) a retrospective alcohol questionnaire in the postnatal period at 3-6 weeks and/or 24 months testing age. At 6, 24 and 42 months, early neurodevelopmental outcomes were assessed using the Bayley-III Scales of Infant Development (BSID-III), the Kaufman Assessment Battery for Children (KABC-II) or the

Peabody Picture Vocabulary Test (PPVT-IV). Conversational turn-taking in mother-child dyads was also assessed at 42 months testing age. Both univariate and multivariate analyses were used to analyse the data.

Results: The findings of this thesis showed that PAE was significantly associated with both fine motor (B=-3.30, 95%CI 0.06-0.46, p=0.001) and gross motor scores (B=-0.30, 95%CI 0.06-0.44 p=0.001) at 6 months (Chapter 4 Manuscript 2). Chapter 5 (Manuscript 3) showed that when accounting for the interaction between prenatal alcohol and tobacco smoking exposure, impaired fine motor functioning occurred up till 24 months (B=-12.59, 95%CI -21.98- -3.19, p=0.01), but these effects attenuated by 42 months. Significant interactions occurred between prenatal alcohol, including tobacco smoking exposure, and impaired receptive vocabulary (B=-2.49, 95%CI -5.24 -0.27, p=0.02) and cognitive functioning at 24 months (B=-3.25, 95%CI -5.98- -0.52, p=0.02) (Chapter 5 Manuscript 3). Finally, when exploring conversational turn-taking in alcohol exposed mother-child dyads and unexposed dyads, PAE was significantly associated with conversational turn-taking i.e. child overlapping utterances (OR=3.25, CI 0.98-10.76, p=0.050) (Chapter 6 Manuscript 4).

Conclusion: The associations of PAE with early neurodevelopmental outcomes shown here expand on the previous literature. Our findings reported that PAE may influence early neurodevelopmental outcomes, however, future studies should include additional longitudinal studies to replicate the findings, and ongoing follow-up of our own cohort may continue clarify the potential association of PAE and additional risk factors on later neurodevelopmental outcomes at school age and beyond. Effective alcohol programmes targeting pregnant women and interventions to address child developmental impairments in this vulnerable cohort are required.

CHAPTER 1

Introduction

1. Introduction

Prenatal alcohol exposure (PAE) may lead to a wide variety of neurodevelopmental disorders collectively known as Foetal Alcohol Spectrum Disorders (FASDs) and is a leading preventable cause of developmental impairments in low- and middle-income countries (LMICs) (1-3). There are many contextual risk factors associated with PAE, which may interact with each other to affect both early and later child developmental outcomes. In cases where both PAE and a combination of risk factors (e.g. poverty, low birth weight or prematurity) have occurred, the risk of neurodevelopmental impairments has been more profound. The Drakenstein Child Health Study (DCHS) (see Chapter 2 - General Methodology, Pg. 37-50), a South African birth cohort study, provides a valuable opportunity to investigate the risk factors associated with early child developmental outcomes, and is situated in a peri-urban, low socioeconomic status (SES) South African community constituting of two suburbs, with a high prevalence of PAE, typical of many populations in LMICs. Despite the relative dearth of PAE research emerging from LMICs such as South Africa, the DCHS provides a useful context for understanding the interrelationships of PAE and associated risk factors on the neurodevelopmental trajectory of very young children. In this chapter, I shall briefly explore the known prevalence rates and risk factors of PAE, as well as the effects of PAE on early neurodevelopmental outcomes. I shall outline how the thesis will attempt to address the gaps in the current literature. Finally, I shall present the research aim and specific objectives of this thesis.

2. Background and literature review

PAE has received widespread attention for four to five decades since seminal papers described the potential physical and cognitive outcomes amongst children. Three initial papers published in the 1970's (1-3) have provided the foundational basis for the diagnosis of foetal alcohol syndrome (FAS), and its association with the physical and cognitive outcomes of those affected. These early studies reported that PAE is a teratogen that may cross the placenta and affect the central nervous system resulting in damage to the brain and other organs of the developing embryo and foetus (1-3).

2.1 Defining foetal alcohol spectrum disorders (FASDs)

The diagnosis of FASDs advanced consistently since its conception in the literature (3, 4). In 1996, the United States' Institute of Medicine (IOM) distinguished between separate diagnostic categories. These included foetal alcohol syndrome (FAS), partial FAS (pFAS), alcohol related neurodevelopment disorders (ARNDs) and alcohol related birth defects (ARBDs). FAS, the most extreme condition, was described by Jones and Smith (6, 7) as a pattern of permanent physical and/or mental birth impairments. These authors (6, 7) initially described the fundamental components of FAS after observing a unique pattern of impaired development (stunted growth, neurobehavioural deficiencies and facial abnormalities) in young children (5-7). PFAS, on the other hand, was described as those who do not meet the full diagnostic criteria for FAS, but have some facial abnormalities, growth problems or central nervous abnormalities. The categories, ARND and ARBD, were defined as those having minimal growth deficiency and minor physical abnormalities. ARND is characterized by deficiencies in cognitive, behaviour and self-regulation, while ARBD may comprise of physical defects (heart, kidneys, bones and hearing) (5). While previous attempts have been made to improve the diagnoses of FASDs (7), there are many biological, social and medical barriers which create significant challenges for those at risk for the disorder.

2.2 Prevalence studies of PAE in the general population and in South Africa

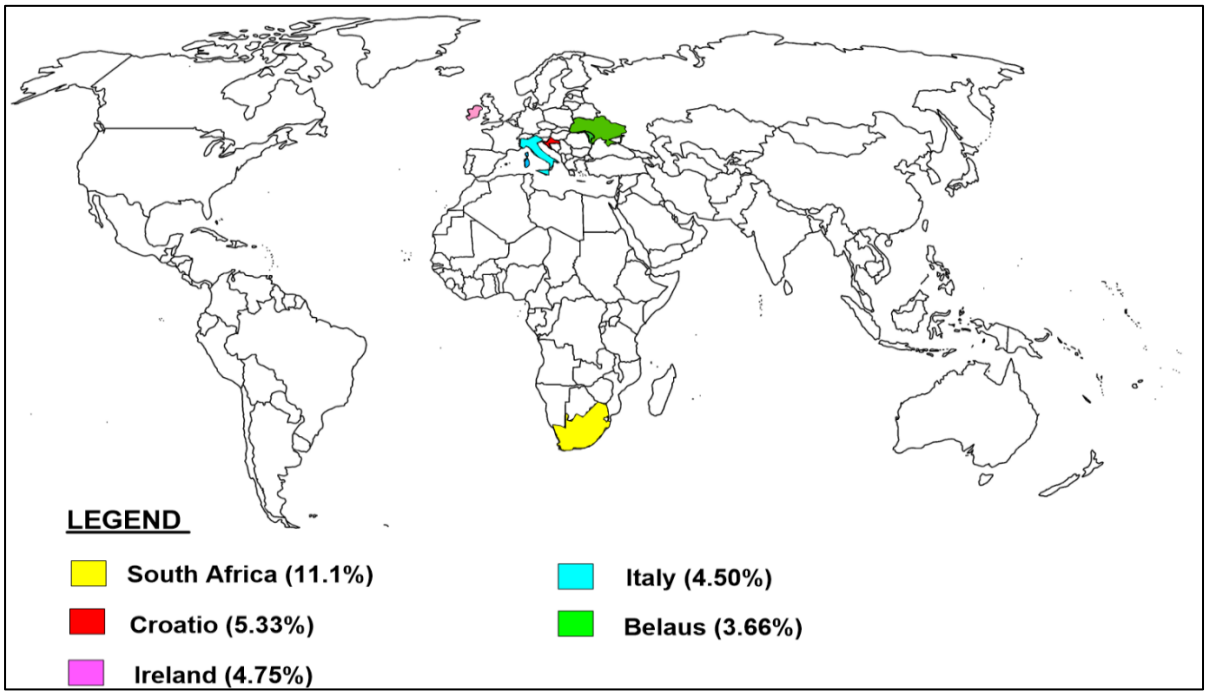
Alcohol consumption during the prenatal period remains a major global concern (8-10). Previous studies have found that globally about 5.7% of women are alcohol dependent and 10% consume alcohol during pregnancy (8, 9). In South Africa, alcohol consumption during pregnancy is highly prevalent in certain regions, and about 3.2-6.5% report alcohol consumption during pregnancy (10). Increasing prevalence of PAE is associated with greater burden of additional behavioural risk factors, including risky sexual behaviour, unemployment, and mental health problems (10).

2.2.1 Prevalence studies of FASDs and FAS globally and in South Africa

Much of the early work on the prevalence of FASDs took place in high-income countries (HICs), where only a comparatively small portion of the world's population resides (Figure 1). Previous studies have reported high incidence of FASDs in countries such as Croatia (5.33%),

Ireland (4.75%), Italy (4.50%) and Belarus (3.66%) and in South Africa (11.10%) (11). Findings by Popova and colleagues (9) have found that FAS (per 10 000 people) is highly prevalent in HICs such as Ireland (89.7), Italy (82.1) and Belarus (69.1) (9, 10). In South Africa, an increasingly broad coverage of population studies for this disorder resulted in the prevalence of FAS (55.42 per 1000 children) being well-described in certain regions of the country (12), however, no single national study has been administered. The documentation of PAE remains contentious due to a lack of sufficient research in South Africa, especially given that there are multiple risk factors that may influence a child's vulnerability to a FASD, including individual vulnerabilities in the genetic background of the mother as well as co-existing physical and environmental factors (13-16).

Figure 1. Foetal Alcohol Spectrum Disorders (FASDs) weighted by the number of live births by region estimated by the WHO (2000–2014)



Source: Lange S, Probst C, Gmel G, Rehm J, Burd L, Popova S. Global prevalence of foetal alcohol spectrum disorder among children and youth: a systematic review and meta-analysis. JAMA paediatrics 2017;171(10):948-956.

2.3 Prenatal alcohol exposure: Maternal risk factors

Alcohol use during pregnancy frequently comorbid with a range sociodemographic, physical and psychosocial factors. Previous studies have identified associations between PAE, poor nutritional status and lower levels of income (12-16). Others have reported that heavy PAE is

associated with poor maternal socioeconomic status (SES) (14), nutrition (17-19), depression (24) and low body mass index (BMI) (20, 21). In addition, May and colleagues (13) have measured the head circumference of mothers who consumed alcohol during pregnancy and found that they were significantly smaller than their counterparts, and some of the mothers had FAS or pFAS themselves. As seen previously, many of the research studies describing risk factors explored sociodemographic factors, (12-16), while less is known about potentially co-occurring mental health factors. Some of these comorbid psychiatric disorders include anxiety disorders, oppositional defiant disorder, bipolar disorder and some features of autism (12-16). More specifically, PAE and comorbid maternal post-traumatic stress disorder (PTSD) may have detrimental consequences on childhood outcomes but have not been well studied (12). There is a need to fully understand and address mental health conditions as these may either aggravate or mitigate the effects of PAE and potentially have serious consequences.

2.4 Prenatal alcohol exposure: Physical and neurodevelopmental outcomes

2.4.1 Prenatal alcohol exposure: Physical outcomes

Over the decades, there has been a large body of evidence evaluating the association between PAE and negative physical outcomes. Previous studies have shown that PAE is established as a risk factor for adverse outcomes including stillbirth (23), spontaneous abortion and premature birth (17-20), intrauterine growth retardation (19), low birth weight (20-23), as well as deficits in brain functioning and other organs of the developing embryo and foetus (28, 29). Others have reported complications during pregnancy, reduced foetal growth and increased perinatal mortality (23, 24). PAE may also cause damage to multiple brain structures resulting in neurodevelopmental impairments and translating into lifelong disabilities and risky health outcomes for the child (27-29).

2.4.2 Prenatal alcohol exposure: Neurodevelopmental outcomes

Infancy and early childhood are the most vulnerable period for motor, language and cognitive development. An extensive body of literature exists on the association between PAE, motor, cognitive and language outcomes (30-33). Some researchers, but not all, have reported that young children show decreased performance in motor functioning, verbal and working memory in the early years (34-42). In a prospective study from Ottawa (Canada), 217 twelve-

month and 153 twenty-four-month-old toddlers who were prenatally exposed to marijuana, alcohol or cigarettes showed associations between PAE and poor early motor functioning at 12 months (36). These authors reported motor function impairments even after adjusting for maternal age, gestation, birth weight and parity. In a follow-up cohort, Fried and colleagues (36, 37) have found no effects on motor outcomes in samples at preschool age (60 and 70 months of age). In high risk environments such as South Africa, Davies and colleagues (24) have reported that heavy PAE was associated with lower locomotor coordination scores at both 7-12 months (n=45 FASD) and 17-29 months (n=35 FASD) in comparison to their non-FASD counterparts. However, this study (24) did not adjust for any potential confounding variables, and further research is needed to clarify and understand the inconsistent findings of previously reported studies taking in consideration confounding factors particularly in high risk environments.

Previous studies have reported that PAE is associated with language impairments in the early years (43-47). A study by O'leary (46) exploring the association of PAE and language skills at age 24 months documented no differences in language abilities between children with PAE and their unexposed counterparts (46). They (46) found that PAE had no significant effects on children's receptive and expressive abilities at 24 months of age. In a similar age window as the study by O'Leary (46), Davies and colleagues (24) found significantly poorer receptive and expressive scores in more heavily exposed toddlers than their unexposed counterparts at 17-29 months. Similarly, a study by Wyper and Rasmussen (48) reported that children with FASDs scored significantly lower than unexposed children on both receptive and expressive communication between 5 and 13 years of age. More specifically, younger children had impairments in vocabulary and sentence imitation, while older children had impairments in word ordering and grammatic comprehension (48). Coggins and colleagues (43) have explored the language outcomes of school-going children with FASDs and found that these children displayed impairments in language and social outcomes between 6-12 years of age (43). They further indicated that children with FASDs demonstrated impairments in specific components of language including referential features (i.e. expression of main story elements) and pragmatic components of language (i.e. expressive vocabulary) in comparison to younger children (5-7 years). Despite the few studies on the association between PAE and language outcomes, more research is needed in this area of study. In a literature review by

Cone-Wesson (47), FAS was associated with areas of impaired language development. However, this review did not include a discussion of the association of PAE with language developmental trajectories across time. A systematic review was important to delineate the long-term effects of PAE on language development across multiple timepoints in the early years.

Very young children with impairments in comprehending or expressing age-appropriate vocabulary may struggle with understanding the foundational skills of conversational interaction (43, 44). Social communication delays associated with PAE may further impact receptive and expressive vocabulary as well as substantial secondary disabilities later on in life (academic failure, communication delays and social interaction problems). While limited research exists on the association between PAE and social communication outcomes, it is hypothesised that these associations may negatively impact scholastic attainment and quality of life, comorbid mental health problems including depression and behavioural problems.

The influence of PAE on cognitive development has been demonstrated by Carter and colleagues (49). These authors described how exposure in the utero may cause brain damage, leading to cognitive impairments including impaired attention and social cognition. Others have identified impairments in preschool years associated with reduced cognitive flexibility, inhibition and executive functioning (49, 50), nonverbal intelligence and social judgement (51-53), and lastly verbal and visual spatial memory (53-56). More specifically, Rasmussen and Bisanz (55) have reported that PAE is associated with cognitive flexibility in young children and decreased abilities in executive functions, nonverbal, attention and social judgment. In addition, research by Pei and colleagues (56) have identified associations between PAE and nonverbal memory in young children. However, most of the above-mentioned studies demonstrated a lack of and had small sample sizes. Longitudinal population-based studies with large sample sizes and comprehensive assessments of cognitive abilities throughout childhood may provide important results.

2.5 Definitions: Motor, language, cognitive outcomes, and conversational turn-taking

Motor development is a broad concept and has been defined in several different ways. It has been described as learned sequences of movements that are joined to produce smooth and well-organized acts in order to successfully complete a particular task (57). There are two different categories of motor tasks: fine and gross motor functions. Fine motor functioning is related to small muscle groups such as those in the hand and wrist (57). These are associated with grasping, writing or drawing and feeding (57). Gross motor functioning, on the other hand, is related to large muscle movements and includes jumping, sprinting and walking (58). Both fine and gross motor skills are crucial for children's growth and development as they may assist with other outcomes such as language and cognitive skills.

Language development is a complex construct, which encompasses a range of components including receptive, expressive and other systems of communication (61). Receptive communication is defined as the internal process and understanding of a language, while expressive communication involves the ability to express a language (62). Previous linguists have demonstrated that language evolves from the structural characteristics of language (receptive and expressive communication) into active and frequent participation of conversational patterns of talk (62, 63). This active participation of talk which includes the rapid switching between receptive and expressive language has come to be known as conversational turn-taking (63). Recent work by Hilbrink and colleagues (63) has shown that when children participate in constant back-and-forth interactions with their mothers, they show increased activity in the part of the brain responsible for receptive production and processing. Hilbrink and colleagues (63) have suggested that in order for smooth conversational turn-taking to occur, speakers need to listen, comprehend and plan their own turn, and simultaneously predict the next turn. Important components of conversational turn-taking include overlapping utterances, utterances preceded by short, moderate pauses and long pauses (63). Hilbrink and colleagues (63) further suggests that smooth conversational turn-taking requires minimal duration of pauses between utterances and overlapping utterances (63). This coordinated system of talk allows for the fluency and continuity of controlled conversational turn-taking between speakers.

Cognitive functioning is defined as the mental activity of obtaining knowledge and understanding thoughts, practices and sensations (59). Previous studies have identified important components of cognitive outcomes including executive functioning, memory and intelligence (59, 60). A component of intelligence is nonverbal intelligence, which has shown to measure of general cognition without the confound of language ability (59). It is the ability to make sense and communicate without using words, and such intelligence allows people to think through, plan or monitor, and execute an activity (60). It takes the notion of eliminating language even further and includes only measures that can be communicated via gestures without any need for verbal expression on the part of the child (60). Achieving efficient nonverbal outcomes at an early age is associated with better intellectual performance and higher education levels and further research on this domain is crucial for early and later child development.

Previous studies have shown that the regions of the brain that are affected by PAE are the basal ganglia, corpus callosum, cerebellum, and, to some degree, the hippocampus (64). Neuronal loss and structural changes occurring in these areas result in disorders in movement, balance, and impairment of learning and memory, especially spatial learning (31). Other affected areas include motor (e.g. sensorimotor motor functioning), cognitive (e.g. executive functioning and attention), language (receptive language and speech) and behavioural outcomes in children up to school years of age (30-33). Thus, further research is needed to clarify and understand the important effects of PAE on these early neurodevelopmental trajectories of very young children (36, 37).

Rationale

The negative impact of PAE on child health and developmental outcomes has become increasingly concerning, especially during early childhood where there may be increased susceptibility. Early child development is a crucial period for rapid brain activity and neurodevelopmental functioning, and any changes leading to impairments as a result of PAE may be detrimental for the lifelong outcomes of young children. There are several reasons as to why early neurodevelopmental outcomes in the context of PAE necessitate particular attention through the preschool years.

First, despite the increasing literature detailing the effects of PAE on young children's neurodevelopment, comparable data across different timepoints are limited in the first years of life. Of these investigations (36, 37, 74-77), much of the work published to date have lacked complex longitudinal analyses (multilevel data), comparable control groups, statistical control of important sociodemographic and psychosocial confounders and larger sample sizes (24, 36, 37). While the detrimental effects of PAE have been well-described previously, early neurodevelopmental trajectories rarely are explored in LMIC populations. Investigating the neurodevelopmental trajectory within a group of alcohol exposed children may be useful to identify delays and find ways to improve the wellbeing of children in this vulnerable cohort.

Secondly, despite the effects that PAE may have on children's neurodevelopmental outcomes (75), there are few studies exploring the association between PAE and conversational interaction in mother-child dyads. Coggins and colleagues (43) have shown that heavy PAE is associated with impairments in conveying meaningful information in children ages 6-12 years. They suggested that children with PAE showed very little interest and engagement with their peers during classroom activities, and could less successfully plan, monitor and evaluate their responses than unexposed children (43). To our knowledge, no previous studies have explored the association between PAE and conversational turn-taking in mother-child dyads. Previous evidence has shown that successful conversational turn-taking has broad neurodevelopmental implications, and this kind of parental input is crucial for obtaining language and social development (66-70). In mother-child conversational turn-taking, the mother is typically skilled at this type of coordination and interaction offering a potential source of input for learning from such interactions. Conversational turn-taking on the part of the mother has been correlated positively with mother-infant attachment (74), as well as more basic functions in speech and language development (71-73). Considering this, early conversational turn-taking in the mother-child dyad is a critical precursor for later development and establishes a basis by which children come to use language with others. Thus, additional research is required to build further consensus around specific aspects of impairment in children's conversational interaction in those exposed to prenatal alcohol consumption.

Thirdly, while previous studies have shown that it is crucial to explore the association of PAE and children's developmental outcomes, these have been primarily undertaken in HICs where sociodemographic and psychosocial risk factors differ from those seen in sub-Saharan African regions. Maternal exposure to violence, low birth weight, childhood under-nutrition and infectious diseases are more prevalent in LMICs, and such factors may be associated with greater risk for negative outcomes. This may be highly relevant to South Africa, where there is a particularly high prevalence of maternal depression, community or intimate partner violence and substance abuse (78).

This current study aimed to address some of these gaps in the literature by examining the associations of PAE and early neurodevelopmental outcomes in the richly characterised population of the DCHS. First, this thesis includes a systematic review of longitudinal studies on the associations between PAE, language, communication and speech outcomes during early childhood. Second, it presents an investigation of the association between PAE, motor, language and cognitive outcomes up to 2 years of age. Third, it explores the associations of PAE, including interactions with tobacco smoking exposure, and early neurodevelopmental trajectories up to 4 years of age. The fourth focus of this thesis includes an investigation of the conversational turn-taking between alcohol exposed mother-child dyads and their unexposed counterparts. The above-mentioned empirical studies formed part of the DCHS which reflects a LMIC population, in which there are high levels of sociodemographic and psychosocial risk factors and thus further work on the effects of PAE on child outcomes is highly relevant in a setting such as this, given the high-risk profile of mothers and children.

4. Structure of the dissertation

This thesis comprises seven chapters. Of these seven chapters, four chapters (Chapters 3 to 6) represent four journal articles which address the four primary objectives of the thesis. For this reason, it is to be expected that there will be some overlap of the literature in the chapters presented as articles.

5. Aim, objectives and hypothesis

This thesis aimed to investigate the association of PAE and early neurodevelopmental outcomes across 6, 24 and 42 months of age as part of the DCHS, a South African birth cohort.

6. Study objectives

6.1 to conduct a systematic review on the available longitudinal studies on the impact of PAE on language, speech and communication development, as well as to explore potential environmental confounders during the preschool period;

6.2 to investigate the association between PAE and early neurodevelopment through two years of age, and to adjust for sociodemographic and psychosocial risk factors;

6.3 to explore the association of PAE, including interactions with tobacco smoking exposure, and longitudinal neurodevelopmental trajectories across the first 4 years of life in the context of high sociodemographic and psychosocial risk factors.

6.4 to compare the conversational turn-taking between mothers and their alcohol exposed children to those between mothers and their unexposed children at the age of 42 months, and to adjust for sociodemographic and psychosocial risk factors

7. Hypotheses

7.1 Children with PAE will show lower neurodevelopmental outcomes than their unexposed counterparts after adjusting for sociodemographic and psychosocial confounders at 6 and 24 months;

7.2 Children with PAE, and comorbid tobacco smoking exposure, will demonstrate lower neurodevelopmental outcomes than their unexposed counterparts on trajectories of 6, 24 and 42 months of age;

7.3 Significant differences will occur between the conversational turn-taking of mothers and their PAE children with that between mothers and their unexposed children at 42 months testing age

The next chapter provides a detailed account of the DCHS, as well as methodological details relevant to the thesis as a whole. Chapters 3 to 6 comprise publications which address a specific research objective (6.1-6.4), as outlined above. Finally, Chapter 7 presents a general discussion and conclusion of the thesis, including research limitations, recommendations for future work in the field and concluding comments.

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CHAPTER 2

Methods

1. Introduction

In the previous chapter, the burden of prenatal alcohol exposure (PAE) and the effects of it on young children's neurodevelopmental outcomes were explored. This chapter presents information regarding a population-based birth cohort study, the Drakenstein Child Health Study (DCHS) (1, 2), which provides data to address the specific questions posed in this thesis. An overview of the DCHS, the sub-study and specific methodological information are discussed below.

2. Study design, context and population - Drakenstein Child Health Study (DCHS)

The DCHS is an ongoing birth cohort study exploring maternal and child health longitudinally in a poor, peri-urban sub-district in the Western Cape, South Africa, with a focus on paediatric lower respiratory tract infections (1, 2). It is located in the Drakenstein sub-district, nearby the Cape Winelands region which is further situated in the Western Cape Province of South Africa (N=1143 live births) (2). This study is situated in the town of Paarl, 60 km outside Cape Town with a population of approximately 200 000 people. This area comprises a centralized hospital, Paarl Hospital, where all births and hospital based paediatric care including admissions occur.

The Paarl Hospital provides obstetric care (including routine ultrasound evaluation at 20-28 weeks' gestation to all pregnant women) and all hospital based paediatric care. Pregnant women were enrolled from 20-28 weeks' gestation during their antenatal visit for ultrasound scanning at Paarl Hospital or at two primary health care clinics to ensure the cohort is representative of the local population (2, 3). The two primary health clinics are TC Newman (mixed-race community) and Mbekweni (a Black[1] African community) (2, 3). Comparable to many low and middle-income countries (LMICs), these communities have a high burden of childhood disease and pneumonia. There is also a high prevalence of maternal risk factors

[1] The racial groups, that is 'Coloured', 'Black', and 'Indian', were employed as racial categories within the Apartheid era to reinforce a segregated society and refer to those who were not afforded the same benefits as 'Whites' in this era. These terms are used here solely for descriptive purposes and does not imply acknowledgement of these terms by the author.

including alcohol consumption, tobacco smoke exposure, maternal depression, post-traumatic stress disorder (PTSD) malnutrition and poverty (3-6).

As described in previous publications (1, 2), pregnant women who were eligible to participate in the DCHS included those who were 18 years or older, had access to one of the two clinics for antenatal care and had intended to remain in the area for at least a year. The exclusion criteria comprised of those who did not live in the region and those who could not be readily followed-up or intended to move out of the district within the following 2 years.

The women forming part of the DCHS were included for screening by study staff at one of the two antenatal clinics in the community (4). They completed written consent forms in their preferred language namely, English, Afrikaans or isiXhosa. Following consent (Appendix E), questionnaires (Appendix B and C) and specimen collection were scheduled for antenatal and postnatal follow-up visits at the clinics.

At postnatal visits, the team ensured that the mothers received adequate information about the testing procedure. A considerable amount of effort was made to ensure a safe, confidential and a supportive environment. A private meeting room was provided for each study site for the administration of measures. Mothers who were willing to participate had an opportunity to ask questions about the testing process and were asked to complete a selection of clinician administered and self-reported questionnaires. Participants had the choice to not answer certain questions and furthermore remained in the study as long as their exposure and diagnostic status were determined reasonably. Maternal and child questionnaire data were uploaded on password protected storage drives under the participants' study number.

2.1 Inclusion and exclusion criteria of sub-study

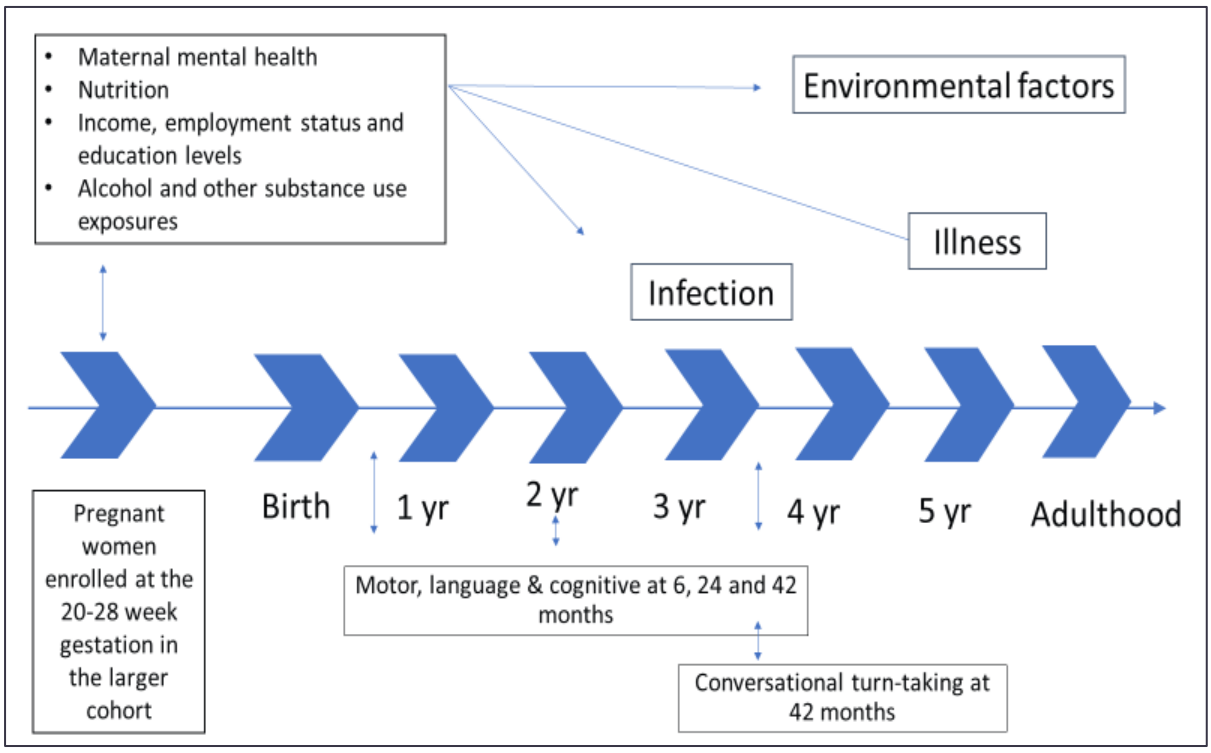
Participants who were included in the sub-study formed part of the DCHS (Figure 1). PAE was assessed using a composite, dichotomous classification (moderate-to-severely exposed vs. unexposed) combining the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) administered in the prenatal period and retrospectively collected data on hazardous alcohol use during pregnancy administered in the postnatal period (22). The inclusion criteria for moderate-to-severe alcohol exposure comprised of: (i) scores greater than moderate

consumption on the ASSIST antenatally (or having a minimum score of 11 on the alcohol questions); (ii) 2 or more drinks a week on the retrospective alcohol questionnaire according to quantity, timing and frequency of alcohol use in pregnancy (3-6 weeks and/or 24 months testing age) regarding alcohol use during pregnancy postnatally. The retrospective questionnaire was used on all mothers in the cohort. Thus, mothers were more likely to respond to questions in the postnatal period than during pregnancy (22). The unexposed control group included mothers with no exposure or a score of less than 11 on the ASSIST antenatally (4). Infants born prematurely or with any other congenital malformations, as well as sets of twins and triplets were excluded from the study.

Tobacco smoking exposure during pregnancy was measured by the ASSIST, antenatally. Women who had a minimum score of 4 on the smoking questions were coded as high-risk smokers while those with less than 4 as low risk smokers.

Women with other drug exposures were not excluded from the study as they comprised of very small sample sizes.

Figure 1. DCHS and sub-study timeline



2.2 Measures included in sub-study

Mothers were asked to complete a battery of measures at both antenatal and postnatal study visits in languages: English, Afrikaans or isiXhosa. These are dominant languages spoken in the Western Cape Province of South Africa. Translation of the measures from English to Afrikaans and isiXhosa included a standard forward and back-translation process (8). The measures were administered by trained fieldworkers and have been described in detail previously (1-3). Some of these antenatal measures include both sociodemographic and psychosocial assessments.

2.2.1 Sociodemographic measures. The social and demographic measures comprised of data on socioeconomic status (SES) (maternal income, education, employment status, and asset sum). Higher scores on this validated composite score indicated higher SES (11). Body mass index (BMI) for the child was also calculated.

2.2.2 Psychosocial measures. The psychological measures included data on maternal smoking (cigarette or cannibas) and psychiatric variables (symptom severity/frequency of PTSD and depression). Maternal smoking was assessed using the ASSIST (24), PTSD was measured using the Modified Posttraumatic Stress Disorder Symptom Scale (MPSS) (14), and depression was evaluated using the Beck Depression Inventory (BDI-II).

2.2.3 Modified Post Traumatic Stress Disorder Symptom Scale. The MPSS is a 17-item measure that was used to assess the frequency and intensity of PTSD symptoms (14). The MPSS measures three symptom clusters of PTSDs including re-experiencing, avoidance or emotional numbing and increased arousal (14). The items on this measure ranges from 0 (absence of a symptom) to 3 (symptom occurred five or more times per week or very much or almost always). This measure had reasonably good diagnostic validity for PTSD (19).

2.2.4 Beck Depression Inventory. This assessment was used to measure depressive symptoms and comprises of 21 items on the severity of symptoms ranging from 0 (absence of symptoms) to 3 (severe, often with functional impairment) (15). A total score was obtained by computing individual item responses, with higher scores indicating an increase in the severity of symptoms. A cut-off score of 20 was used to dichotomize participants into “probable moderate or severe clinical cases” versus “probable sub-threshold participants”

(17). The BDI-II has shown good validity and internal consistency when used in both psychiatric and non-psychiatric populations (15) and in South Africa (18).

2.2.5 Alcohol, Smoking and Substance Involvement Screening Test. As discussed above, the ASSIST was used to detect alcohol and/or substance use disorders in the primary care setting (Appendix D). This measure includes seven items with scores from 0-10 for alcohol and 0-3 for illicit drugs indicating low-risk, 11-26 for alcohol and 4-26 for illicit drugs indicating moderate-risk, and scores above 26 indicating high risk with the likelihood of alcohol or substance dependence. The higher the score, the greater the alcohol or substance-related risk. The ASSIST has good reliability scores and has been validated in numerous populations as well as primary care settings (16, 20). For the purpose of this study, the ASSIST was administered to women in the antenatal period, and although this measure provides retrospective data on alcohol consumption (over the last 3 months and lifetime use), it describes categories of alcohol use risk while the women are pregnant.

In addition to the ASSIST, alcohol data was collected from all the mothers in the postnatal period (i.e. at 3-6 weeks and/or 24 months testing age). As mentioned above, this retrospective measure gathered data from the World Health Organizations (WHOs) classification of moderate-to-severe levels of alcohol use on quantity, timing and frequency of alcohol use during pregnancy (moderate-to-severe use was described as having an average of 2 or more drinks a week in all of the three trimesters of pregnancy) (Appendix F).

The Bayley-III Scales of Infant Development (BSID-III) was administered at 6, 24 months and fine motor subscale at 42 months, while Peabody Picture Vocabulary Test (PPVT-IV) and Kaufman Assessment Battery for Children were administered at the 42-months.

2.2.6 Bayley Scales of Infant Development. The BSID-III was used to measure developmental functioning in young children and includes five scales: cognitive, language, motor, socio-emotional and adaptive behaviour scales. The motor scale is divided into gross and fine motor; the language scale is divided into receptive and expressive communication; and the adaptive behaviour scale into ten subscales including community use, communication, health, safety, self-care, pre-academics, leisure, self-care, self-direction and social (21). For this study, the motor, language and cognitive scales were directly administered to the infant or toddler.

Composite scores were created on the equivalents of the scaled scores, (based on scores with a mean of 10 and standard deviation of 3 ranging from 1-19). The BSID-III has been standardized in a sample of 1000 children from a United States population, stratified with respect to gender, race/ethnicity, geographic region, and parent education level (21). This measure has shown to be reliable for use in the South African population (22).

As mentioned previously, the motor scale includes fine and gross motor development (21). The fine motor subscale includes 66 items that assesses prehension, perceptual-motor integration, motor planning, speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills and responses to tactile information. The gross motor subset includes 72 items that assesses movement of the limbs, static positioning (e.g. sitting and standing), and dynamic movement including locomotion, coordination, balance and motor planning. For this study, the motor assessments were measured using directly observed items administered at 6, 24 and 42 months of age.

Receptive communication includes 49 items that assesses pre-verbal behaviours, vocabulary development (identifying objects and pictures), understanding morphological development (pronouns and prepositions), morphological markers (e.g. plural, tense markings and the possessive), social referencing and verbal comprehension (21). The expressive communication subscale includes 48 items that assesses pre-verbal communication (babbling and gesturing), vocabulary development (naming objects, pictures or naming attributes) and morpho-syntactic development (21). For this study, these subscales were directly administered at 6 and 24 months of age.

The cognitive scale assesses the child's performance in several areas, such as, visualization, memory and attention (21). This scale included items such as attention to familiar and unfamiliar objects, looking for a fallen object and pretend play. This was administered at 6 and 24 months of age.

2.2.7 Peabody Picture Vocabulary Test. The PPVT-IV was designed to assess children's receptive vocabulary according to Standard American English and provides an estimate of verbal ability or scholastic aptitude (23). It contains 228 full colour stimulus items, displaying 4 pictures per page and is arranged in 12 sets of generally increasing difficulty (17). The examiner reads out a word and thereafter the child responds by pointing to the picture they

think corresponds to the word the assessor has given. The test was untimed and takes approximately 10-15 minutes to complete. The PPVT-IV includes test-retest reliability correlations between 0.92 and 0.96, and internal consistency correlations between 0.94 and 0.95 (24). This measure was administered at 42 months of age.

2.2.8 Kauffman Assessment Battery. The KABC-II (psychological diagnostic test) is a clinical instrument of general cognitive function (25). It was developed to incorporate a wide age range of abilities and was designed to be a culture-fair tool measuring cognitive function in children aged 3.0 - 18.11 years (25-27). For the purpose of this study, the cognitive skills (nonverbal index) and expressive vocabulary subscales were utilized at 42 months of age. This measure showed good reliability with an alpha level of 0.78 (27).

Piloting of the measures above took place prior to data collection to ensure fidelity to specific instructions for test administration and data scoring. Trained paediatric occupational therapists and/or physiotherapists administered the measures at 6, 24 or 42 months of age. Research assistants (MA Neuropsychology students) were also trained to administer the measures. Assessors had background experience in paediatric clinical and research environments and were blinded to the exposure status of the infants and toddlers. Assessors were trained by a paediatric neurologist (K.A. Donald) at 6- and 24-months testing. KAD attended regular site visits to confirm compliance with test administration and techniques. At 42 months of testing, assessors were trained by a neuropsychologist (C. du Plooy). CD attended regular site visits to approve compliance with test supervision and procedures. After completion, the assessors checked measures to ensure scoring precision.

Conversational turn-taking was assessed between mothers and their children at 42 months of age, as described below.

2.2.9 Conversational turn-taking. Conversational turn-taking was assessed for both mothers and their children using audio-video tape recordings. It included four elements developed by Kaye and Charney (29): an utterance from the first speaker, an overlapping or alternating utterance, and an utterance from the second speaker (28). Conversational turn-taking comprised of utterances involving at least one switching pause or an overlapping utterance by the mother or child (28-30).

i. Utterance. An utterance unit is defined as a stretch of one child's speech separated by the speech of either the child or mother. An utterance is a word sequence that is terminated by an overlap ([ ]), a short pause period (..), or a long pause period (…). If there was an overlap or pause in between the word sequence, they were separated into two utterances by either the mother or child.

ii. **Overlapping**. Overlapping utterances are defined as a short period of overlapping speech, occurring either when the mother or child utter words over each other.

iii. **Utterances preceded switching pauses**. Switching pauses occurred between either the mother or child's utterances. These were presented as moderate or long pauses. Moderate pauses ranged between 0-3000ms and long pauses were greater than 3000ms.

2.2.10 Procedure for video-audio recordings. After discussing and completing informed consent forms, the mother and child were settled in a comfortable position facing each other. The video and microphone were placed near the mother and child. For the video-audio recordings, the assessors informed the mother to engage in play with the toddler as they normally would by using a set of blocks, dolls or additional toys for approximately 10 minutes. In the first 5 minutes, blocks were provided, and in the following 5 minutes dolls and animal toys were provided. These were age appropriate toys for toddlers at 42-month assessments. Conversational turn-taking was manually coded using the Computerized Language Analysis (CLAN) program of the Child Language Data Exchange System (31). This was applied to derive the frequencies of conversational turn-taking sequences. The data was stored in a safe and secure place.

2.3 Statistical analysis

The Statistical Package of Social Sciences (SPSS-25) and R (version 3.6.1) were used to analyse the data which included both descriptive and multivariate statistics (32).

Descriptive statistics included frequencies, percentages, means (SD) and medians (IQR) were presented for either normal or not normally distributed data. For comparisons between alcohol and unexposed groups, chi-squared tests were used for categorical variables (sociodemographic and psychosocial variables). For normally distributed data, independent sample t-tests were used to explore differences between groups regarding the developmental

outcomes. In the case of data that were not normally distributed, Mann-Whitney U tests were used to compare the alcohol and unexposed groups.

2.3.1 Cross-sectional analyses

Multiple linear regression was used to explore the associations between PAE, motor, language and cognitive development at 6 and 24 months (at each age). The model adjusted for the maternal sociodemographic and psychosocial confounding variables, which are known to be associated with child neurodevelopment (motor, language and cognitive). The confounding variables included maternal SES, smoking, BMI, and risk of PTSD and depression. Significance was set at 0.05 and 95% CIs were reported for all estimates.

Multiple logistic regression was used to explore the associations between PAE, overlapping utterances and utterances preceded by pauses at 42 months. This model adjusted for potential sociodemographic and psychosocial confounders (maternal clinic, SES, BMI, smoking, PTSD and depression). Significance was set at 0.05 and 95% CIs were reported for all estimates. The data was reshaped to a long format. Hence, the predictor and outcome variables contain information of the baseline and follow-up visits.

2.3.2 Longitudinal analyses

Longitudinal multilevel linear models were applied to examine the associations of PAE and fine motor, language and cognitive outcomes at 6, 24 and 42 months of age. These models were constructed to compare the alcohol exposed and unexposed groups on a longitudinal growth of child neurodevelopment. The model adjusted for potential confounding variables (SES, smoking, PTSD or depression)

2.4 Ethics and procedure

The DCHS study was approved by the Faculty of Health Sciences Human Research Ethics Committees of the University of Cape Town (UCT) and Stellenbosch University in South Africa, as well as by the Western Cape Department of Health Provincial Research Committee. The sub-study was further approved by the Faculty of Health Sciences Human Research Ethics Committees of the University of Cape Town (Appendix A). Pregnant mothers provided informed written consent at enrolment and re-consented annually following childbirth. Only

those who provided informed consent were included in the study. Individuals who did not participate in the study were assured that this would not bias them or be held against them in the future. Confidentiality and anonymity were reassured at each timepoint. A private meeting room was provided at each clinic site. Mothers had the opportunity to choose not to answer certain questions and remained in the study as long as their exposure and diagnostic status could be determined reasonably. Mothers had the opportunity to withdraw from the study without affecting their participation in the larger project at a later stage. The assessors were blinded to the exposure status of the mothers and toddlers in order to maintain confidentiality. The team ensured that the mothers received enough information about the study and testing process and provided answers to any questions that they had. Maternal and child questionnaire data and video-audio recordings were uploaded on password protected storage drives under the participant's study number. The material was securely stored, and only researchers' that were part of the DCHS had direct access to it.

The next chapter (based on Publication 1) addresses the first objective of this thesis and will delineate a systematic review of longitudinal studies exploring the associations between PAE, speech, language and communication outcomes.

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CHAPTER 3

Publication 1

Effects of prenatal alcohol exposure on language, speech and communication outcomes: a review of longitudinal studies

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Counts: Abstract = 225 words; Text = 4319; References = 30; Tables = 4; Figure = 1

Synopsis

In the previous chapter, an overview of the Drakenstein Child Health Study (DCHS) and the methodology of the sub-study were discussed. This chapter presents existing longitudinal studies on the effects of PAE on language, speech and communication development during the preschool period. This is presented in Research Objective 1 of this thesis (see Chapter 1, Section 6.1 - p 27):

To conduct a systematic review on the available longitudinal studies on the impact of PAE on language, speech and communication development, as well as to explore potential environmental confounders during the preschool period.

The manuscript on which this chapter is based was submitted to the Acta Neuropsychiatrica journal which focuses on translational neuropsychiatry and is currently published.

Abstract

Introduction: Previous studies have shown that prenatal alcohol exposure (PAE) results in increased risk for poor health, cognitive and language outcomes and is a major contributing factor to the global burden of disease. The aim of this paper was to provide a systematic review and update on the available longitudinal studies on the impact of PAE on language, speech and communication development, as well as associated potential environmental confounders during the preschool period.

Methods: A literature search was restricted to English, full-text, peer reviewed, longitudinal studies in from 1970 until present: PUBMed, Scopus, Web of Science {C-e Collection, Biological Abstracts, KCI-Kean Journal Database, Russian Science Citation Index, SciELO Citation Index, Zoological Rec-d}, Academic Search Premier (Africa-Wide Information, CINAHL, MEDLINE and PsycINFO. Keywords included: prenatal alcohol exposure; speech or language or communication outcomes; neurocognitive or neurodevelopment or neurobehavioral or neurobehavioural; infant or baby or toddler or pre-schooler; and longitudinal or follow-up. The inclusion criteria included (i) cohorts with at least 2 timepoints; (ii) association of light, moderate or heavy PAE on language, speech or communication delay, development or disorder; (iii) environmental confounders; (iv) infants up to preschool age.

Results: Six studies satisfied the threshold for inclusion. Three studies reported that PAE was significantly associated with receptive or expressive communication. These studies demonstrated lower scores on either receptive or expressive communication in the alcohol group in comparison to the unexposed group, even after controlling for environmental factors up to 36 months.

Conclusion: Evidence from the longitudinal studies reviewed suggest that PAE influenced delays in receptive and expressive communication up to 36 months. Contextual risk factors played a significant role in language development over time and especially as children approached school age.

Keywords: child, communication, language, prenatal alcohol exposure, preschool, speech

1. Introduction

As shown in chapter 1, prenatal alcohol exposure (PAE) is an important public health concern that results in increased risk for poor general health and cognitive outcomes and is a major contributing factor to the global burden of disease. Globally, about 10% of women consume alcohol during pregnancy (1), and one of every 67 of these women delivered a child with foetal alcohol syndrome (FAS) (2-3). Recent data from the World Health Organization (WHO) reported a global prevalence of FAS ranging between 2 and 7 per 1000 (4), and foetal alcohol spectrum disorders (FASD) between 0 and 176.77 per 1000 (5). In South Africa, there are regional variations in prevalence rates, often linked to high risk populations with 55.42 per 1000 affected by FAS and 113.22 per 1000 affected by FASD in certain communities (6). Adding to this concern is the growing literature demonstrating that moderate and severe PAE is a significant risk factor for cognitive, language, speech or communication impairments, which may in turn have far-reaching effects on subsequent cognitive, socioemotional and educational outcomes.

The umbrella term, FASD, included a range of effects that can occur when an individual is prenatally exposed to alcohol. These included FAS, which is described as the teratogenic effects of alcohol on the developing foetus and includes growth retardation; central nervous system impairment; and facial features of short palpebral fissures, thin upper lip, and elongated, flattened mid-face (6). Foetal alcohol effects (FAE), on the other hand, may be evident in infants and children with exposure to alcohol, but who have only some of the characteristics of FAS. Alcohol related neurodevelopmental disorder (ARND) and alcohol-related birth defects (ARBD) are only diagnosed in children without physical features of FAS or PFAS (6).

Exposure to alcohol in foetal development has been associated with a range of cognitive delays, as well as language or speech deficits among children (7-9). As early as the 1970s, Iosub and colleagues (10) identified four generic communication disturbances including speech, language, voice and fluency delays. Language impairments in children with FASDs which have been described to date include problems in speech discrimination, comprehension, syntax development, prosodic features, rules of dialogue poor performance in sentence combining (11) expressive delays (12), working memory deficits (13) and

disturbances in semantic elaboration (14). Previous research by Schonfeld and colleagues (15) suggests that while a child with FAS or FAE may present with delays in speech, they may also display impaired pragmatic language use which may result in poor peer relationships and thus significant social and communicative problems. Preschool children with PAE have reported to exhibit social communication deficits and adverse social interactive experiences (16, 17). These behavioural deficits have been postulated to be due to the impact of PAE on frontal lobe development and function. For the child with a FASD, speech and language disorders appear to have been reported as a part of their impairment profile commonly, while sociocommunicative outcomes have been investigated and reported less frequently (16, 17). Previous research has also shown that these children may be particularly vulnerable to language deficits as a result of both the specific effects of PAE as well as the associated impact of both intrinsic and extrinsic risk factors such as maternal depression, poor nutrition and other sociodemographic factors (11-15,18).

Studies exploring the impact of PAE on the progression of language, speech or communication, which include consideration of the impact of environmental factors remain less common. Several additional questions remain about the nature of the associations of PAE, risk and protective factors on language development of young children across the course of the first years of life.

When considering the current literature on the impact of PAE on cognitive and language functioning, most published studies report on cross-sectional studies and convenience samples of modest size. Although such studies have been valuable in advancing research in this area, they have an important limitation - they prevent making strong causal inferences on language development over time. Only through longitudinal designs may cause and effect be determined, whilst acknowledging any confounding factors which may exert important additional influences on outcomes in these children.

To our knowledge, only two reviews with a specific focus on PAE and child language development have been carried out (19, 20). Both reviews included cross-sectional studies (19, 20) which may provide a weaker level of evidence than that of available longitudinal studies (20). Because cross-sectional studies occur at a single point in time, this form of research is prone to potential bias. Selection bias may occur should the range of children be

restricted to a combination of retrospective accounts of alcohol and other drug exposure resulting in high or low susceptibility for developing language delay and, additionally, if the exposure differs in alcohol or other drug use, it may differ in predicting language outcomes (20). Cone-Wesson (20) defined areas of language impairment specific to FAS only, while Abkarian (19) explores the effects of FAS and FAE on the communication abilities of children. As a result of their specific focus, these reviews may have been less inclusive of the available literature than a systematic review approach would have allowed and likewise, did not include a discussion of the association of PAE with language developmental trajectories across time. The aim of this paper was to provide a systematic review and update on the available longitudinal studies on the impact of PAE on language, speech and communication development, as well as associated potential environmental confounders during the preschool period.

2. Methods

2.1 Article search strategy

Full-text, peer-reviewed, electronic resources for the following databases were restricted in English from the year 1970 until present: PUBMed, Scopus, Web of Science {C-e Collection, Biological Abstracts, KCI-Kean Journal Database, Russian Science Citation Index, SciELO Citation Index, Zoological Rec-d}, Academic Search Premier (Africa-Wide Information, CINAHL, MEDLINE, PsycINFO). Keywords included: Prenatal Alcohol Exposure (PAE) or Foetal Alcohol Syndrome or Fetal Alcohol Syndrome (FAS) or Partial Foetal Alcohol Syndrome or Partial Fetal Alcohol Syndrome (pFAS) or Alcohol-Related Birth Defects or Alcohol-Related Neurodevelopmental Disorder (ARND) or Foetal Alcohol Effects or Fetal Alcohol Effects (FAE); speech or language or communication outcomes; neurocognitive or neurodevelopment or neurobehavioral or neurobehavioural; infant or baby or toddler or preschooler or pre-schooler; longitudinal or follow-up.

Titles, abstracts and articles were screened to determine if publications met the following inclusion criteria. Inclusion criteria for the review were (i) cohort studies with at least 2 timepoints; (ii) association of light, moderate or heavy PAE on language, speech or communication delay, development or disorder; (iii) from infancy (birth to 2 years old) up to

preschool age (6 years). Longitudinal research included data that was collected for each variable for two or more distinct periods, same or similar measures, a comparable group, and the analysis involved some comparison of data between or among periods (21). Articles that met the inclusion criteria were examined against a list of exclusion criteria. Studies presenting solely animal, cross-sectional analyses, clinical studies, case reports, comments, letters, and reviews were excluded. Studies reporting other cognitive or developmental outcomes were excluded, unless language, speech or communication was specifically explored as an outcome measure.

2.2 Quality assessment

The articles included in this review were rigorously assessed using the quality assessment tool for systematic reviews of observational studies (QATSO) (Tables 1 and 2) (22). The checklist was developed based on epidemiological principles, reviews of study designs, and existing checklists for the assessment of observational studies (22). The QATSO covers the following aspects (i) external validity (1 item) - addresses the extent to which the findings from the study can be generalized to the population from which the study subjects are derived; (ii) reporting (2 items) - assesses whether the information provided in the paper is sufficient to allow a reader to make an unbiased assessment of the findings of the study; (iii) bias (1 item) - addresses bias in the measurement of the outcomes in a study; and (iv) confounding (1 item) - addresses whether studies have applied adjustment for confounders in the analysis. This item is specific to studies concerning association of risk factors. Although the QATSO score consists of five items, users may select four to five items depending on the type of studies.

2.3 Data synthesis

In this review, a narrative synthesis of the findings from the primary studies was completed to deliver a textual answer to the review question. The following steps delineated by Popay and colleagues include (i) preliminary analysis (ii) and thematic analysis (23). Preliminary synthesis comprised of extracting descriptive characteristics of the studies into a table and producing a textual summary of the results. This method synthesized the selected studies for the review according to the following: the domains of interest, sample size, context, age, method of data collection and data analysis, and main results (Tables 3 and 4) (23). Thematic

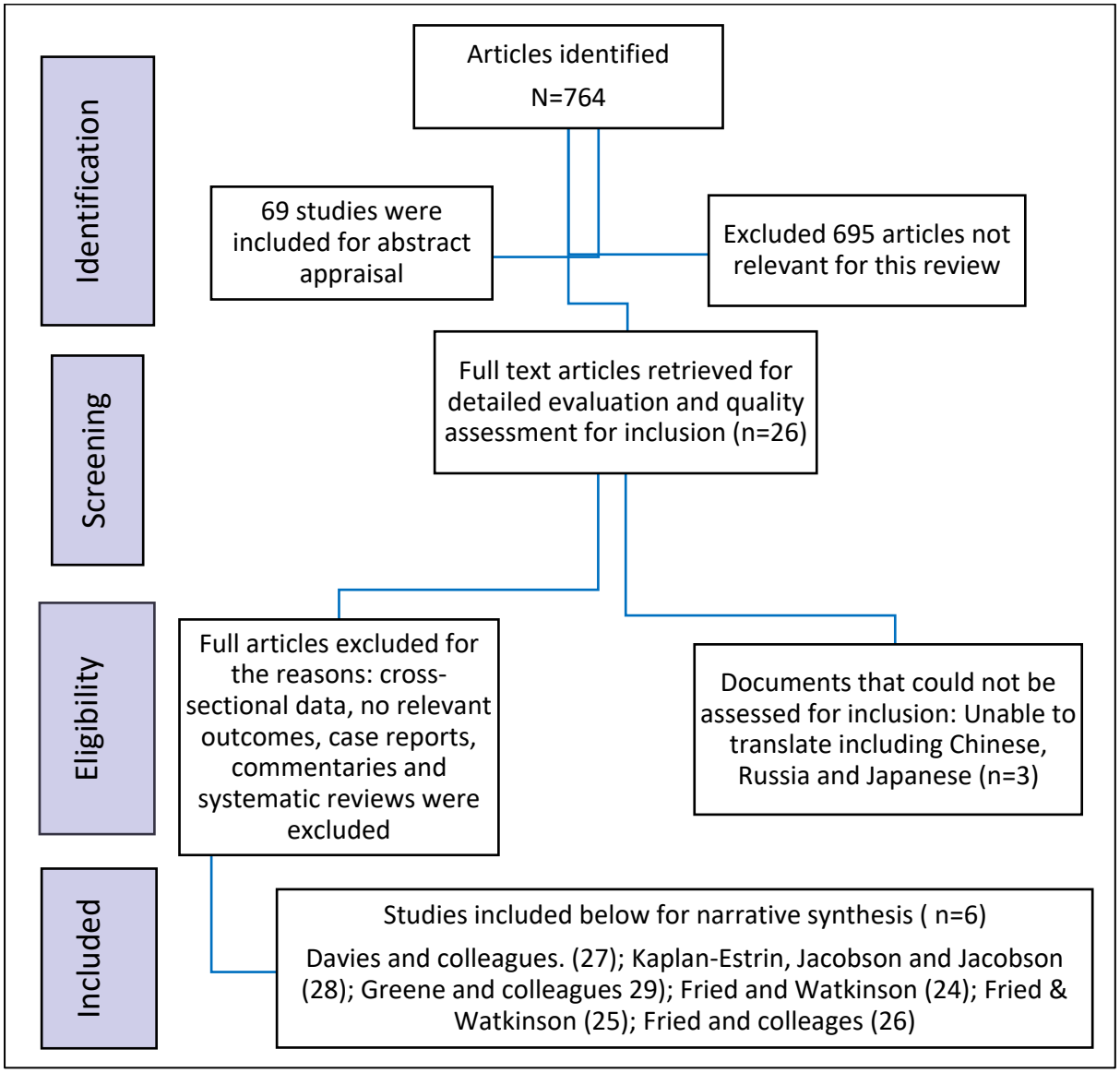
analysis was then used to extract the main themes from the studies. The two themes developed in the results represent the main areas of longitudinal knowledge available on early language development in the context of PAE. Due to the heterogeneous nature of the exposure and outcomes across the studies included, it was not deemed appropriate to conduct a meta-analysis.

3. Results

3.1 Article search procedure

The search for the review produced a total of 764 articles from the specified databases (Figure 1). Based on a subsequent title search, 69 studies were included for abstract appraisal, excluding 695 studies as they did not meet the inclusion criteria. Screening of titles, abstracts and articles for the inclusion and exclusion criteria produced a total of 26 relevant publications. Of the 26 studies, 20 studies did not have data on language, speech or communication outcomes on two timepoints that could be extracted and were therefore excluded. Six studies met all the eligibility criteria for the review and scored above 67% on the QATSA indicating adequate quality for inclusion (Figure 1 and Table 2). The descriptive characteristics of the six studies that were selected are displayed in Table 4, including the domains of interest, sample size, context, age, method of data collection and data analysis, and main results. A systematic account of how the studies were conceptualized, their focus of research, context, method and findings are provided below.

Figure 1: Article search and appraisal process



Two themes were extracted from the literature selected: (i) the effect of PAE on receptive, expressive delay and speech delay; (ii) the contextual risk factors associated with PAE on early receptive, expressive communication and speech acquisition. The content of the themes from the studies was achieved by the themes that emerged from an inductive analysis of study findings to answer the review question. These themes provided an overview of the main areas of longitudinal knowledge and a comparison of data between or among periods on early language development in the context of PAE.

The broad themes identified in this review include (Table 4):

(1) Effect of PAE on receptive, expressive development and speech acquisition.

(2) Contextual risk factors associated with PAE on early receptive, expressive communication and speech acquisition.

Theme 1: The effect of PAE on receptive, expressive development and speech acquisition

This theme comprised of six studies exploring the association of PAE with receptive, expressive communication and speech acquisition over at least two timepoints in the preschool age (Table 3 and 4). Three studies from one cohort investigated the neurodevelopmental outcomes (motor, language and cognitive development) of children with prenatal exposure to marijuana, cigarettes or alcohol (24-26), while others explored the impact of PAE on developmental performance (locomotor, personal, social, hearing and language, eye and hand movements) (27). Other previous studies also examined the impact of PAE on neurobehavioral outcomes (including communication) (28), language and speech acquisition specifically (29).

The studies in this review were primarily undertaken in well-resourced countries, such as, North America and Canada in comparison to one study in South Africa. The Ottawa Prenatal Prospective Study, for example, included cohorts ranging between 12 and 24 months (24), 36 and 48 months (25), 60 and 72 months (26), 7-12 months and 17-21 months (27). The rest of the studies included age categories namely 6.5, 13 and 26 months (28); and 12, 24 and 36 months of age (29). Three studies recruited women from public hospitals (27-29), and three prospective studies recruited women through the media or at voluntary sign-up locations (24-26). In addition, sample sizes of these studies were relatively small. All the studies had two assessment timepoints at follow-up visits, with low attrition between the first and last assessment. In four studies, mothers were interviewed once in each trimester during pregnancy, and alcohol consumption was broken down into beer, wine and liquor with both the quantity and pattern of consumption recorded and then converted to average ounces of absolute alcohol[2] (AA) per day (24-26, 29). None or light alcohol consumption contained less than 0.14 oz AA daily, while heavy alcohol consumption comprised more than *0.85 oz AA* daily

[2] Absolute alcohol (AA) is a common name for the chemical compound ethanol.

(24-26). Control groups were categorized as having none or light to low levels of alcohol exposure (24-26). One study under this theme utilized two specialist clinicians with dysmorphology trained personnel to diagnose FASD (FAS and pFAS) amongst infants and children (27). In this study (27), infants who did not meet criteria for a FAS or pFAS diagnosis formed the non-FASD comparison group. Child measures included the Bayley Scales of Infant Development (BSID) (24), the Griffiths Mental Developmental Scales (GMDS) (27), the Reynell Developmental Language Scales (RDLS), the McCarthy Scales of Children's Abilities (MSCA) (25, 26); and the Peabody Picture Vocabulary Test (PPVT) (26). For child speech acquisition, data was coded from a taped language production to determine the Mean Length of Utterance (MLU) (28), and the Early Language Milestone scale (ELM) was used to assess the intelligibility of the child's utterances (29). Three studies adjusted for at least maternal age, sex and home environment (24-26). The Home Observation for Measurement of the Environment inventory included information collected by observation of the child's home environment. Statistical analyses techniques varied from study to study and included Wilcoxon rank-sum test (27), linear regression (24, 28, 29) approaches and discriminant function analysis (25, 26). Most of the studies measured alcohol consumption using self-report measures.

Six studies investigated PAE as a predictor and language as an outcome allowing for a longitudinal assessment of the association between the variables and exploring sociodemographic and home environment as confounders up to 24 months. Three of the studies formed part of the Ottawa Prenatal Prospective Study where 700 women were interviewed regarding their prenatal alcohol use, from which small embedded cohorts were selected for follow-up. These studies reported heavy PAE as significantly associated with language, and that the alcohol exposed group scored lower than the unexposed group (24, 25, 27). According to Fried and Watkinson, there were significant associations between heavy PAE, vocalization and the onset of words at 12 months (24). By time two (24 months), language outcomes were significantly lower in the exposed group in comparison to the control group (24). After adjusting for the child's home environment, language impairments among prenatally alcohol exposed children did not continue to persist. While PAE had a significant impact on very early language development, the authors also recommended

assessing alcohol and cigarette use together in subsequent studies (23, 24). Additional studies explored language development as a result of PAE up to 72 months. In the follow-up sample by Fried and Watkinson (25), heavy alcohol exposure was significantly associated with receptive communication delay by time one (36 months). Lower scores on language outcomes in the PAE group than the control group were reported, even after adjusting for home environmental factors and other prenatal substance exposures such as cigarette and marijuana use. When heavy alcohol consumption interacted with heavy nicotine consumption, more severe delays occurred in language performance at 36 months than when looking at PAE alone. In addition, adjusting for home environment, exposure to other substance use reported and parental education did not alter this relationship in that specific cohort (25). The authors concluded that although there appeared to be an association between both maternal cigarette and alcohol use during pregnancy with language performance, this did not necessarily predicate a causal connection. However, they reported that the evidence does justify a careful assessment of the possible role of the combined effect of PAE and prenatal exposure to other substances in the prevention of a child reaching his or her full developmental potential.

Davies and colleagues (27) reported that the FASD (FAS and pFAS) group had significantly lower scores on language performance in comparison to non-FASD group at both time 1 (7-12 months) and time 2 (17-21 months) (27). At time one, the greatest decrease in performance was in the locomotor abilities, and by time two, the greatest decrease in performance was in the receptive and expressive communicative abilities in comparison to the other domains. While this study had the shortest-term outcome measurement, and the smallest cohort of alcohol exposed children (n=45 at time 1; n=35 at time 2), the authors evaluated the associated socioeconomic (SES) factors as having a potential role in putting children with PAE at further risk for poor developmental outcomes (27). The findings showed decrease language performance among the heavily exposed groups**.**

The results of two studies (25, 26) examining the effects of heavy PAE on language abilities showed no significant associations. Fried and Watkinson (25) found that by 48 months of age, no effect on language outcomes was found in their cohort despite reporting poorer language outcomes at an earlier timepoint as described above. Similarly, Fried and colleagues (26)

found no significant effect of PAE on comprehension at 60 and 72 months in their group. The strongest predictor for productive vocabulary development was the child's age at testing and home environment.

Light or moderate PAE was not significantly associated with language development and speech acquisition up to 3 years of age (28, 29). However, neither of the studies (28, 29) were designed to clarify the characteristics of a control group. When control groups in a longitudinal study are not discussed, there is a potential for important confounding variables that may invalidate the study results and would require complicated statistical analyses to make effective inferences about the sample.

Theme 2: Contextual risk factors associated with PAE and the effect it has on early receptive, expressive communication and speech acquisition

The studies in this thematic domain focused on a cohort exploring the risk factors associated with PAE, and the effect these had on language and speech acquisition in this group of children (Table 4). Three of the included studies explored demographic, physical and environmental factors as potential confounders in the association between PAE and language development (see Table 4). The pattern of results suggested that birth weight and quality of the caretaking environment are more sensitive indicators than PAE on subsequent language development, as well as for successful receptive and expressive communication (26) and speech acquisition (28, 29) especially as children approach school age. Findings from Davies and colleagues (27) indicated that children scored within the normal range on language assessments during early infancy, however, as they grew older, their language proficiency became significantly impaired and their home environment had a significant impact on their language outcomes rather than PAE. Most of the studies described here explored only sociodemographic and home environmental factors, and not more intrinsic psychosocial factors such as maternal depression or post-traumatic stress disorder (PTSD) that may have an important impact on language development.

4. Discussion

The aim of this review was to examine the evidence from a well-defined set of studies that have taken a longitudinal approach to the research of PAE in relation to language, speech and communication development up to preschool age. The search strategy identified a relatively small group of six studies, which highlights the general paucity of longitudinal research in this crucial area. There were somewhat mixed results. Some groups demonstrated significant, negative associations between moderate-to-heavy levels of PAE and language development over the first three years of life (24, 25, 27). This was observed after adjusting for a wide variety of sociodemographic, physiological and environmental variables. Studies reporting on language outcomes over time in slightly older children approaching school age did not demonstrate the same effects.

Data from previous studies reinforced the point that in many parts of the globe, young children face considerable language challenges in the early phases of life as a result of PAE. Longitudinal examinations on PAE demonstrate a negative effect on the trajectory of language development over the first 3 years of life. These studies revealed that there was a delay in receptive and expressive communication among heavy PAE children in comparison to their unexposed counterparts after adjusting for a wide variety of sociodemographic, physiological and environmental variables. In addition, as children grew older, the strongest significant predictor for receptive vocabulary was the child's age at testing and home environment. Further support for this finding is reported in the work by Melhuish and colleagues (30) on home learning as a powerful predictor of language production and reading skills. The evidence further suggests that home environment supports language acquisition in a number of ways but in particular, by providing children with opportunities for communicative experiences, which further motivates the language acquisition process.

Exploring the association between factors such as PAE and low SES, may be particularly important for the development of language in the early years of life. The high levels of both PAE and reported low SES observed is consistent with previous literature which demonstrated a clear association between alcohol use and poverty. Coggins and colleagues (16) particularly noted the impact of negative caregiving environments and heavy PAE on the evolving

sociocommunicative abilities in children. Studies such as these highlight the complex cumulative model of risk according to which a single factor may not be influential by itself, whereas its predictive value might be moderated by the association with other risk factors. The effects of PAE on the developing language trajectory argues for the prioritization of studies which include an extensive longitudinal approach to understanding potential risk and protective factors. Additional inclusion of factors such as SES, maternal psychopathology, parenting styles and conversational patterns are also likely to be relevant.

It is also important to note that the amount of alcohol consumed in the Ottawa Prenatal Prospective study was relatively low. The average amount of alcohol consumed by pregnant mothers in this sample was *0.14 oz* (alcohol per day) and of the 70 women who consumed alcohol, only four drank three times a day. There is a considerable body of literature that PAE-associated effects are potentiated by high risk environments such as those found in many low- and middle-income countries (LMICs) (27), including South Africa, and one must be cautious in extrapolating the results of these studies to other populations.

4.1 Assessment of quality

Prior publications provide a solid base for conclusions concerning some of the more general effects of PAE on developmental performance in young children. Only one study explicitly explored the effects of PAE on the language outcomes in their original design (29). This meant that the other five studies included language as one of the many variables of interest for the outcomes examined.

Methodological limitations within some of the longitudinal studies included lack of control groups (28, 29), use of small sample sizes and skewed selections of advantaged families (24-26), as well as self-report tools (24-28). The lack of unexposed control groups in some of the studies meant that researchers were not fully able to examine the differing developmental trajectories between groups over time. Second, the use of small, high SES samples may not be generalizable to disadvantaged communities or countries. Third, majority of studies measured alcohol exposure using diagnostic assessments. The criteria for PAE in these studies include women who meet the criteria for alcohol abuse and dependence, with a lack of information on the alcohol exposure effects in the children. Further, while there is a large

amount of self-report maternal measures on language development in the children and there is a paucity of investigation of the additional contextual risk and protective factors in the mother-child dyad.

Despite the small number of studies, the longitudinal association between PAE and language outcomes described in this review may highlight important factors to consider in future studies investigating this area: (i) including adequate unexposed control groups from similar community backgrounds, (ii) larger sample sizes, (iii) samples to include children from socioeconomically disadvantaged communities and from low- and middle-income countries, (iv) diagnostic assessments of FASD's where possible, (v) additional data collection on risk and protective factors which may additionally impact developmental outcomes in this high-risk group of children, and (vi) more complex longitudinal analyses.

4.2 Limitations of the study

The current study was not without limitations. Only studies published in English were included in the review, possibly biasing results. Moreover, three of the reviewed studies were conducted in Canada (on the same cohort of children), two in the United States of America and one in South Africa (potentially reducing the relevance to other countries). The strategies identified by the current review hope to provide other researchers with guidance for fully exploring the impact of PAE on language development in very young children.

The findings of this review indicated that (i) children with PAE experienced language delay up to 3 years of age (ii) additional contextual risk factors played a significant role in the language development over time and especially as children approached school age. Further research on child neurodevelopment in children with PAE, as well as the risk or protective factors in alcohol exposed children are needed and may be important for designing more effective and preventive interventions for young children.

The next chapter (based on Publication 2) will address the second research objective of this thesis and will explore the association between PAE and early neurodevelopmental outcomes as part of the Drakenstein Child Health Study (DCHS).

Table 1. Quality appraisal tool

Checklist for PAE, speech, language and communication outcomes			
1. Sampling method: Was it representative of the population intended in the study?			
Non-probability sampling (including purposive, quota, convenience and snowball sampling)			0
Probability sampling			`1
2. Was the measurement of prenatal alcohol exposure explored as objective, and the measurement tool valid and reliable? (or were psychometric properties provided?)			
No			0
Yes			1
3. Was a response rate mentioned in the study? If the reported response rate is below 60%, the question should be answered 'No'.)			
No			0
Yes			1
4. Did the investigator(s) control for confounding factors (e.g. stratification/ matching/restriction/adjustment) when analysing the associations			
No			0
Yes			1
5. Was privacy or sensitivity of the nature of prenatal alcohol exposure considered when the survey was conducted e.g. if conducted in a general clinic setting?			
No			0
Yes			1
Methodological appraisal score			
Poor (%)	Fair (%)	Good (%)	
0–33	34–66	67–100	

Table 2. Quality appraisal of included studies

Authors of publications	Q1	Q2	Q3	Q4	Q5	%	Outcome
Fried and Watkinson (24)	1	1	1	1	1	100	Include
Fried and Watkinson (25)	1	1	1	1	1	100	Include
Fried and colleagues (26)	1	1	1	1	1	100	Include
Davies and colleagues (27)	1	1	1	0	1	80	Include
Greene and colleagues (29)	1	1	1	1	1	100	Include
Kaplan-Estrin and colleagues (28)	1	1	1	1	1	100	Include

Table 3. Type of research articles

Article type	Authors	Number of studies
Receptive, expressive communication and alcohol exposure in the prenatal period	i.Fried and Watkinson (24); ii. Fried and Watkinson (25); iii. Fried and colleagues (26); v. Davies and colleagues 27)	4
Speech acquisition and alcohol exposure in the prenatal period	vi. Greene and colleagues (29); vii. Kaplan-Estrin and colleagues (28)	2

Table 4. Summary of studies on prenatal alcohol exposure, receptive, expressive communication and speech acquisition

Author	Domains	n = PAE	n = control	Context	Age	Method of data collection and analysis	Findings
Fried and Watkinson (24)	Maternal use of marijuana, cigarettes and alcohol; motor, mental and language development N=700 (large study cohort)			Ottawa, Canada		Maternal interviews on alcohol, cigarette and marijuana; Home Observation for Measurement of the Environment (HOME)	
						Analysis: Multiple regression	
		n=203 (12 months)	n=166		12 months	Bayley Scales of Infant Development (BSID)	Significant association between heavy PAE and cognitive performance.
		n=146 (24 months)	n=30		24 months	BSID & Reynell Developmental Language Scales (RDLS); HOME	Significant association between heavy PAE, expressive abilities. Significantly lower scores in the exposed group in comparison to the CON group. Significant receptive communication delay after controlling for socio-demographic, physical and environmental factors.

						Comment: PAE predicted lower levels of vocalization in comparison to the CON group.	
Fried and Watkinson (25)	Maternal use of marijuana, cigarettes and alcohol; motor, mental and language development	n=698 (large study cohort)		Ottawa, Canada		Maternal interviews on alcohol, cigarette and marijuana; HOME	
						Analysis: Discriminant function analysis	
		n=133	n=50		36 months	McCarthy Scales of Children's Abilities; Reynell Developmental Language Scales (RDLS) Tactile Form Recognition Task	Significant association between receptive vocabulary performance and heavy PAE. Significantly lower scores in the exposed group in comparison to the CON group at 36 months only. Significant receptive vocabulary delay after controlling for socio-demographic, physical and environmental outcomes.
		n=130	n=59		48 months	Peabody Vocabulary Test Reynell Developmental Language Scales	No significant association between PAE, expressive and receptive abilities.
						Comment: PAE predicted no change in language development at 48 months.	

Fried,O'Connell and Watkinson(26)	Maternal use of marijuana, cigarettes and alcohol Cognitive and language development	n=698 (large study cohort)		Ottawa, Canada		Maternal interviews on alcohol, cigarette and marijuana. HOME	
						Analysis: Discriminant function analysis	
		N = 135	N = 64		60 months	McCarthy Scales of Children's Abilities; Peabody Picture Vocabulary Test (PVT)	No significant association between PAE, expressive and receptive abilities.
		N = 136	N = 70		72 months	McCarthy Scales of Children's Abilities; PVT	No significant association between PAE, expressive and receptive/comprehension.
						Comment: PAE predicted no change in language development at 72 months.	
Davies and colleagues (27)	FASD (FAS and PFAS; locomotor, personal, social, hearing and language, eye and hand movements N = 500 (large study cohort)			Northern Cape, South Africa		Maternal interviews on demographic, socio-economic status and alcohol use. Beck Depression Inventory	
						Analysis: Chi-square test, Student's t-test, Wilcoxon rank-sum test	
		n=45 FASD	n=347		7-12 months	Griffiths Mental Developmental Scales (GMDS)	FASDs children scores were significantly lower than CON group

		n=35 FASD	n=48		17-21 months	Griffiths Mental Developmental Scales (GMDS)	FASDs children scores were significantly lower than CON group. The greatest significant decrease in performance occurred hearing and language. Significant developmental deprivation as children grow older in disadvantaged environments.
						Comment: PAE predicted lower levels of language performance than the CON group, with decrease in language performance in 7-12 and 17-21 months.	
Greene and colleagues (29)	Foetal alcohol exposure (without FAS); language and speech acquisition N = 359 (large study cohort)	N=359	No control group	Cleveland, Ohio North America	1, 2, and 3 years	Michigan Alcoholism Screening Test (MAST) – first antenatal visit. retrospective estimate of AA/Day at 5 years; HOME	
						Analysis: Multiple regression	
		n=279			1 years	Sequenced Inventory Communication Development (SICD) Data coded from a taped language production sample – 2 years	No significant association between PAE, expressive, and receptive outcomes

		n= 275			2 years	Mean Length of Utterance (MLU) - number of morphemes; Type/Token Ratio – number of intelligible words; Number of intelligible utterances; Spontaneity – ability to initiate utterances	No significant association between PAE, expressive, receptive performance and speech acquisition
		n=269			3 years	Sequenced Inventory Communication Development (SICD)	No significant association between PAE, expressive and receptive outcomes

Kaplan-Estrin and colleagues (28)	PAE (low and high exposure); Neurobehavioural; N = 92 (large study cohort)		No control group stated	Michigan, United States, North America		Oral interviews on drinking patterns, Urine samples. Peabody Picture Vocabulary Test—Revised (PPVT-R); HOME, the Beck Depression Inventory, and current maternal drinking – 6 months	
						Multiple regression	
		n=92			13 months	Bayley Scales of Infant Development (BSID)	Significant associations between PAE and MDI (Mental Dev. Index) at 6/5 and 13 months The strongest relationships for the MDI at 13 was age and HOME environment
		n=91			26 months	Bayley Scales of Infant Development (BSID) Communication Development Inventory (CDI) The Noncanonical Commands Test The Early Language Milestone (ELM)	No significant association between PAE (low and high levels) and speech acquisition at 26 months

				Comment: PAE predicted no change in speech acquisition over time.

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CHAPTER 4

Publication 2

Prenatal alcohol exposure is associated with early motor, but not language development in a South African cohort

Authors

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Synopsis

In the previous chapter, a review of longitudinal studies was presented exploring the association between prenatal alcohol exposure (PAE), language, speech and communication outcomes. This chapter describes the association between PAE and early neurodevelopmental outcomes at 6 and 24 months of age, as well as explores additional sociodemographic and psychosocial risk factors in the Drakenstein Child Health Study (DCHS). This manuscript directly addresses Research Objective 2 (see Chapter 1, Section 6.2 - p27):

To investigate the association between PAE and early neurodevelopment through two years of age, and to adjust for sociodemographic and psychosocial risk factors.

This manuscript on which this chapter is based was submitted to the Journal of Acta Neuropsychiatrica and is currently published.

Abstract

Introduction: Prenatal alcohol exposure (PAE) has been recognized as a major global public health concern. This study aimed to investigate the association between PAE and early neurodevelopment in the first 2 years of life, adjusting for maternal sociodemographic and psychosocial factors, in the Drakenstein Child Health Study (DCHS), a South African birth cohort study.

Methods: The DCHS comprises a population-based birth cohort of 1143 children, of which, a subsample completed the Bayley Scales of Infant Development-III (BSID-III) at 6 (n=260) and 24 months of age (n=734). A subset of alcohol exposed, and unexposed children was included in this analysis at age 6 months (n=52 exposed; n=104 unexposed) and 24 months (n=92 exposed; n=184 unexposed). Multiple hierarchical regression was used to explore the associations of PAE, motor and language development.

Results: PAE was significantly associated with decreased gross motor (β=-0.30, 95%CI 0.06-0.44 p=0.001) or fine motor (β=-3.30, 95%CI 0.06-0.46, p=0.001) functioning after adjusting for maternal sociodemographic and psychosocial factors at 6 months of age only. No significant effects were found in either receptive or expressive communication and cognitive outcomes at either timepoint.

Conclusion: PAE has potentially important consequences for motor development in the first 2 years of life, a period during which the most rapid growth and maturation occurs. These findings highlight the importance of identifying high-risk families in order to provide preventive interventions, particularly in antenatal clinics and early intervention services.

Keywords: prenatal alcohol exposure[1], motor development[2], language development[3], neurodevelopment[4],

1. Introduction

As noted in Chapter 3, prenatal alcohol exposure (PAE) has been recognized as a major global public health concern. A recent study estimated 9.8% of mothers consumed alcohol during pregnancy and 4.3% were heavy drinkers (defined as an average of two or more drinks per day) (1). Estimated global prevalence rates of foetal alcohol spectrum disorders (FASDs) have been reported at 7.7 (4.9 - 11.7) per 1000 children (2). In low and middle-income countries (LMICs), such as South Africa, the estimated prevalence of FASDs is as high as 111.1 per 1000 children in some communities (2). The majority of previous studies exploring the impact of PAE on child development in the context of sociodemographic and psychosocial factors have been performed in high-income countries, even though higher rates of PAE, poverty, post-traumatic stress disorder (PTSD) and depression exist in LMICs (3-5). The research taking into account contextual factors such as those cited above, underscores the importance of examining the adverse effects of PAE in young children (3, 4), within the broader context of psychosocial and environmental risk factors that may additionally influence not only early neurodevelopmental outcomes, but also lifelong health trajectories.

The adverse effects of PAE manifest a continuum of disorders, namely, FASDs. Foetal alcohol syndrome (FAS) is a pattern of often irreversible physical and mental birth deficiencies (6, 7), while alcohol related neurodevelopmental disorder (ARND) and alcohol related birth defects (ARBDs) are only diagnosed in children without the physical features of FAS or PFAS (8). Previous studies have shown that FAS and ARND were associated with a range of impairments in motor functioning, reading comprehension or executive functioning in the early school years (9-13). Safe and colleagues (6), for example, have reported that children with FAS displayed both motor function and language impairments at 12 years of age, while Coggins and colleagues (16) found that school-aged children with FASDs often exhibit clinically meaningful deficits in language and social communication between 6-12 years of age. Previous work by Viholainen and colleagues (17) reported that impaired language development was also a precursor of problems with motor functioning in the school years.

While there is a rapidly growing literature detailing the effects of PAE on neurodevelopmental outcomes in school-going children, comparable data across motor and language functioning are limited in very young children. A previous cross-sectional study assessed specific

developmental domains and found PAE deficits at 12 months of age: motor coordination and gross motor functioning (19). Other cross-sectional analyses found that FAS was associated with abnormal walking and balance (12-15, 20-23), and deficits in receptive and expressive communication through 2 years (24-26). However, very few studies included data at different timepoints in the first two years of life (18). Of the few studies exploring developmental impairments over time, heavy PAE was significantly associated with delayed motor functioning in toddlers between 12-17 months but not at 24 months of age (27-29). However, the heterogeneity in designs and methodologies of previous studies limit the ability to interpret results across different age cohorts. For example, the impact of maternal alcohol consumption on child outcomes using a clinical diagnosis of FAS without a focus on children who do not meet the FASD criteria was reported in only one study (27).

Much of the longitudinal research describing the developmental outcomes in early childhood has been conducted in well-resourced settings (20, 30, 31), less is known about the effects of PAE on early neurodevelopmental outcomes at different timepoints in LMICs, and much of the work published to date has lacked control groups and or has adjusted for very few confounders (maternal age, gestation, birth weight and parity) (28-31). Few studies have adjusted for additional psychosocial factors, such as maternal PTSD, which frequently co-occurs with PAE and which may have detrimental effects on young children's neurodevelopmental outcomes. This study aimed to investigate the association between PAE and early neurodevelopment through two years of age, adjusting for sociodemographic and psychosocial factors in the Drakenstein Child Health Study (DCHS), a South African birth cohort study.

2. Materials and methods

2.1 Design and setting

This study formed part of the DCHS, a multidisciplinary birth cohort study investigating the early determinants of child health (32-34). The DCHS enrolled pregnant women (20 to 28 weeks' gestation) from two primary health care clinics, Mbekweni (a predominantly black African community) and TC Newman (a mixed-ancestry community) in the Western Cape, South Africa. Both communities are characterized by low socioeconomic status (SES) and a

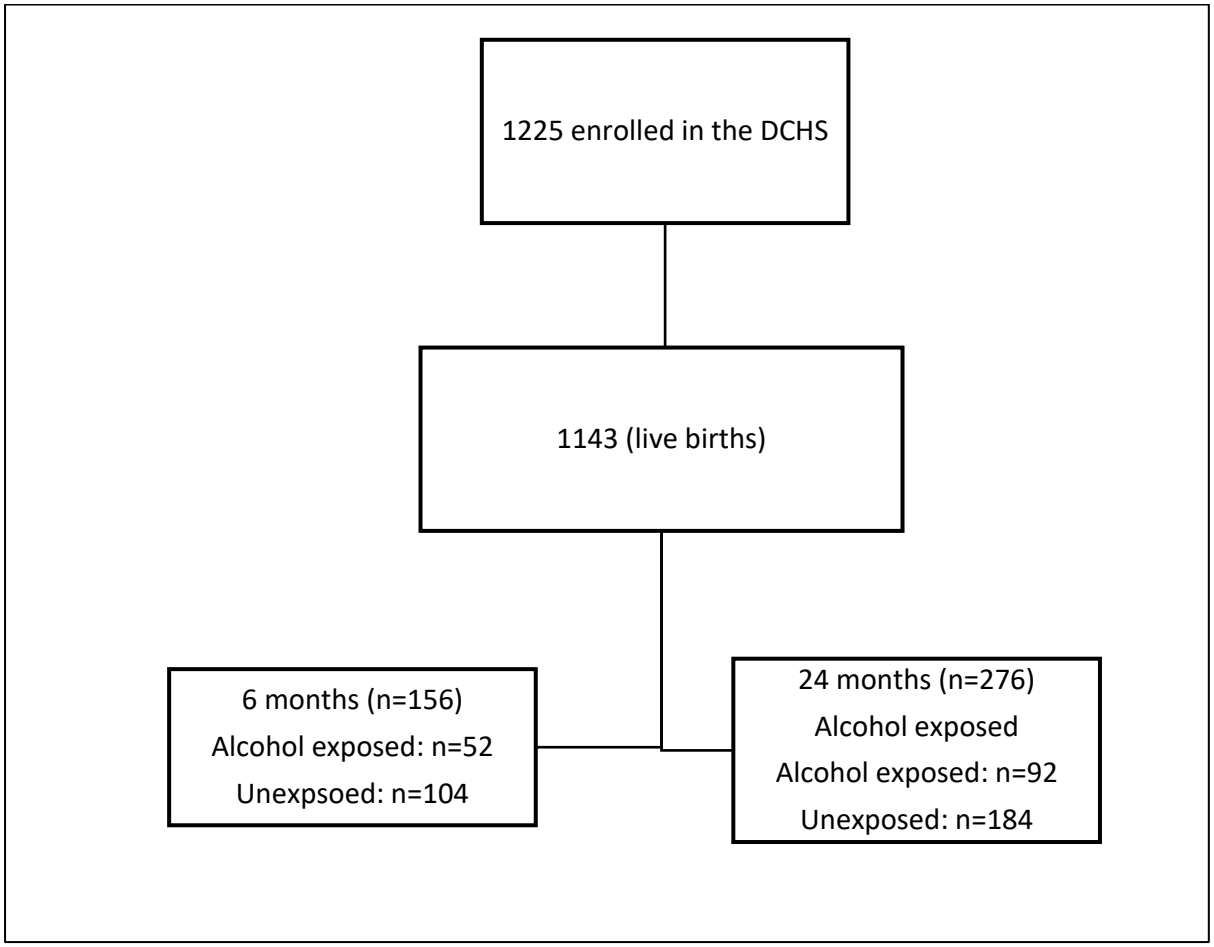
high prevalence of multiple psychosocial risk factors (34). Pregnant women were eligible to participate if they were 18 years or older, had access to one of the two primary health care clinics for antenatal care and had stated no intention to move out of the district within the following year. Mother-child dyads were followed longitudinally until children are at least 5 years of age.

2.2 Participants

This study utilized a subgroup from the DCHS. The alcohol group comprised of a composite, dichotomous category which included i. a minimum score of 11 on the alcohol questions of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) (Appendix D) (35, 36) antenatally and ii. 2 or more drinks a week on average during any of the three trimesters reported by all mothers on a retrospective questionnaire [(World Health Organizations (WHOs) classification of moderate-to-severe alcohol use)] based on quantity, timing and frequency at 3-6 weeks and/or at 24 months testing age (Appendix F). Infants born prematurely or with any other congenital malformations, as well as sets of twins and triplets were excluded from the study.

In total, there were 1143 live births in the DCHS. A subsample of the larger cohort completed the BSID-III at 6 months (n=260), whereas the full cohort was invited to participate at 24 months (n=734) making a larger sample available. At 6 and 24 months of age, a subset of infants and toddlers were selected whose mothers reported moderate-to-severe levels of alcohol consumption and for whom Bayley Scales of Infant Development (BSID-III) data were available. Of the 260 infants, 52 were exposed to alcohol at 6 months, and of the 734 toddlers, 92 were exposed to alcohol at 24 months. The unexposed group comprised of 104 infants at 6 months and 184 toddlers at 24 months. Unexposed control children were randomly matched for maternal education and clinic site in a 1:2 ratio (Figure 1).

Figure 1. Study sample selection



2.3 Measures

Participants were asked to complete self-reported and clinician-administered measures at antenatal and postnatal study visits in their preferred language, English, Afrikaans or isiXhosa. At the point of assessments (6 and 24 months), every effort was made to ensure a safe, anonymous, confidential and supportive environment. Translation of the measures from English to Afrikaans and isiXhosa included a standard forward and back-translation process (33). Prior to the administration of the measures, adult mothers or legal guardians of the children received enough information about the study and were asked to complete an informed consent form in their preferred language.

Maternal sociodemographic, psychosocial and infant measures for this study have previously been described (32, 33) which include:

2.3.1 Sociodemographic measures. Measures included data on maternal SES (income, education, employment status and asset sum), marital status and HIV status (37). Higher scores on this validated composite score indicated higher SES. Child sex, birth weight, gestational age and body mass index (BMI) were also included.

2.3.2 Psychosocial measures. Measures included data on composite scores of maternal smoking exposure (cigarette and cannabis use) and psychiatric variables (severity and frequency of PTSD and depression) administered antenatally. Maternal smoking risk was assessed using the ASSIST (36), PTSD was assessed using the Modified Posttraumatic Stress Disorder Symptom Scale (MPSS) (38) and depression was measured using the Beck Depression Inventory (BDI-II) (39).

Composite scores were created for maternal smoking and psychological variables. The indicators for SES included maternal income, education, employment status and asset sum; smoking risk included cigarette and cannabis use; and psychological variables included assessing symptom of PTSD and depression. Composite variables were used to combine data into a single score as they are considered more robust than a unidimensional measure (42).

2.3.3 Alcohol, Smoking and Substance Involvement Screening Test. As above, the ASSIST assessed alcohol or substance use. This measure included seven items with scores from 0-10 for alcohol and 0-3 for illicit drugs indicating low risk: 11-26 for alcohol and 4-26 for illicit drugs indicating moderate risk and above 26 as high risk of severe problems, with the likelihood of alcohol dependence (35). The higher the score, the greater the alcohol-related risk. The ASSIST has good reliability and validity in several countries including Australia, Brazil, Ireland, India, Israel, the Palestinian Territories, Puerto Rico, the United Kingdom and Zimbabwe (35) and in South Africa (36).

2.3.4 Bayley-III Scales of Infant Development. The BSID-III was conducted at the 6- and 24-month visits, to assess child development in infants and toddlers between 0-42 months (40). This is an international, well-validated test that was used to measure language, motor and cognitive development. The BSID-III has been standardized with a stratified sample of 1000 children ranging from 0-42 months that was representative of the US population with respect to gender, race/ethnicity, geographic region, and parent education level having high reliability

and validity (40). The BSID-III has been shown to be a reliable tool for use among the South African population (41).

The motor scale evaluated early fine and gross motor development (40). The gross motor subset included 72 items that assessed movement of the limbs, static positioning (e.g., sitting, standing), dynamic movement, including locomotion, coordination, balance and motor planning. The fine motor subtest included 66 items that assessed prehension, perceptual-motor integration, motor planning, speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, and responses to tactile information. The motor assessments were administered using directly observed items for the infant and toddler (40).

The language scale assessed receptive and expressive communication and was directly administered to the infant or toddler (40). The receptive communication subtest included 49 items that assessed pre-verbal behaviour, vocabulary development (identifying objects and pictures), understanding morphological development (pronouns and prepositions), morphological markers (e.g. plural, tense markings, the possessive), social referencing and verbal comprehension (40). The expressive communication subtest included 48 items that assessed pre-verbal communication (babbling, gesturing), vocabulary development (naming objects, pictures or naming attributes) and morpho-syntactic development. Composite scores were based on the composite equivalents of the scaled scores. Scaled scores were based on scores with a mean of 10 and standard deviation of 3 and range from 1-19. The assessors were trained by a paediatric neurologist who ensured quality control and scoring precision. A trained paediatric occupational therapist or physiotherapist administered the BSID-III scales in the home language of the infants and toddlers. The assessors had background experience in paediatric clinical and research environments and were blinded to the exposure status of the children.

The DCHS was approved by the Faculty of Health Sciences Human Research Ethics Committees of the University of Cape Town (UCT) and Stellenbosch University in South Africa, and by the Western Cape Department of Health Provincial Research Committee. All study participants provided written informed consent.

2.4 Statistical analysis

The data were analysed using descriptive statistics which included frequencies and percentages for categorical data while means (SD) were presented for normally distributed data. Medians (IQR) were presented for data that were not normally distributed and for all BSID-III scores. For comparisons between alcohol exposed and unexposed children, chi-squared tests were used for categorical variables while t-tests or, in the case of data that were not normally distributed, Mann-Whitney U test were used. Variables that were associated with PAE at an alpha level of 0.05 (95%CI) or less were included in the final model to determine whether the outcome measures that were significantly associated with PAE remained significant after adjusting for potential confounders (Table 5 and 6). Multiple hierarchical regression was used to explore the associations of PAE with motor, language and cognitive development. The model adjusted for the maternal sociodemographic and psychosocial confounding variables, which are known to be associated with child neurodevelopment (motor, language and cognitive outcomes). Potential confounding variables included composites of SES (28), smoking tobacco risk (cigarette and cannabis) and psychological variables (30), and child body mass index (BMI) z-score, child's nutritional status according to their gender and age. Significance was set at 0.05 and 95% CIs were reported for all estimates, were applicable.

3. Results

The maternal and child sociodemographic and psychosocial characteristics are presented in Table 1. At 6 months, the median maternal age at enrolment was 24 years (IQR 21-30). In the alcohol group, 15.4 % of the mothers were HIV infected, 46.2% were classified as having PTSD and depression risk and in the unexposed group, 14.4% of the mothers were HIV infected and 55.8% had PTSD and depression risk. At 24 months, the median maternal age at enrolment was 26 years (IQR 22-31). In the alcohol group, 16.3% of the mothers were HIV infected and 44.6% were classified as having PTSD and depression risk and in the unexposed group, 16.8 % of the mothers were HIV infected and 43.1% were at risk of PTSD and depression. There were no differences across the groups in sociodemographic or psychosocial variables, except for smoking risk, where mothers who consumed alcohol were more likely to smoke at both 6

months (65.4% versus 37.5%, respectively, p=0.001) and 24 months of age (69.6 versus 37.5 respectively, p=0.001). There were no significant differences between the exposed and unexposed groups regarding infants' birth weight and BMI.

Table 2 compares the median scores of the alcohol group and unexposed groups for motor and language development at both 6 and 24 months of age. At 6 months, the alcohol exposed group had significantly lower median scores for gross and fine motor functioning compared to the unexposed infants [gross: median scores- 9.0 (IQR 7.2-11.0) versus 11.0 (IQR 9.0-12.0), respectively, p=0.01; fine: median scores- 11.5 (IQR 10.0-13.0) versus 13.0 (IQR 12.0-15.0), respectively, p=0.001)]. At 24 months, there were no significant differences, although there remained a trend toward impairment for fine motor functioning in exposed children [(median scores- 8.0 [(IQR 7.0-11.0) versus 9.0 (IQR 8.0-11.0), respectively, p=0.07)]. There were no significant differences in language and cognitive functioning at 6 or at 24 months.

The final model explained a significant amount of variance in gross motor functioning [F(4) =3.98, p=0.002, adj R^2=0.10], and the R^2 showed that the amount of variance increased from 5% (BMI, SES, smoking exposure, PTSD and depression) to 14% after adding PAE into the model (Table 3). Similarly, the final model explained a significant amount of variation in fine motor functioning, [F(4)=3.66, p=0.004, adj R^2=0.13], and the R^2 showed that the explained variance accounted for an extra 9% (0.04 to 0.13) in fine motor functioning (Table 3).

Table 4 demonstrates the regression analysis for gross and fine motor functioning. PAE was significantly associated with gross motor functioning (β=-0.30, 95%CI 0.06-0.44 p=0.001) and fine motor functioning (β=-3.30, 95%CI 0.06-0.46, p=0.001) after adjusting for BMI, SES, smoking exposure and psychological variables at 6 months of age. BMI was significantly associated with both gross (β=-0.08, 95%CI 0.57-1.21, p=0.001) and fine motor functioning (β=-0.18, 95%CI 0.46-0.97, p=0.004) while SES was significantly associated with gross motor functioning (β=0.23, 95%CI 1.24-4.19, p=0.001) at 6 months.

4. Discussion

This study comprehensively assessed motor, language and cognitive functioning in a population-based cohort over the first 2 years of life. The findings of our study indicated that

PAE is associated with both gross and fine motor functioning at 6 months of age, even after adjusting for maternal sociodemographic and psychosocial factors. While PAE was not associated with receptive and expressive communication nor cognitive performance at either timepoint in this group, there remained a trend towards significance for poorer fine motor functioning at 24 months of age.

Our findings showed that PAE was associated with deficits in motor functioning across the first two years of life. This is consistent with previously reported cohort studies at preschool age (27-29). In particular, Fried and Watkinson (28) found a significant association between PAE and motor functioning in early infancy (12 months), even after adjusting for maternal age, gestation, birth weight and parity, but found the effect to wane at later ages. These same investigators continued to report a lack of association between PAE and motor outcomes in a follow-up of these children into school age but reported associations between PAE and language comprehension at 36 months (30). Important differences between our study and the cohort in these studies include middle- and -high income samples, no control groups and the authors adjusted for primarily physical confounders (maternal age, gestation, birth weight and parity), but not psychosocial factors. Our study adds to the growing body of scientific evidence implicating PAE in motor functioning impairment at 6 months of age even after adjusting for important psychosocial factors such as severity of PTSD and depression when compared to a matched control group.

In our cohort, PAE was not found to be associated with receptive or expressive communication or cognitive functioning at the age of 24 months. Previous studies have reported impairments in language and cognitive functioning in toddlers between the ages of 12 and 24 months (27, 28), however, reports indicated that as children grew into the school years, PAE was not significantly associated with language or cognitive outcomes (16) using standard measures. Lack of associated impact of PAE on early language outcomes in this study may, in part, be a result of language impairments being subtle in infancy and it therefore, being more difficult to identify these outcomes than in other domains. It may be useful for future studies to consider the extent to which specific language outcomes affect the pragmatic or conversational patterns of children affected by PAE (not just general categories of receptive or expressive communication).

Additional limitations deserve consideration. Firstly, the sub-study comprised a small sample size which may have limited the power to detect differences between the groups. Secondly, despite assurances of confidentiality, an additional limitation included some women may have chosen not to disclose or minimize reporting alcohol use to the research teams, and the low reported alcohol consumption may therefore represent an element of response bias. Thirdly, the BSID-III tool measures general ability in completing a given task but may have low sensitivity for detecting minor developmental impairments especially during infancy. Although this tool has been validated for use in South Africa, this study may not be generalizable to other populations. Additionally, sensory information (vision and hearing) was not explored in the study.

A study strength of this study included the ability to investigate the possible effects of PAE at levels typically seen in the general population on offspring in a large birth cohort study, using measures of both exposure and outcome as well as additional measures to address the problem of confounding. Additionally, assessors were blinded to the groups assigned in the study.

A large proportion of very young children in LMICs do not reach their developmental potential due to a wide variety of sociodemographic and psychosocial factors that may impact early developmental outcomes. Our study, reporting the association of PAE and early motor functioning, is one of only a few studies that have additionally addressed important potential psychosocial confounders which frequently co-occur with alcohol use in these communities. These findings highlight the importance of identifying high-risk families in order to provide preventive interventions, particularly in antenatal clinics and early intervention services.

The next chapter – based on the third publication – will explore the association of PAE and neurodevelopmental trajectories (motor, language and cognitive outcomes) at 6, 24 and 42 months of age.

Table 1. Maternal and child baseline sociodemographic and psychosocial characteristics assessed on BSID-III at 6 and 24 months

	6 months			24 months		
	Alcohol exposed n=52 (33.3)	**Unexposed n=104 (66.7)**	**p-value**	**Alcohol exposed n=92 (33.3)**	**Unexposed n=184 (66.7)**	**p-value**
Maternal variables						
Age n(%)						
18-29	36 (69.2)	79 (76.0)	0.19	59 (64.1)	126 (68.5)	0.84
30-39	15 (28.8)	22 (24.0)		30 (32.6)	51 (27.7)	
40-49	1 (1.9)	0 (0)		3 (3.3)	1 (3.8)	
Study site n(%)						
Mbkweni	19 (36.5)	38 (36.5)	1.00	32 (34.8)	66 (35.9)	0.48
TC Newman	33 (63.5)	66 (63.5)		60 (65.2)	118 (64.1)	
SES n(%)						
Low levels of SES	16 (31.4)	34 (33.3)	0.45	29 (31.9)	65 (35.7)	0.60
Low-medium levels SES	14 (27.5)	31 (30.4)		29 (31.9)	54 (29.7)	
Medium-to-high levels of SES	16 (31.4)	21 (20.6)		22 (24.2)	34 (18.7)	
High SES	5 (9.8)	16 (15.7)		11 (12.1)	29 (15.9)	
Education n(%)						
Primary	4 (7.7)	7(6.7)	0.95	13 (14.1)	24 (13.0)	0.97

Secondary	61 (92.3)	97 (93.3)		79 (85.9)	160 (87.0)	
Tertiary	0 (0)	0 (0)		0 (0)	0 (0)	
Marital status n(%)						
Married or cohabiting	22 (42.3)	33 (31.7)	0.19	54 (58.7)	107 (58.2)	0.52
Other	30 (57.7)	71 (68.3)		38 (41.3)	77 (41.8)	
HIV status n(%)						
Uninfected	44 (84.6)	89 (85.6)	0.87	77 (83.7)	153 (83.2)	0.53
Infected	8 (15.4)	15 (14.4)		15 (16.3)	31 (16.8)	
Smoking risk (cigarette and cannibas risk) n(%)						
No	18 (34.6)	65 (62.5)	0.001*	28 (30.4)	115 (62.5)	0.001**
Yes	34 (65.4)	39 (37.5)		64 (69.6)	69 (37.5)	
Psychological variables (PTSD-depression risk) n(%)						
Absent	28 (53.8)	46 (44.2)	0.26	51 (55.4)	103 (56.9)	0.46
Present	24 (46.2)	58 (55.8)		41 (44.6)	78 (43.1)	
Child variables						
Sex n(%)						
Male	26 (50.0)	60 (57.7)	0.36	50 (54.3)	102 (55.4)	0.48

Female	26 (50.0)	44 (42.3)		42 (45.7)	82 (44.6)	
Birth weight n(%)						
<1500	1 (1.9)	0 (0.0)	0.41	3 (3.3)	2 (1.1)	0.09
1500>2500	8 (15.4)	11 (10.6)		14 (15.2)	29 (15.8)	
2500>3500	36 (69.2)	76 (73.1)		66 (71.7)	117 (63.6)	
>3500	7 (13.5)	17 (16.3)		9 (9.8)	36 (19.6)	
BMI z-score, Median (IQR)	-0.005 (-0.75-0.77)	0.22 (-0.533.30)	0.12	0.18 (-0.48-0.97)	0.50 (-0.42-1.38)	0.11
Maternal age, years Median (IQR)	25 (21-31)	24 (21-29)	0.33	26 (22-31)	26 (22-31)	0.59
Gestational age, weeks Median (IQR)	39 (37-39)	39 (38-40)	0.14	39 (37-40)	39 (37-40)	0.81

* $p<0.05$
** $p<0.01$

Table 2: Mean differences in BSID-III domain scaled scores at 6 months and 24 months according to exposed and unexposed group

6 months					24 months			
BSID-III	**Alcohol exposed Median (IQR) n=52**	**Unexposed Median (IQR) n=104**	**95% CI**	**p-value**	**Alcohol exposed Median (IQR) n=92**	**Unexposed Median (IQR) N=184**	**95% CI**	**p-value**
Gross motor	9.0 (7.2-11.0)	11.0 (9.0-12.0)	0.003-0.006	0.01**	8.0 (7.0 - 9.8)	9.0 (7.0-10.0)	0.20-0.19	0.20
Fine motor	11.5 (10.0-13.0)	13.0 (12.0-15.0)	0.001-0.001	0.001**	8.0 (7.0 -11.0)	9.0 (8.0-11.0)	0.06-0.07	0.07
Receptive communication	9.0 (8.0-11.0)	10.0 (8.3-12.0)	0.60-0.62	0.61	7.0 (5.0 - 8.0)	7.0 (6.0-9.0)	0.85-0.84	0.84
Expressive communication	10.0 (7.0-13.0)	10.0 (8.0-13.0)	0.99-0.99	0.99	7.0 (6.0 - 9.0)	7.0 (6.0-9.0)	0.74-0.75	0.74
Cognitive functioning	9.0 (7.0-11.0)	10.0 (8.0-11.0)	0.23-0.24	0.24	7.0 (6.0-8.0)	8.0 (6.0-8.0)	0.52-0.51	0.52

* $p<0.05$
** $p<0.01$

Table 3. Regressing gross and fine motor functioning onto BMI, SES, smoking risk, psychological variables risk, PAE at 6 months of age

Gross motor	**R**	**R Square**	**Adjusted R Square**	**F**	**p-value**
1	0.07	0.005	-0.001	0.79	0.375
2	0.12	0.01	0.001	0.99	0.371
3	0.18	0.03	0.01	1.46	0.229
4	0.21	0.04	0.01	1.44	0.225
5	0.36	0.13	0.09	3.66	0.004**
Fine motor	**R**	**R Square**	**Adjusted R Square**	**F**	**p-value**
1	0.07	0.005	-0.001	0.79	0.375
2	0.12	0.01	0.001	0.99	0.371
3	0.18	0.03	0.01	1.46	0.229
4	0.21	0.04	0.01	1.44	0.225
5	0.36	0.13	0.09	3.66	0.004**

1. Predictors; BMI
2. Predictors: BMI, SES
3. Predictors: BMI, SES, smoking risk
4. Predictors: BMI, SES, Smoking risk, Psychological variables
5. Predictors: BMI, SES, Smoking risk, Psychological variables, PAE
*p<0.05
** p<0.01

Table 4. Coefficients for predictors in final model of gross motor functioning on the BSID-III at 6 months of age (after adjusting for SES, smoking risk, PTSD and depression)

	Gross motor			Fine motor		
Variables	**Beta**	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**
BMI	-0.08	0.57-1.21	0.001**	-0.18	0.46-0.97	0.04*
SES	0.23	1.24-4.19	0.009*	-0.003	0.54-1.80	0.97
Smoking risk	0.05	0.49-3.74	0.57	-0.07	0.25-1.88	0.47
Psychological variables	-0.06	0.27-1.87	0.49	-0.13	0.18-1.25	0.13
PAE	-0.30	0.06-0.44	0.001**	-3.30	0.06-0.46	0.001**

* $p<0.05$
** $p<0.01$

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CHAPTER 5

Publication 3

Prenatal alcohol exposure is associated with early neurodevelopmental trajectories in a South African birth cohort: the first 4 years

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Synopsis

In the previous chapter, the association between prenatal alcohol exposure (PAE) and early neurodevelopmental outcomes at 6 and 24 months were explored. In this chapter, the longitudinal data is presented on the effects of PAE, including tobacco smoking exposure, on motor, language and cognitive outcomes at 6, 24 and 42 months. This addresses Research Objective 3 of this thesis (see Chapter 1, Section 6.3 - p 28):

Prenatal alcohol exposure is associated with early neurodevelopmental trajectories in a South African birth cohort: the first 4 years

This manuscript on which this chapter is based on is currently ready to be submitted to the journal of Developmental and Behavioural Paediatrics.

Abstract

Background: Prenatal alcohol exposure (PAE) is a significant public health concern. Several previous studies have reported that children with PAE are at increased risk for poor outcomes including impaired neurodevelopment, though few have explored its comorbidity with tobacco smoking exposure. We investigated the associations of PAE, including comorbid tobacco smoking exposure, and the neurodevelopmental trajectory of children over 6, 24 and 42 months of age in the context of high sociodemographic and psychosocial risk in the Drakenstein Child Health Study (DCHS), a South African birth cohort.

Method: Pregnant women were recruited antenatally in a peri-urban community outside Cape Town. Children were assessed using the Bayley Scales of Infant Development (BSID-III-fine motor: n=144) at 6, 24 and 42 months, and with the Peabody Picture Vocabulary Test (PPVT-IV receptive vocabulary: n=129) and Kauffman Assessment Battery (KABC-II expressive: n=112 and nonverbal index: n=134) at 42 months. Longitudinal, mixed linear modelling (MLM) was used to explore the associations of PAE on neurodevelopmental trajectories over 6, 24, and 42 months of age.

Results: The analysis revealed a significant association between PAE and fine motor functioning impairments at 6 months after statistically controlling for confounding variables (β=-4.54, 95%CI -6.91- -2.17, p=0.001), and these effects decreased by 42 months. When accounting for the interaction between prenatal alcohol and smoking exposure, impaired fine

motor functioning occurred at both 6 months (β=9.16, 95%CI 1.70-16.61, p=0.02) and 24 months (β=-12.59, 95%CI -21.98- -3.19, p=0.01), but improved by 42 months. The interaction between prenatal alcohol and tobacco smoking exposure resulted in impaired receptive vocabulary (B=-2.49, 95%CI -5.24 -0.27, p=0.02) and cognitive functioning (B=-3.25, 95%CI -5.98- -0.52, p=0.02) at 24 months.

Conclusion: Comorbid prenatal alcohol and smoking exposure increases the risk for impaired motor, receptive language and cognitive outcomes in the preschool years. These findings have important implications for young children, and continued follow-up can inform the development of preventive intervention programs.

Keywords: prenatal alcohol exposure[1], fine motor[2], language[3], cognitive[4], mixed linear modelling[5]

1. Introduction

As noted in earlier chapters, prenatal alcohol exposure (PAE) remains a significant public health concern. Estimated global incidence of foetal alcohol spectrum disorder (FASD) is 7.7 per 1000 births and in low- and middle-income countries (LMCs) estimated incidence rates is 111.1 per 1000 children in certain regions such as South Africa (1, 2). As also noted, studies have reported that PAE is a significant risk factor for foetal growth and development (3-7), however, the described impact on child development is driven in part by the interplay of associated contextual risk factors. UNICEF, in their Sustainable Development Goals, has argued that a wide variety of risk factors impair growth and development of young children and may have important long-term consequences especially among the vulnerable and marginalised (8). To date, few studies have explored the association between PAE and additive risk factors with neurodevelopmental outcomes in vulnerable cohorts (3-6), and longitudinal analyses over multiple timepoints in the preschool years are lacking.

There is a strong body of evidence suggesting that PAE is associated with a wide range of neurodevelopmental impairments increasing the risk for poor long-term motor, language and executive functions. Previous studies have reported associations between PAE and deficits in locomotor performance in the first two years of life (5) and with gross motor abilities by four years of age (13), while others have found no associations with fine motor performance at 24 months of age (9-11, 17). Most of these previous studies may have been limited by a lack of comparable unexposed control groups and limited information on potentially important confounding variables [(e.g. socioeconomic status (SES), smoking exposure, and maternal depression)] which may confer additional risk for delayed neurodevelopment. It has been previously reported that both prenatal alcohol and tobacco exposure have been associated with adverse growth, cognitive and behavioural problems (18-20). While these studies have reported significant cross-sectional associations (12-17), inconsistent findings demonstrate a need for data on comorbid prenatal substance exposures on longitudinal neurodevelopmental trajectories. Understanding the effects of both prenatal alcohol and tobacco smoking exposure would be crucial to explore characteristics that are uniquely associated with child neurodevelopmental trajectories across multiple timepoints.

We aimed to explore whether PAE, including interactions with tobacco smoking exposure, was associated with the trajectory of motor, language and cognitive development in children through their first 4 years in the Drakenstein Child Health Study (DCHS), a South African birth cohort.

2. Methods

2.1 Design and setting

This study formed part of a multidisciplinary birth cohort study investigating the early determinants of child health (21-24). The DCHS enrolled pregnant women aged 18 years or older (20 to 28 weeks' gestation) from two primary health care clinics in the Paarl region, Western Cape, South Africa. These communities are characterised by low SES and feature a high prevalence of multiple psychosocial risk factors, including single parent households, high rates of post-traumatic stress disorder (PTSD) and depression, exposure to community violence, human immunodeficiency virus (HIV) and alcohol abuse (23).

The total sample for the DCHS comprised 1143 live births, as indicated in Figure 1. For the purpose of this analysis, the subsamples for the alcohol and unexposed groups included complete-case datasets of developmental data resulting in a total sample size for each outcome (Figure 1). These datasets omitted children that had missing data in any of the variables (exclusion of participants occurred at the point of analysis) (Figure 1).

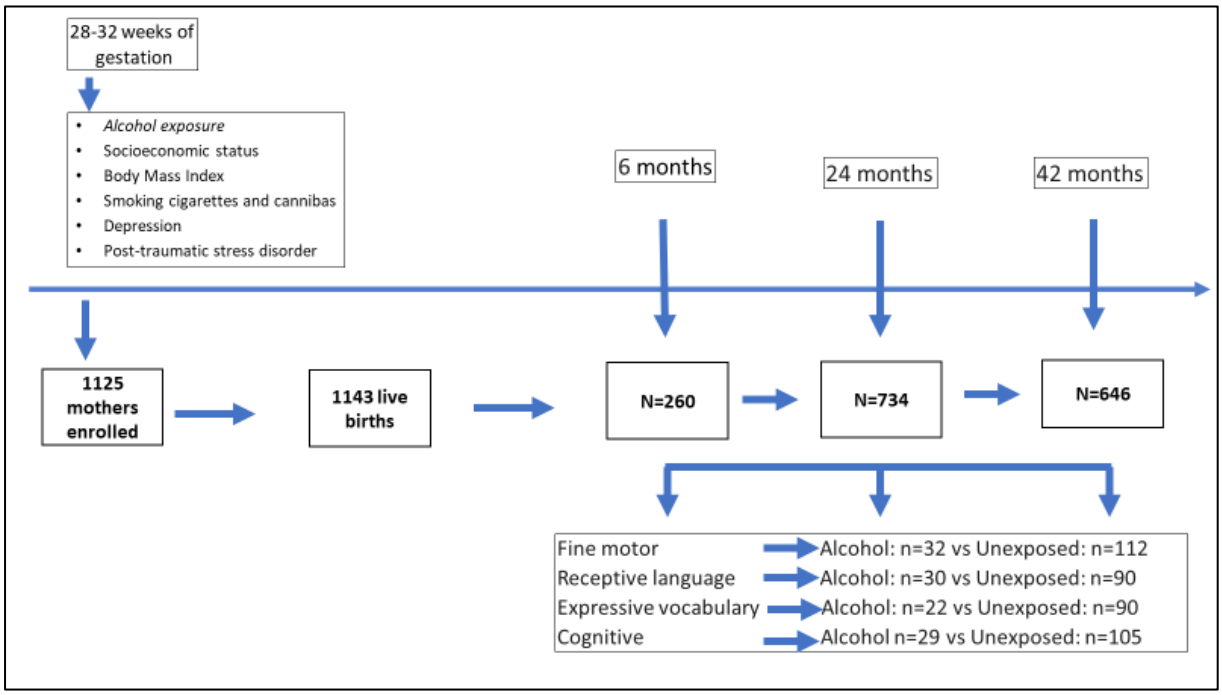
2.2 Participants - exposed versus unexposed

The data was prospectively collected and included a secondary data analysis. Alcohol use during pregnancy was assessed using a composite, dichotomous variable (exposed versus unexposed) combining the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) (Appendix D) antenatally and retrospectively collected data (postnatally) (Appendix F) (22). The inclusion criteria for moderate-to-severe PAE comprised of: (i) scoring greater than moderate exposure on the ASSIST antenatally (or having a minimum score of 11 on the alcohol questions); (ii) 2 or more drinks on average a week on a retrospective alcohol questionnaire administered to all mothers in the postnatal period (to further quantify alcohol use) at 3-6 weeks and 24 months testing age regarding alcohol use during pregnancy (mothers

may be more likely to respond to a retrospective measure after pregnancy) (22). The unexposed control group included mothers with a score of less than 11 on the ASSIST antenatally. Infants born prematurely or with any other congenital malformations, as well as sets of twins and triplets were excluded from the study.

Tobacco smoking exposure during pregnancy was measured by the ASSIST, antenatally. Participants who had a minimum score of 4 on the smoking questions were coded as high-risk smokers while those with less than 4 as low risk smokers.

Figure 1. Study sample selection



2.3 Measures

Participants who participated in the study completed self-report and clinician-administered measures in their preferred language, English, Afrikaans or isiXhosa. On administration of the measures, adult mothers or legal guardians of the children received information about the study and completed an informed consent form.

Sociodemographic and psychosocial measures for this study are described in detail elsewhere (23) and include:

2.3.1. Sociodemographic measures. The sociodemographic measures included data on SES (maternal income, education, employment status and asset sum) (25). Higher scores on this validated composite score indicated higher SES. Child sex, birth weight, gestational age, HIV status and body mass index (BMI) z-score were also included.

2.3.2. Psychosocial measures. Psychosocial measures included data on maternal smoking (tobacco use) and psychiatric disorders (PTSD and depression). Maternal tobacco smoking was assessed using the ASSIST (participants were categorized as either low, moderate or high risk) (24). PTSD severity and frequency (no exposure vs. suspected PTSD vs. trauma-exposed) were measured using the Modified Posttraumatic Stress Disorder Symptom Scale (MPSS) (26). Participants with scores of 29 or greater on the MPSS were classified as having PTSD (26). Severity of depression (below vs. above threshold) was assessed using the Beck Depression Inventory (BDI-II) (27). Participants with scores 20 or greater on the BDI-II were described as clinical depressed (27).

2.3.3 Alcohol, Smoking and Substance Involvement Screening Test. This measure comprised of seven items with scores from 0-10 for alcohol use and 0-3 for illicit drug use indicating low risk; 11-26 for alcohol and 4-26 for illicit drugs indicating moderate risk, and above 26 as high risk of severe problems with the likelihood of substance dependence (29) (Appendix D). The higher the score, the greater the alcohol or substance-related risk. This measure has good reliability and validity in several countries including South Africa (28-30). For the purpose of this study, the ASSIST provided data on alcohol consumption (over the last 3 months and lifetime use), and it describes categories of alcohol use risk while the women are pregnant. The retrospective questionnaire, administered to the all mothers postnatally, gathered data according to the World Health Organizations (WHOs) classification of moderate-to-severe levels of alcohol use according to quantity, timing and frequency of alcohol use during pregnancy (2 or more drinks a week during any of the three trimesters of pregnancy) (Appendix F).

The child outcomes are described below:

2.3.4 Bayley Scales of Infant Development. This measure was administered at the 6, 24- and 42-month visits to assess child development. This is an international, well-validated measure

used to assess motor, language and cognitive development. While no overall (total) developmental score provided by the BSID-III, it does provide four types of norm-referenced scores across the subscales: scaled scores, composite sores, percentile ranks, and growth scores. The BSID-III has been standardised with a sample between 0-42 months among 1000 children from a US population stratified with respect to gender, race/ethnicity, geographic region, and parent education level, having high reliability and validity (31). The BSID-III has been shown to be a reliable tool for use among the South African population (32).

Fine motor subtests were used across all three timepoints, namely, 6, 24- and 42-month visits. The fine motor subtest includes 66 items which assess prehension, perceptual-motor integration, motor planning, speed, visual tracking, reaching, object grasping, object manipulation, functional hand skills, and responses to tactile information. These outcomes were directly administered to the infant and toddler (31). Language subtests, which comprised of receptive and expressive domains, were administered at 6- and 24-month visits. The receptive language subtest includes 49 items that assesses pre-verbal behaviour, vocabulary development (identifying objects and pictures), understanding morphological development (pronouns and prepositions), morphological markers (e.g. plural, tense markings, the possessive), social referencing and verbal comprehension (31). The expressive communication subtest includes 48 items which assesses pre-verbal communication (babbling, gesturing), vocabulary development (naming objects, pictures or naming attributes) and morpho-syntactic development (31). The cognitive scale (or nonverbal scores) assessed the child's performance in several areas, such as, visualization, memory and attention. It included items such as attention to familiar and unfamiliar objects, looking for a fallen object and pretend play (31). This subtest was administered at 6 and 24 months of age.

2.3.5 The Peabody Picture Vocabulary Test. The PPVT-IV was administered at the 42-month visit to assess receptive verbal ability. It comprises 228 full colour stimulus items which displays four pictures per page arranged in sets of generally increasing difficulty. The examiner reads out a word, and then the child responds by pointing to the picture they think corresponds to the word (33). The test is untimed and takes approximately 10-15 minutes to complete. The test was developed for standard American English speakers but a modified, translated, Afrikaans and isiXhosa version of the PPVT-IV was used (34). The PPVT-IV has yet

to be standardised in the South African context, and for this reason, raw unstandardized scores were calculated and utilised. This measure included test-retest reliability correlations between 0.92 and 0.96 and internal consistency correlations between 0.94 and 0.95 (33).

2.3.6. The Kaufman Assessment Battery for Children. The KABC-II was administered at 42-month visits to assess general cognitive functioning (34). For this study, general cognitive functioning was measured using the following subtests: triangles, hand movements, conceptual thinking and face recognition. Raw scores for each test were converted to age dependent scaled scores, and these scaled scores were converted into an age-dependent non-verbal index (NVI). In addition to the non-verbal index scores, expressive vocabulary was used to assess children's ability to say the correct names of objects and illustrations. The above-mentioned subtests were standardised using normative data derived from a United States (US) reference population with a mean of 100 and standard deviation of 15. It was designed as a culture-fair tool in children aged 3 to 18 years of age (35, 36), and showed good reliability with an alpha value of 0.78 (38).

Research assistants (RA's) were trained by a neuropsychologist who ensured quality control and scoring precision. The RA's had background experience in paediatric clinical and research environments. These measures were administered in the child's home language.

The DCHS was approved by the Faculty of Health Sciences Human Research Ethics Committees of the University of Cape Town (UCT) and Stellenbosch University in South Africa, and by the Western Cape Department of Health Provincial Research Committee.

2.4 Statistical analysis

To determine if there were significant differences between the original dataset and those used in the analyses, t-tests and chi-square tests were used. The complete dataset was analysed using descriptive statistics which included frequencies and percentages for categorical data while means (SD) were presented for normally distributed data. Medians (IQR) were presented for data that were not normally distributed. For comparisons between the alcohol exposed and unexposed children, chi-squared tests were used for categorical variables while t-tests, analysis of variance (ANOVA) or, in the case of data that were not normally distributed, Mann-Whitney U tests were used.

2.4.1 Mixed linear models' longitudinal analyses

Separate analyses were conducted for each of the four developmental outcomes. Initial procedures included a visual examination of the frequency distributions and longitudinal plots for individual subjects to evaluate the distributions of all variables. The longitudinal association of PAE was assessed in relation to the selected measures of the various developmental outcomes at 6, 24, and 42 months using mixed linear modelling (MLM) with restricted maximum likelihood estimation. The coefficient estimates (b-values), 95% CIs and p-values were estimated using the *lme4* (39) package in R version 3.6.1.

All MLM models in this analysis included a dummy coded indicator of PAE and dummy-coded terms to index the assessment visits. To account for variability between children, the intercept for each child was treated as a random effect. The effects of PAE were presented with and without consideration of possible maternal confounding variables. The confounding variables, selected *a priori*, included SES, smoking, PTSD and depression scores. To account for variability in the effects of the various predictor variables over time, the interaction between confounders and assessment time were examined in the models. The interaction effects of PAE and smoking over time were also examined.

To allow for modelling across different age-appropriate language and cognitive measures, scores for each measurement were internally standardised to create *z*-scores. In doing this, the interpretation of the language and cognitive outcomes are different to that of the motor skills. For language and cognitive measures, the models were used to estimate the association between PAE groups at the three timepoints. For the motor outcome, the models were used to estimate the effects of PAE on rate of change in motor performance over time.

The following model-building strategy was used for each developmental outcome: First, assessment time, alcohol versus unexposed, and the interaction between assessment time and PAE were included in the model. Second, the model was modified to also include maternal SES (lower or higher levels of SES), maternal smoking tobacco smoking (none, low smoking risk, moderate smoking risk or high smoking risk), maternal depression levels (below threshold versus BDI-II above threshold), exposure to PTSD (no exposure, suspected PTSD, trauma-exposed), and the interaction between each of these confounders with assessment

time. Third, the model was further modified to include the interaction between assessment time, PAE, and tobacco smoking exposure.

3. Results

3.1. Sociodemographic and psychosocial characteristics

Table 1 provides the maternal characteristics of children who provided data for the longitudinal analyses. No significant differences occurred between alcohol and unexposed groups for all maternal sociodemographic and psychosocial variables except for maternal smoking in subsamples of children who completed the expressive (59.1% versus 31.1%, p=0.04) and cognitive measures (58.6% versus 30.5%, p=0.02). Table 2 includes maternal and child characteristics measured at birth for the entire sample. The median maternal age was 25 years (IQR 17-44). Higher proportions of mothers who consumed alcohol during pregnancy resided in TC Newman than Mbekweni (67.2% versus 32.8%, p=0.001). Most mothers who consumed alcohol formed part of the lower SES group in comparison to the higher SES group (60.3% versus 38.2% respectively, p=0.01). Higher proportions of mothers who consumed alcohol formed part of the higher-risk smoking group than those in lower-risk smoking group (16.8% versus 2.3 % p=0.001). Significant differences occurred between alcohol and unexposed groups for PTSD and depression (p=0.001 for both). The median child age at testing was 6 months (IQR6-8), 25 months (IQR21-29) and 42 months (IQR39-27).

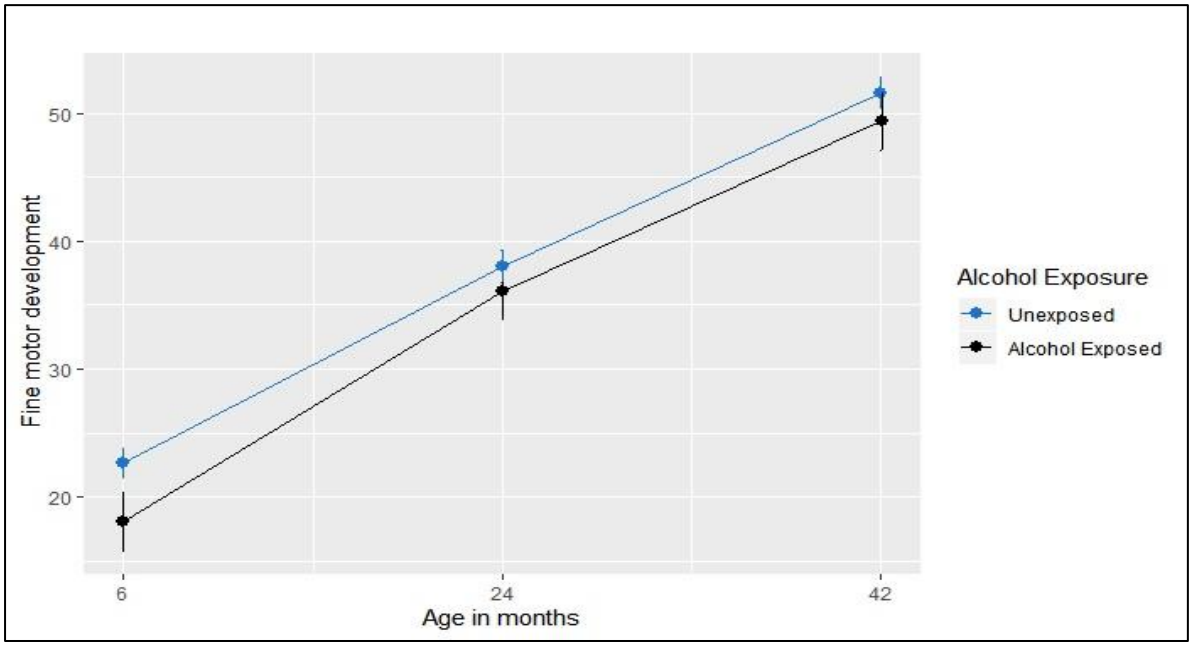
Univariate analyses showed that at 6 months of age, the alcohol group had significantly lower mean scores for fine motor outcomes compared to the unexposed group [fine motor: mean scores- 20.00 (SD=2.78) versus 22.40 (SD=3.30), respectively, p=0.001)] (See Appendix G). No significant differences were found between alcohol and unexposed groups for the remainder of the outcomes at 6, 24 and 42 months. When comparing moderate and heavy exposed groups, no significant differences occurred between the groups for all outcomes except for cognitive skills [moderate exposure mean scores=76.19 (SD=14.45) versus heavy exposure mean scores=66.19 (SD=15.22), p=0.04)] (See Appendix H).

3.2 Longitudinal Analyses

3.2.1 Longitudinal mixed linear models for fine motor scores at 6, 24 and 42 months

In the initial model (Table 3, Model 1), PAE was associated with fine motor functioning[3] at 6 months, with the exposed group having a score of 2.40 lower than their unexposed counterparts (B=-2.40, 95%CI -3.76- -1.04, p=0.001). The intra-class correlation (ICC) for Model 1 indicated that subject-specific intercepts accounted for 22% of the random variation observed in the population. After adjusting for confounders, PAE was significantly associated with deficits in fine motor performance at 6 months (B=-2.38, 95%CI-3.78- -0.98, p=0.001) (Table 3 Model 2). The gap between the fine motor performance of children with alcohol exposure compared to their unexposed counterparts had narrowed by 42 months. Inclusion of the interaction between assessment time, PAE, and smoking risk resulted in a 2-fold increase in the effect of alcohol on fine motor scores at 6 months (Table 3 Model 3). Specifically, Figure 2 demonstrates that the alcohol exposed group shows lower fine motor performance than their unexposed counterparts at all timepoints. When accounting for the interaction of alcohol and smoking exposure, impaired fine motor functioning occurred over timepoints at 6 months (β=-9.16, 95%CI 1.70-16.61, p=0.02), and 24 months (β=-12.59, 95%CI -21.98- -3.19, p=0.01), but recovered by 42 months.

Figure 2. Model-based longitudinal fine motor performance by prenatal alcohol exposure group (for observations taken at age 6, 24 and 42 months)



[3] Bayley Scales of Infant Development (BSID-III) were used to assess fine motor functioning at 6, 24 months and 42 months.

3.2.2 Longitudinal mixed linear models for receptive language scores at 6, 24 and 42-months

There was no evidence of PAE-associated receptive language skills[4] at any timepoint in this cohort (Table 4 Model 1). The ICC for Model 1 indicated that subject-specific intercepts accounted for 24% of the random variation observed in the population. At 24 months, children with both alcohol and tobacco smoking exposure had poorer receptive vocabulary compared to those who were unexposed (B=-2.49, 95%CI -5.24 -0.27, p=0.02) (Table 4 Model 3). No significant interaction effect was observed at 6 and 42 months.

3.2.3 Longitudinal mixed linear models for expressive language scores at 6, 24 and 42 months

No evidence exists for an association between PAE alone, or the interaction of alcohol and tobacco smoking exposure, with expressive vocabulary[5] at any timepoints in this cohort (Table 5 Model 1 and 2). The ICC for Model 1 indicated that subject-specific intercepts accounted for 10% of the random variation observed in the population.

3.2.4 Longitudinal mixed linear models for cognitive scores at 6, 24 and 42 months

As shown in Table 7 (Model 1), there was no evidence of PAE-associated cognitive deficits[6] at any of the timepoints. Subject-specific intercepts accounted for 20% of the random variation observed in the population. A significant interaction effect was found between alcohol and tobacco smoking exposure with lower cognitive scores at 24 months (B=-3.25, 95%CI -5.98 - -0.52, p=0.02) (Table 6 Model 3). When looking at children whose mothers reported tobacco smoking exposure, the findings showed that the mean cognitive scores at 24 months were significantly lower in the alcohol group compared to those in unexposed group.

4. Discussion

Our study adopted a novel approach to investigating neurodevelopmental trajectories after exploring comorbid prenatal alcohol and smoking exposure among children up to 42 months of age. Longitudinal, multivariate analyses investigating the association of PAE and early

[4] Peabody Picture Vocabulary Test (PPVT-IV) was used to assess receptive language at 42 months.
[5] Kauffman Assessment Battery (KABC-II) was used to measure expressive vocabulary at 42 months.
[6] Kauffman Assessment Battery (KABC-II) was used to assess cognitive skills at 42 months.

neurodevelopmental trajectories suggested that although the rate of change in fine motor performance improved by 24 months, comorbid prenatal alcohol and tobacco smoking exposure increased impairments in fine motor performance after controlling for confounders through 24 months, and these effects dissipated only by 42 months. Additionally, comorbid prenatal alcohol and tobacco smoking exposure was significantly associated with impaired receptive language and cognitive scores at 24 months of age.

Our initial findings demonstrated that PAE was associated with deficits in fine motor development at 6 months even after controlling for maternal SES, tobacco smoking exposure, PTSD and depression. These findings are consistent with our previous study of a smaller sample in the same cohort (41) and extended to exploring the interaction of alcohol and tobacco smoking on early neurodevelopmental outcomes.

Our longitudinal findings showed that children exposed to comorbid prenatal alcohol and tobacco smoking exposure lagged behind in their fine motor performance in the first 2 years of life, but in this cohort, exposed children caught up to their unexposed counterparts at 42 months. Previous cross-sectional research on the severe effects of co-occurring prenatal alcohol and smoking exposure has demonstrated worse developmental outcomes in comparison to children exposed to alcohol or to tobacco alone in the early years (48, 49). Kalberg (8), for example, reported that combined prenatal alcohol and smoking had negative consequences for motor development among children in the age group 20-68 months (N=14 FAS children). While previous studies have found associations between prenatal alcohol, including smoking, and impaired developmental outcomes, it is also noteworthy that others have reported that the impact of PAE on motor performance may in fact dissipate as children mature even amongst those exposed to a wide variety of risk factors (42-45). Barr and colleagues (45) found no combined effects of prenatal alcohol and tobacco smoking exposure on early developmental outcomes though they reported smoking alone predicted deficits in cognitive processing among 4-year-old children. Mother-child dyads in this study (45), however, reside in middle-class communities in a North American community, and were at very low risk for adverse environmental exposures while a strength of our study was that dyads reside in low SES communities and were at far higher risk for smoking and other substance exposures, intimate partner violence and have limited access to resources (50). Our

findings add to the body of research indicating that prenatal alcohol and smoking exposures restricted fine motor development up to 24 months. Further research is crucial to ascertain whether differences in fine motor patterns performance re-emerge in the school years.

An additional finding observed in our cohort was that even though PAE had shown impairments in fine motor performance in the very early years (6 months of age), they reflected a staged recovery by the time they reached 42 months. Previous studies have shown that a range of social risk factors (e.g. education and income levels, family size, access to resources) may predict long-term motor performance beyond PAE (42-47). This was evident in South African children from the DCHS cohort with SES as one of the strongest predictive factors for motor delays across the entire cohort of vulnerable children at 24 months (50). Evaluation of the longitudinal patterns of other cohorts, as well as the ongoing follow-up of our own cohort will continue to clarify the potential impact of PAE and additional contextual factors on later developmental outcomes at school age and beyond.

Our results showed that comorbid prenatal alcohol and tobacco smoking exposure were significantly associated with impaired language and cognitive outcomes among toddlers at 24 months. This was consistent with previous literature on the associated PAE and smoking effects on cognitive outcomes among infants with significant differences between exposed and unexposed groups (51). However, different measures used in our study limited our ability to determine whether the comorbid effects of prenatal alcohol and tobacco smoking exposure on language and cognitive development changed over different timepoints.

The DCHS has many strengths. First, this study was able to recruit from a population group with similar SES and age at pregnancy thereby decreasing between-group confounding effects. Second, prospective study enrolment and collection of detailed prenatal and postnatal maternal factors and repeat child outcome measures over years, is unusual in high-risk environments such as this and allowed for a novel analysis of developmental trajectory in critical early years. Third, the alcohol data specifically included mothers who were users of alcohol with moderate-to-severe risk (versus just the severe users). We assessed the differences between developmental trajectories in children of mothers with drinking patterns of none or low risk compared to those with moderate-heavy alcohol and smoking patterns.

Fourth, we used directly administered assessment tools which assessed a range of important domains foundational for later learning, cognitive and academic outcomes.

Several limitations of this study deserve emphasis. First, mothers may have minimised reporting alcohol use, thus introducing an element of response bias. Second, we only included motor, language and cognitive outcomes, and there may be other developmental or behavioural elements not adequately captured by this battery. Third, we did not explore the effects of home environment, including parenting style, which may have played a further mediating role in early neurodevelopmental outcomes in these children. An additional limitation of this study was the use of different measures at the 42-month timepoint for receptive, expressive and cognitive outcomes. Multilevel modelling is most often discussed in the context of repeated measures data (39). However, the preschool period exhibits a time for rapid language and cognitive development, and few measures are available to adequately assess these skills spanning 6 to 42 months of age. An additional limitation of this study was that sensory information was not explored in the study.

This longitudinal study explored the important association between PAE, including comorbid tobacco smoking exposure, and early neurodevelopmental trajectories in children aged 6, 24 and 42 months in a South African birth cohort. Ongoing research could answer questions related to the impact of PAE in the context of potential mediating or moderating risk factors, on motor, language and cognitive outcomes amongst children into school age. This may provide an opportunity for development of interventions aimed at supporting particularly vulnerable and at-risk children in these communities.

The next chapter - based on the last and final publication will explore the association between PAE and conversational turn-taking in mother-child dyads as part of the DCHS, and thereby addressing the fourth objective of this thesis

Table 1: Maternal sociodemographic and psychosocial characteristics of participants assessed

	Fine motor			Receptive			Expressive			Cognitive		
Maternal variables	**Alcohol exposed n=32**	**Unexposed n=112**	**p-value**	**Alcohol exposed n=30**	**Unexposed n=99**	**p-value**	**Alcohol exposed n=22**	**Unexposed n=90**	**p-value**	**Alcohol exposed n=29**	**Unexposed n=105**	**p-value**
SES n(%)												
Lower SES	20 (62.5)	58 (51.8)	0.14	19 (63.3)	53 (53.6)	0.21	15 (68.2)	46 (51.1)	0.05	17 (58.6)	56 (53.4)	0.30
Higher SES	12 (37.6)	66 (45.8)		11 (36.7)	46 (46.4)		7 (31.8)	44 (48.8)		12 (41.4)	49 (46.7)	
Smoking (n(%)												
No smoking	10 (31.3)	58 (51.8)	0.06	9 (30.0)	52 (52.5)	0.12	7 (31.8)	50 (55.6)	0.04**	8 (27.6)	57 (54.3)	0.02**
Lower risk	1 (3.1)	10 (8.9)		1(3.3)	6 (6.1)		0 (0.0)	8 (8.9)		1 (3.4)	9 (8.6)	
Moderate risk	18 (56.3)	36 (32.1)		17 (56.7)	33 (33.3)		13 (59.1)	28 (31.1)		17 (58.6)	32 (30.5)	
High risk	3 (9.4)	8 (7.1)		3 (10.0)	8 (8.1)		2 (9.1)	4 (4.4)		3 (10.3)	7 (6.7)	
PTSD n(%)												

Absent	24 (75.0)	84 (75.0)	0.62	23(76.7)	73 (73.7)	0.66	18 (81.8)	70 (77.8)	0.797	21 (72.4)	79 (75.2)	0.67
Suspected exposure	5 (15.6)	12 (10.7)		4(13.3)	10 (10.1)		2 (9.1)	7 (7.8)		5 (17.2)	12 (11.4)	
Trauma exposed	3 (9.4)	16 (14.3)		3 (10.0)	16 (16.2)		2 (9.1)	13 (14.4)		3 (10.3)	14 (13.3)	
Depression n(%)												
Absent	19 (59.4)	74 (66.1)	0.49	18 (60.0)	63 (63.6)	0.71	11 (50.0)	60 (66.7)	0.146	16 (55.2)	67 (63.8)	0.40
Present	13 (40.6)	38 (33.9)		12 (40.0)	36 (36.4)		11 (50.0)	30 (33.3)		13 (44.8)	38 (36.2)	

*** p<0.05* (Comparing the sample of mothers whose children had outcome data for the longitudinal analyses to those who provided data in the antenatal period (entire sample) displayed no statistically significant differences in the samples for PAE, SES and smoking).

Table 2: Maternal and infant baseline sociodemographic and psychosocial characteristics of the DCHS sample

	Alcohol exposed	**Unexposed**	**p-value**
Variable	**N = 131 (13.2)**	**N = 860 (86.8)**	
Maternal age, years			
Median (IQR)	25.4 (30.8-21.5)	25.8 (30.8-21.9)	0.73
Maternal age, years			
Study site n(%)			
Mbkweni	43 (32.8)	493 (57.3)	0.001**
TC Newman	88 (67.2)	367 (42.7)	
SES n(%)			
Lower levels of SES	79 (60.3)	423 (49.2)	0.01**
Higher SES	50 (38.2)	429 (49.9)	
Marital status n(%)			
Married or cohabiting	52 (39.7)	345 (40.1)	0.92
Other	79 (60.3)	514 (59.8)	
Smoking (n(%)			
No smoking	42 (32.1)	602 (70.0)	
Lower risk, n (%)	3 (2.3)	65 (7.6)	0.001**
Moderate risk n (%)	54 (48.9)	162 (18.8)	
Higher risk, n (%)	22 (16.8)	31 (3.6)	
PTSD n(%)			
Absent	99 (75.6)	635 (73.8)	0.001**
Suspected PTSD	14 (10.7)	114 (13.3)	
Trauma exposed	18 (13.7)	103 (12.0)	
Depression n(%)			
Absent	82 (62.6)	679 (79.0)	0.001**
Present	49 (37.4)	179 (20.8)	
Child variables			
Sex n(%)			
Male	72 (55)	441 (51.3)	0.43

Female	59 (45)	419 (48.7)	
HIV status n(%)			
Uninfected exposed	20 (15.3)	193 (22.4)	1.06
HIV unexposed	111 (84.7)	667 (77.6)	
Birth weight, Median (IQR)			
Gestational age, weeks Median (IQR) or Mean (SD)	39 (40.0-37.0)	39 (40-38)	0.24
BMI z-score, Median (IQR)	0.13 (0.87-0.58)	0.30 (1.07-0.46)	0.05

** $p<0.05$

Table 3: Estimated effects under longitudinal model specifications of prenatal alcohol exposure on fine motor standard scores in children 6, 24 and 42 months

	Model 1				Model 2				Model 3			
	B	**std. error**	**95% CI**	**p-value**	**B**	**std. error**	**95% CI**	**p-value**	**B**	**std. error**	**95% CI**	**p-value**
Intercept	22.40	0.33	21.76 – 23.04	<0.001**	22.23	0.61	21.04 – 23.42	<0.001**	22.61	0.62	21.39 – 23.83	<0.001**
24 months	15.46	0.41	14.67 – 16.26	<0.001**	15.57	0.76	14.08 – 17.06	<0.001**	15.43	0.79	13.89 – 16.97	<0.001**
42 months	28.84	0.41	28.04 – 29.64	<0.001**	29.03	0.76	27.54 – 30.52	<0.001**	28.97	0.79	27.43 – 30.51	<0.001**
PAE	-2.40	0.69	-3.76 – -1.04	0.001**	-2.38	0.71	-3.78 – -0.98	0.001**	-4.54	1.21	-6.91 – -2.17	<0.001**
24 months x PAE	1.41	0.86	-0.28 – 3.10	0.10	1.62	0.90	-0.14 – 3.37	0.07	2.55	1.52	-0.44 – 5.53	0.10
42 months x PAE	1.63	0.86	-0.06 – 3.32	0.06	1.99	0.90	0.24 – 3.74	0.03**	2.30	1.52	-0.68 – 5.29	0.13
lower smoking risk					-1.93	1.16	-4.20 – 0.33	0.10	-2.87	1.20	-5.22 – -0.52	0.01**
moderate smoking risk					-0.26	0.65	-1.54 – 1.02	0.69	-0.95	0.74	-2.41 – 0.51	0.20
high smoking risk					-1.54	1.14	-3.77 – 0.69	0.18	-1.83	1.31	-4.40 – 0.74	0.16

high SES					0.82	0.60	-0.35 – 2.00	0.17	0.64	0.60	-0.53 – 1.81	0.29
suspected PTSD					0.32	0.92	-1.48 – 2.12	0.72	0.47	0.91	-1.32 – 2.27	0.61
Trauma-exposed					-0.17	0.89	-1.92 – 1.59	0.85	-0.37	0.89	-2.12 – 1.38	0.68
BDI above threshold					0.39	0.62	-0.83 – 1.60	0.53	0.52	0.62	-0.69 – 1.73	0.40
24 months x lower smoking risk					1.19	1.45	-1.66 – 4.03	0.42	2.35	1.51	-0.61 – 5.31	0.12
42 months x lower smoking risk					1.71	1.45	-1.13 – 4.55	0.24	2.31	1.51	-0.66 – 5.27	0.13
24 months x moderate smoking risk					-0.67	0.82	-2.28 – 0.93	0.41	-0.72	0.94	-2.56 – 1.12	0.44
42 months x moderate smoking risk					-0.40	0.82	-2.01 – 1.21	0.63	-0.27	0.94	-2.11 – 1.56	0.77

24 months x high smoking risk					0.60	1.43	-2.20 – 3.39	0.68	1.30	1.65	-1.94 – 4.54	0.43
42 months x high smoking risk					-1.13	1.43	-3.93 – 1.66	0.43	-1.79	1.65	-5.02 – 1.45	0.28
24 months x high SES					0.12	0.75	-1.36 – 1.59	0.88	0.26	0.75	-1.22 – 1.74	0.73
42 months x high SES					-0.16	0.75	-1.64 – 1.32	0.83	-0.11	0.75	-1.59 – 1.37	0.88
24 months x suspected PTSD					0.37	1.15	-1.89 – 2.62	0.75	0.25	1.15	-2.01 – 2.50	0.83
42 months x suspected PTSD					-1.08	1.15	-3.33 – 1.18	0.35	-1.01	1.15	-3.27 – 1.25	0.38
24 months x Trauma-exposed					-0.42	1.12	-2.62 – 1.78	0.71	-0.37	1.12	-2.57 – 1.84	0.74

42 months x Trauma-exposed					1.51	1.12	-0.69 – 3.71	0.18	1.47	1.12	-0.73 – 3.68	0.19
24 months x BDI above threshold					-0.21	0.78	-1.73 – 1.31	0.79	-0.41	0.78	-1.93 – 1.11	0.60
42 months x BDI above threshold					-0.46	0.78	-1.98 – 1.07	0.60	-0.49	0.78	-2.01 – 1.03	0.53
PAE x lower smoking risk									9.16	3.80	1.70 – 16.61	0.02**
PAE x moderate smoking risk									3.14	1.57	0.07 – 6.21	0.05**
PAE x high smoking risk									1.95	2.66	-3.27 – 7.16	0.47
24 x PAE x lower smoking risk									-12.59	4.79	-21.98 – -3.19	0.01**

42 x PAE x lower smoking risk									-6.47	4.79	-15.86 – 2.92	0.18
24 months x PAE x moderate smoking risk									-0.30	1.97	-4.16 – 3.57	0.88
42 months x PAE x moderate smoking risk									-0.54	1.97	-4.40 – 3.33	0.79
24 months x PAE x high smoking risk									-2.86	3.35	-9.43 – 3.71	0.39
42 months x PAE x high smoking risk									2.26	3.35	-4.31 – 8.83	0.50
Random Effects												
σ^2	9.29				9.38				9.25			
τ_{00}	2.68 $_{subject}$				2.54 $_{subject}$				2.41 $_{subject}$			

ICC	0.22	0.21	0.21
N	144 $_{subject}$	144 $_{subject}$	144 $_{subject}$
Observations	432	432	432
Marginal R^2 / Conditional R^2	0.923 / 0.940	0.923 / 0.940	0.925 / 0.941

** $p<0.05$

Table 4: Estimated effects under longitudinal model specifications of prenatal alcohol exposure on receptive communication scores in children 6, 24 and 42 months

	Model 1				Model 2				Model 3			
	B	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**
Intercept	0.05	0.10	-0.15 – 0.24	0.64	-0.07	0.18	-0.43 – 0.29	0.69	0.03	0.19	-0.34 – 0.41	0.87
24 months	0.03	0.12	-0.21 – 0.27	0.80	-0.07	0.23	-0.52 – 0.37	0.75	-0.12	0.24	-0.59 – 0.35	0.62
42 months	-0.02	0.12	-0.26 – 0.23	0.90	-0.19	0.23	-0.64 – 0.26	0.40	-0.23	0.24	-0.69 – 0.24	0.35
PAE	-0.20	0.21	-0.61 – 0.21	0.33	-0.11	0.21	-0.52 – 0.30	0.60	-0.68	0.36	-1.39 – 0.03	0.06
24 months x PAE	-0.14	0.26	-0.64 – 0.37	0.60	-0.23	0.26	-0.75 – 0.28	0.37	0.02	0.45	-0.87 – 0.91	0.97
42 months x PAE	0.07	0.26	-0.43 – 0.57	0.78	0.08	0.26	-0.43 – 0.59	0.76	0.29	0.45	-0.60 – 1.18	0.52
lower smoking risk					-0.05	0.40	-0.84 – 0.74	0.90	-0.16	0.43	-1.00 – 0.69	0.71
moderate smoking risk					-0.32	0.20	-0.71 – 0.06	0.10	-0.51	0.22	-0.95 – -0.07	0.03**
high smoking risk					-0.14	0.33	-0.78 – 0.50	0.70	-0.40	0.38	-1.14 – 0.35	0.30
high SES					0.32	0.18	-0.04 – 0.68	0.08	0.29	0.18	-0.07 – 0.64	0.12

suspected PTSD					0.27	0.29	-0.29 – 0.83	0.35	0.31	0.29	-0.25 – 0.87	0.28
Trauma-exposed					0.00	0.26	-0.50 – 0.51	0.99	-0.08	0.26	-0.59 – 0.44	0.77
BDI above threshold					0.19	0.19	-0.18 – 0.55	0.32	0.20	0.19	-0.16 – 0.56	0.28
24 months x lower smoking risk					0.92	0.50	-0.06 – 1.90	0.07	-1.26	0.54	0.21 – 2.32	0.02**
42 months x lower smoking risk					-0.09	0.50	-1.07 – 0.89	0.86	-0.04	0.54	-1.09 – 1.02	0.95
24 months x moderate smoking risk					0.54	0.24	0.06 – 1.01	0.03**	0.55	0.28	0.00 – 1.10	0.05
42 months x moderate smoking risk					0.20	0.24	-0.28 – 0.68	0.41	0.21	0.28	-0.34 – 0.76	0.45
24 months x high smoking risk					0.51	0.41	-0.29 – 1.30	0.22	0.65	0.47	-0.28 – 1.58	0.17
42 months x high smoking risk					-0.06	0.41	-0.85 – 0.74	0.89	0.26	0.47	-0.67 – 1.19	0.58

24 months x high SES					-0.10	0.23	-0.54 – 0.34	0.66	-0.06	0.23	-0.51 – 0.38	0.78
42 months x high SES					0.25	0.23	-0.19 – 0.70	0.26	0.27	0.23	-0.17 – 0.72	0.23
24 months x suspected PTSD					-0.48	0.36	-1.17 – 0.22	0.18	-0.50	0.36	-1.20 – 0.20	0.16
42 months x suspected PTSD					-0.27	0.36	-0.96 – 0.43	0.46	-0.32	0.36	-1.02 – 0.38	0.38
24 months x Trauma-exposed					-0.02	0.32	-0.65 – 0.61	0.95	-0.00	0.33	-0.64 – 0.64	1.00
42 months x Trauma-exposed					0.33	0.32	-0.30 – 0.96	0.31	0.37	0.33	-0.27 – 1.01	0.26
24 months x BDI above threshold					-0.20	0.23	-0.65 – 0.25	0.39	-0.23	0.23	-0.68 – 0.22	0.32
42 months x BDI above threshold					-0.07	0.23	-0.53 – 0.38	0.75	-0.10	0.23	-0.55 – 0.36	0.68
PAE x lower smoking risk									0.73	1.13	-1.47 – 2.94	0.52
PAE x moderate smoking risk									0.82	0.47	-0.10 – 1.74	0.08

PAE x high smoking risk									1.19	0.77	-0.32 – 2.70	0.13
24 x PAE x lower smoking risk									-2.49	1.41	-5.24 – 0.27	0.02**
42 x PAE x lower smoking risk									-0.36	1.41	-3.12 – 2.39	0.80
24 months x PAE x moderate smoking risk									-0.17	0.58	-1.31 – 0.98	0.78
42 months x PAE x moderate smoking risk									-0.14	0.58	-1.29 – 1.01	0.81
24 months x PAE x high smoking risk									-0.63	0.96	-2.52 – 1.26	0.51
42 months x PAE x high smoking risk									-1.23	0.96	-3.12 – 0.65	0.20
Random Effects												
σ^2	0.76				0.75				0.75			

τ_{00}	0.24 $_{subject}$	0.22 $_{subject}$	0.21 $_{subject}$
ICC	0.24	0.23	0.22
N	129 $_{subject}$	129 $_{subject}$	129 $_{subject}$
Observations	387	387	387
Marginal R^2 / Conditional R^2	0.010 / 0.247	0.084 / 0.293	0.111 / 0.306

** p<0.05

Table 5: Estimated effects under longitudinal model specifications of prenatal alcohol exposure on expressive communication scores in children 6, 24 and 42 months

	Model 1				Model 2				Model 3			
	B	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**
Intercept	0.04	0.11	-0.16 – 0.25	0.69	-0.00	0.19	-0.38 – 0.38	1.00	0.08	0.21	-0.32 – 0.49	0.68
24 months	0.02	0.14	-0.25 – 0.30	0.86	-0.15	0.26	-0.66 – 0.36	0.56	-0.12	0.28	-0.67 – 0.43	0.67
42 months	0.02	0.14	-0.25 – 0.30	0.86	-0.06	0.26	-0.57 – 0.44	0.80	-0.07	0.28	-0.62 – 0.47	0.80
PAE	-0.21	0.24	-0.68 – 0.25	0.37	-0.20	0.25	-0.69 – 0.29	0.42	-0.64	0.41	-1.44 – 0.17	0.12
24 months x PAE	-0.13	0.32	-0.75 – 0.50	0.69	-0.12	0.33	-0.76 – 0.53	0.72	-0.22	0.55	-1.31 – 0.86	0.69
42 months x PAE	-0.12	0.32	-0.75 – 0.50	0.70	0.00	0.33	-0.65 – 0.65	1.00	0.02	0.55	-1.06 – 1.11	0.97
lower smoking risk					-0.40	0.38	-1.15 – 0.35	0.30	-0.45	0.38	-1.20 – 0.30	0.24

moderate smoking risk					-0.24	0.21	-0.66 – 0.18	0.30	-0.44	0.24	-0.91 – 0.04	0.07
high smoking risk					0.08	0.44	-0.77 – 0.94	0.90	0.32	0.52	-0.70 – 1.33	0.54
high SES					0.28	0.20	-0.10 – 0.67	0.15	0.27	0.20	-0.12 – 0.65	0.18
suspected PTSD					-0.02	0.35	-0.71 – 0.67	0.95	-0.02	0.35	-0.71 – 0.67	0.95
Trauma-exposed					-0.44	0.29	-1.01 – 0.12	0.13	-0.49	0.29	-1.06 – 0.08	0.09
BDI above threshold					0.24	0.20	-0.16 – 0.63	0.24	0.18	0.20	-0.21 – 0.58	0.36
24 months x lower smoking risk					1.44	0.51	0.44 – 2.44	0.01**	1.42	0.52	0.41 – 2.44	0.01**
42 months x lower smoking risk					0.66	0.51	-0.34 – 1.66	0.20	0.66	0.52	-0.35 – 1.67	0.20
24 months x moderate smoking risk					0.64	0.28	0.09 – 1.20	0.03**	0.64	0.33	-0.00 – 1.28	0.05
42 months x moderate smoking risk					0.18	0.28	-0.38 – 0.74	0.53	0.18	0.33	-0.46 – 0.82	0.58

24 months x high smoking risk					0.27	0.58	-0.87 – 1.41	0.65	0.00	0.70	-1.37 – 1.37	1.00
42 months x high smoking risk					-0.10	0.58	-1.24 – 1.03	0.86	-0.05	0.70	-1.42 – 1.32	0.94
24 months x high SES					-0.23	0.26	-0.74 – 0.28	0.37	-0.26	0.27	-0.79 – 0.26	0.33
42 months x high SES					0.05	0.26	-0.46 – 0.56	0.84	0.06	0.27	-0.47 – 0.58	0.83
24 months x suspected PTSD					-0.16	0.47	-1.08 – 0.76	0.74	-0.13	0.48	-1.07 – 0.80	0.78
42 months x suspected PTSD					0.43	0.47	-0.49 – 1.35	0.36	0.42	0.48	-0.51 – 1.36	0.37
24 months x Trauma-exposed					0.75	0.39	-0.01 – 1.50	0.05	0.72	0.39	-0.05 – 1.49	0.07
42 months x Trauma-exposed					0.56	0.39	-0.20 – 1.31	0.15	0.56	0.39	-0.21 – 1.33	0.16
24 months x BDI above threshold					-0.44	0.27	-0.96 – 0.09	0.10	-0.44	0.27	-0.97 – 0.10	0.11
42 months x BDI above threshold					-0.48	0.27	-1.00 – 0.05	0.08	-0.48	0.27	-1.01 – 0.06	0.08

PAE x moderate smoking risk									0.87	0.53	-0.17 – 1.92	0.10
PAE x high smoking risk									-0.36	0.97	-2.27 – 1.54	0.71
24 months x PAE x moderate smoking risk									0.05	0.72	-1.36 – 1.47	0.94
42 months x PAE x moderate smoking risk									-0.01	0.72	-1.42 – 1.40	0.99
24 months x PAE x high smoking risk									0.88	1.31	-1.68 – 3.45	0.50
42 months x PAE x high smoking risk									-0.17	1.31	-2.74 – 2.40	0.90
Random Effects												
σ^2	0.89				0.87				0.88			
τ_{00}	0.10 $_{subject}$				0.11 $_{subject}$				0.08 $_{subject}$			
ICC	0.10				0.12				0.09			
N	112 $_{subject}$				112 $_{subject}$				112 $_{subject}$			
Observations	336				336				336			

Marginal R^2 / Conditional R^2	0.014 / 0.114	0.081 / 0.187	0.109 / 0.187

** p<0.05

Table 6: Estimated effects under longitudinal model specifications of prenatal alcohol exposure on cognitive scores in children 6, 24 and 42 months

	Model 1				Model 2				Model 3			
	B	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**	**B**	**std. Error**	**95% CI**	**p-value**
Intercept	0.08	0.10	-0.11 – 0.27	0.41	-0.19	0.18	-0.54 – 0.16	0.29	-0.13	0.18	-0.48 – 0.23	0.48
24 months	-0.00	0.12	-0.24 – 0.24	0.98	0.38	0.23	-0.06 – 0.83	0.09	0.37	0.23	-0.08 – 0.83	0.10
42 months	-0.07	0.12	-0.31 – 0.17	0.60	0.25	0.23	-0.20 – 0.69	0.28	0.24	0.23	-0.21 – 0.69	0.30
PAE	-0.37	0.21	-0.78 – 0.04	0.08	-0.37	0.21	-0.78 – 0.05	0.09	-0.85	0.38	-1.58 – -0.11	0.03**
24 months x PAE	0.01	0.26	-0.51 – 0.53	0.96	0.04	0.27	-0.50 – 0.57	0.89	0.24	0.48	-0.71 – 1.18	0.63
42 months x PAE	0.31	0.26	-0.21 – 0.83	0.25	0.39	0.27	-0.14 – 0.93	0.15	0.35	0.48	-0.59 – 1.30	0.46
lower smoking risk					0.09	0.34	-0.58 – 0.77	0.78	0.05	0.35	-0.65 – 0.74	0.90
moderate smoking risk					0.01	0.19	-0.37 – 0.39	0.95	-0.14	0.22	-0.57 – 0.29	0.52

high smoking risk					-0.58	0.34	-1.24 – 0.08	0.08	-0.75	0.39	-1.52 – 0.02	0.06
high SES					0.41	0.18	0.06 – 0.76	0.02**	0.38	0.17	0.04 – 0.72	0.03**
suspected PTSD					0.40	0.26	-0.11 – 0.91	0.13	0.46	0.26	-0.05 – 0.97	0.08
Trauma-exposed					-0.12	0.27	-0.65 – 0.40	0.64	-0.18	0.26	-0.69 – 0.34	0.50
BDI above threshold					0.21	0.18	-0.14 – 0.56	0.25	0.24	0.18	-0.11 – 0.59	0.18
24 months x lower smoking risk					-0.10	0.44	-0.96 – 0.76	0.81	0.22	0.45	-0.67 – 1.11	0.63
42 months x lower smoking risk					0.25	0.44	-0.61 – 1.11	0.57	0.24	0.45	-0.65 – 1.13	0.60
24 months x moderate smoking risk					-0.01	0.25	-0.50 – 0.47	0.96	-0.04	0.28	-0.59 – 0.51	0.88

42 months x moderate smoking risk					0.01	0.25	-0.47 – 0.50	0.95	0.10	0.28	-0.46 – 0.65	0.74
24 months x high smoking risk					0.29	0.43	-0.56 – 1.13	0.50	0.40	0.51	-0.59 – 1.39	0.43
42 months x high smoking risk					0.24	0.43	-0.60 – 1.09	0.57	-0.19	0.51	-1.18 – 0.80	0.71
24 months x high SES					-0.34	0.23	-0.78 – 0.10	0.14	-0.32	0.22	-0.76 – 0.12	0.15
42 months x high SES					-0.04	0.23	-0.49 – 0.40	0.85	-0.05	0.22	-0.49 – 0.39	0.83
24 months x suspected PTSD					-0.47	0.33	-1.12 – 0.19	0.16	-0.49	0.33	-1.15 – 0.16	0.14
42 months x suspected PTSD					-0.28	0.33	-0.94 – 0.37	0.40	-0.23	0.33	-0.88 – 0.42	0.49

24 months x Trauma-exposed					-0.24	0.34	-0.91 – 0.42	0.48	-0.25	0.34	-0.91 – 0.41	0.46
42 months x Trauma-exposed					0.01	0.34	-0.66 – 0.67	0.99	-0.00	0.34	-0.66 – 0.66	1.00
24 months x BDI above threshold					-0.41	0.23	-0.86 – 0.04	0.07	-0.47	0.23	-0.92 – -0.02	0.04**
42 months x BDI above threshold					-0.84	0.23	-1.29 – -0.39	0.001**	-0.81	0.23	-1.25 – -0.36	0.001**
PAE x lower smoking risk									0.43	1.08	-1.69 – 2.56	0.69
PAE x moderate smoking risk									0.71	0.48	-0.22 – 1.65	0.14
PAE x high smoking risk									0.79	0.78	-0.74 – 2.32	0.31

24 x PAE x lower smoking risk									-3.25	1.39	-5.98 – -0.52	0.02**
42 x PAE x lower smoking risk									0.17	1.39	-2.57 – 2.90	0.91
24 months x PAE x moderate smoking risk									-0.02	0.61	-1.22 – 1.18	0.97
42 months x PAE x moderate smoking risk									-0.22	0.61	-1.42 – 0.98	0.72
24 months x PAE x high smoking risk									-0.42	1.00	-2.39 – 1.55	0.68
42 months x PAE x high smoking risk									1.43	1.00	-0.54 – 3.40	0.16

Random Effects			
σ^2	0.80	0.78	0.76
τ_{00}	0.20 $_{subject}$	0.17 $_{subject}$	0.16 $_{subject}$
ICC	0.20	0.18	0.17
N	134 $_{subject}$	134 $_{subject}$	134 $_{subject}$
Observations	402	402	402
Marginal R^2 / Conditional R^2	0.015 / 0.209	0.102 / 0.264	0.149 / 0.297

** p<0.05

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CHAPTER 6

Publication 4

Prenatal alcohol exposure impacts conversational turn-taking between young children and their mothers in a South African birth cohort study

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Synopsis

In the previous chapter, the association between prenatal alcohol exposure (PAE) and the early neurodevelopmental trajectory of young children at 6, 24- and 42-months were explored. As the fourth manuscript included in this thesis, this is the first to explore the association between PAE and conversational turn-taking of both mothers and their children (see Chapter 1 - Research Objective 6.4, p 28):

To compare the conversational turn-taking between mothers and their alcohol exposed children to those between mothers and their unexposed children at the age of 42 months and adjust for sociodemographic and psychosocial risk factors.

This manuscript was submitted to the journal *Alcohol* which focusses on biomedical research and the effects of alcohol on the nervous system or other organs. This publication is currently under review.

Abstract

Introduction. Previous studies have demonstrated that prenatal alcohol exposure (PAE) is associated with a range of impairments in language development, including receptive and expressive communication. However, no previous studies have explored the association between PAE and conversational turn-taking, which is important for successful language and social interactions. We aimed to compare the conversational turn-taking between mothers and their alcohol exposed children with that between mothers and their unexposed children at the age of 42 months as part of a South African birth cohort, the Drakenstein Child Health Study (DCHS).

Methods. Thirty children were alcohol exposed and 60 were unexposed controls. Unexposed control dyads were matched for maternal age, education and clinic site in a 1:2 ratio. Conversational turn-taking was video-audio recorded from 90 face-to-face, mother-child interactions for 10 minutes each at 42 months testing age. Multiple logistic regression was used to explore the associations between PAE, overlapping utterances and utterances preceded by pauses, and adjusted for potential sociodemographic and psychosocial confounders (parity, clinic, BMI, smoking risk, psychological variables, SES, child age and maternal age).

Results. Alcohol exposed children had significantly higher median scores than their unexposed counterparts for child overlapping utterances [(median scores: 13.20 (IQR 6.12-24.17) versus 5.64 (IQR 0-16.32), p=0.016)]. There was a trend toward significance for maternal overlapping utterances [(median scores: 14.81 (IQR 8.06-24.79) versus 7.70 (IQR 1.91-18.71), p=0.057)]. After multiple logistic regression and adjusting for sociodemographic and psychosocial confounders, PAE was significantly associated with child overlapping utterances (OR=3.25, CI 0.98-10.76, p=0.050). No significant associations were found for either maternal overlapping utterances or maternal utterances preceded by long pauses after adjusting for confounders.

Conclusions. Our findings offer new and important insight on the association of PAE and conversational turn-taking in a sample of mother-child dyads from a sub-Saharan African setting. The results of this study showed that PAE children showed increased difficulty with turn-taking and topic manipulation in a conversation in comparison to their unexposed counterparts at preschool age. These findings suggest the importance of assessment and intervention for improving turn-taking strategies especially among children with PAE.

Keywords: prenatal alcohol exposure$_1$, conversational turn-taking$_2$, mother-child dyad$_3$, South Africa$_4$

1. Introduction

As discussed in Chapter 5, prenatal alcohol exposure (PAE) may cause a wide variety of neurodevelopmental effects, including cognitive, language and social deficits. Previous studies have shown that PAE was associated with impaired receptive or expressive communication throughout the preschool years (1-8). Few studies, however, exist on the conversational patterns of talk in children, and of these, Coggins and colleagues (9) have shown that heavy prenatal alcohol consumption resulted in impairments in conveying meaningful information in children between ages 6-12 years. To our knowledge, no previous studies have explored the association between PAE and conversational turn-taking, even though, an exploration of this aspect is important for examining the language and social interaction between young children and their mothers

Conversational turn-taking between speakers entails attentive listening, planning and predicting the launch of a turn (10-13). Previous animal studies have demonstrated that, in both natural and lab-controlled environments, marmosets and Campbell's monkeys displayed turn-taking behaviours through vocal exchanges (14, 15). In human studies, Gratier and colleagues (16) have reported that infants (8-21 weeks) actively engaged in turn-taking with their mothers using nonverbal exchanges, vocalizations and facial expressions. These authors further reported that infants engaged in turns from the earliest age and that mothers adjusted their turn-taking formats according to their infants' turn-taking styles. Hilbrink and colleagues (11) have further reported that, as children begin to interact with their mothers using expressive language, they begin not only to ask questions and make comments but predict the content of a turn as well as the timing for when it may occur.

Conversational turn-taking can be achieved in one of the following three ways, i.e. overlapping utterances, utterances preceded by moderate or by long pauses (17-19). Sacks and colleagues (20), for example, have suggested that for smooth turn-taking, there should be neither overlaps nor long pauses between utterances. This understanding of neither overlaps nor long pauses has been supported by the claim that turn-taking relies entirely on the ability to predict upcoming turn-endings, and if violated, may result in poorly modulated conversation (21-25). A previously reported study by Levinson and Torreira (18) have

indicated that short to moderate pauses (0-300ms) were common for transitioning between turns. They suggested that for an appropriate response to occur on time (approximately 0-300ms after the end of a turn), mutual attention between speakers was required. Additional work by Lindsay and colleagues (26) have found that preschool children start predicting a question's ending, and can leave shorter gaps before responding, suggesting that they eventually optimize the timing of their conversational turns, as adults do. In addition to pauses between utterances, a speaker who uses fast turn-taking sequences, may end up overlapping utterances while long pauses between utterances may delay conversation (17, 27). Previous studies have reported that failing to coordinate turn-taking at a young age may lead to interactions becoming awkward, the child being potentially viewed as a poor communicator and eventually this may contribute to long-term social isolation (28-30). To our knowledge, no studies have explored the association between PAE and conversational turn-taking, which is important for examining the language and social interaction between young children and their mothers. The Drakenstein Child Health Study (DCHS) provides a unique opportunity to study conversational turn-taking in mothers and their children. We aimed to provide new empirical evidence on conversational turn-taking (overlapping utterances and utterances preceded by long pauses) between mothers and their alcohol exposed children compared to those between mothers and their unexposed children at 42 months testing age in a sub-Saharan African context.

2. Methods

2.1 Design and setting

This study was a nested sub-study that included mother-child dyads from the larger DCHS. The DCHS is a multidisciplinary birth cohort study investigating the early determinants of child health over time, which has been described fully elsewhere (31-33). In brief, pregnant women were enrolled in the 20 to 28-week gestation period from two primary health care clinics, Mbekweni (a predominantly Black African community) and TC Newman (a mixed-ancestry community) in the Western Cape province, South Africa. Although the Western Cape is traditionally a high-risk area for alcohol use disorders, this community was chosen primarily for its relative stability and representativeness of the general population.

2.2 Participants - exposed versus unexposed

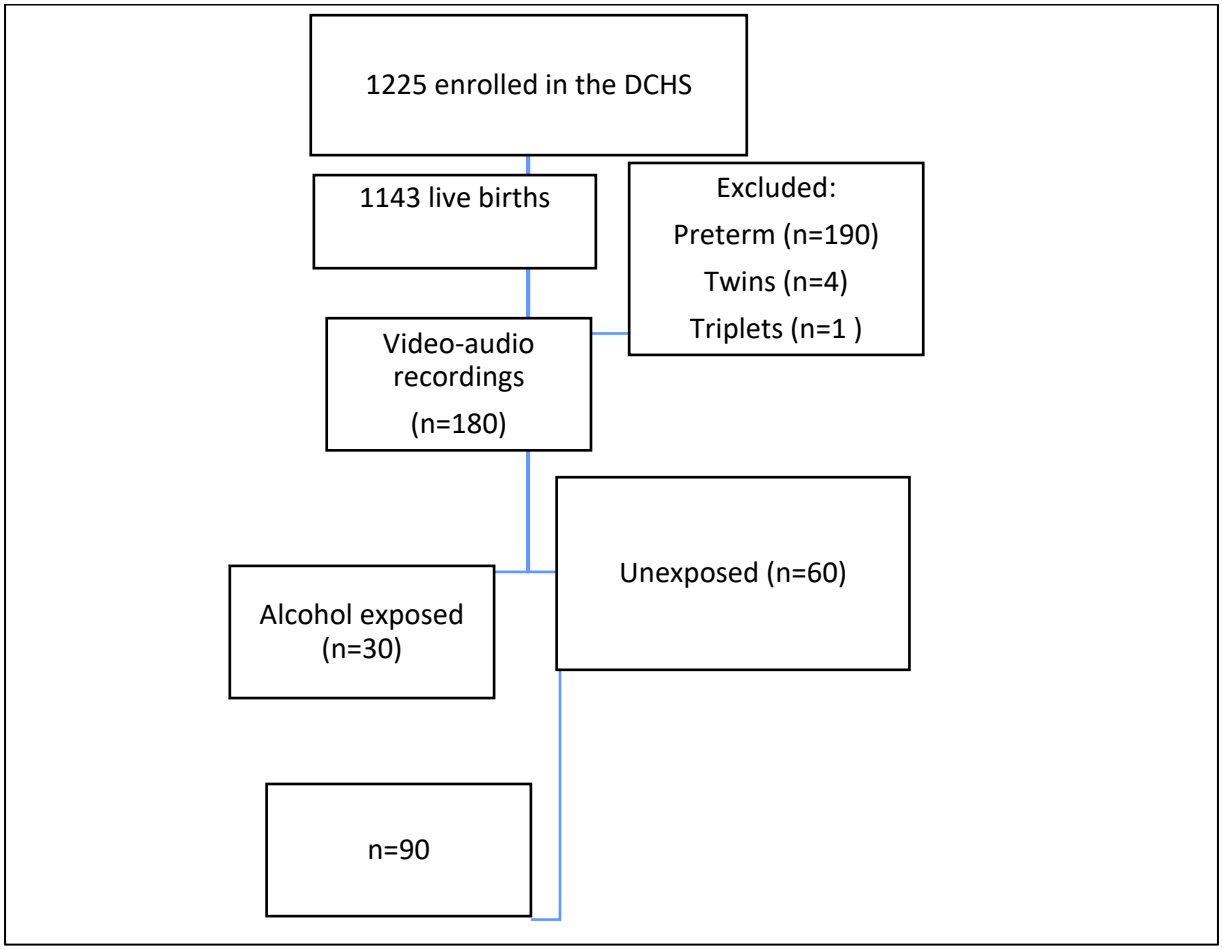
The DCHS comprised a total of 1143 live births (Figure 1). Of this, a total of 90 mothers had moderate-to-severe levels of alcohol exposure antenatally, defined as having a minimum score of 11 on the alcohol questions of the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) and/or retrospectively collected data (31). PAE comprised of a composite, dichotomous classification of both antenatal and retrospectively collected data.

The following inclusion criteria was used in order to optimise numbers: (i) scoring greater than moderate alcohol consumption on the ASSIST antenatally and/or (ii) 2 or more drinks a week on a retrospective alcohol questionnaire reported by all the mothers in the cohort regarding their alcohol use during pregnancy at 3-6 weeks and/or 24 months testing age (31).

For the purpose of this study, the ASSIST was administered to women in the prenatal period, and although this measure provides retrospective data on alcohol consumption (over the last 3 months and lifetime use), it describes categories of alcohol use risk while the women are pregnant. In addition to the ASSIST above, data was also collected in the postnatal period (i.e. at 3-6 weeks and/or 24 months testing age as mentioned above) to further quantify alcohol use in pregnancy.

For our study, mother-child dyads with available video-audio recordings at 42 months were included. This comprised a total of 180 mother-child dyads at the time of the assessment. Thirty mother-child dyads were exposed to alcohol, and 60 were unexposed (mothers had a score less than 11 on the ASSIST antenatally). Unexposed dyads were randomly matched according to maternal age, education and clinic site in a 1:2 ratio. Infants born prematurely or with any other congenital malformations, as well as sets of twins and triplets, were excluded from the study.

Figure 1. Study sample selection



2.3 Measures

Participants were asked to complete self-report and clinician-administered measures at antenatal and postnatal study visits in their preferred language, English, Afrikaans or isiXhosa. Maternal sociodemographic, psychosocial and infant measures for this study have been described elsewhere (32) and include the following:

2.3.1 Sociodemographic measures. The measures included data on socioeconomic status (SES) which included maternal income, education, employment status and asset sum (34). Higher scores on this validated composite score indicated higher SES. Child sex, birth weight, gestational age and body mass index (BMI) z-score were also included.

2.3.2 Psychosocial measures. Composite scores were created for smoking exposure (cigarette and cannabis use), and psychiatric variables [(post-traumatic stress disorder (PTSD)

and depression). Maternal smoking was assessed using the ASSIST (35), PTSD was assessed using the Modified Posttraumatic Stress Disorder Symptom Scale (MPSS) (36) and depression was assessed using the Beck Depression Inventory or BDI-II (37).

The indicators for smoking exposure included cigarette and cannabis use, and for psychological variables included assessments of symptoms of PTSD and depression. For maternal smoking exposure, participants were classified either as low-risk or moderate-to-high risk. For PTSD and depression, participants were categorised as either with the presence or absence of symptoms. Composite variables were used to combine data into a single score as they are considered more robust than a unidimensional variable (38).

2.3.3 Alcohol, Smoking and Substance Involvement Screening Test. As mentioned above, the ASSIST was part of the assessment of alcohol or substance use (Appendix D). This measure includes seven items with scores from 0-10 indicating low-risk, 11-26 indicating moderate risk, and above 26 as high risk or substance dependence (35). The higher the score, the greater the substance-related risk. The ASSIST has good reliability and validity in several countries including South Africa (35, 39).

The retrospective questionnaire, administered to all mothers postnatally, gathered data on the World Health Organizations (WHOs) classification of moderate-to-severe use based on quantity, timing and frequency of alcohol use in pregnancy (moderate-to-severe use was described an average of 2 or more drinks a week in any of the three trimesters of pregnancy). (Appendix F).

A description of conversational turn-taking is described below.

2.3.4 Conversational turn-taking. This, for both the mother and child, was transcribed and coded from the video-audio tape recordings according to procedures developed by Kaye and Charney (17). Conversational turn-taking included four elements during interactions between the mother and child: an utterance from the mother, an utterance from the child, overlapping utterances and switching pauses (separated by long pauses) by either the mother or child (40, 41). The following components for turn-taking were included:

i. **Utterances**. An utterance unit was defined as a stretch of one child's speech separated by the speech of either the child or mother. An utterance was pragmatically defined as a word sequence that is terminated by an overlap ([ ]), a moderate pause period (..) and a long pause period (…). If there was an overlap or pause in between the word sequences, they were separated into two utterances.

ii. **Overlapping utterances.** Overlapping utterances were defined as a short period of speech occurring when either the mother or child uttered words over each other. For each mother and child, separately, the ratio of overlapping utterances to the total turn-taking was calculated and expressed as a percentage. Further, participants with a percentage of 20% or more were classified as "poor" overlapping utterances, as defined by Hilbrink and colleagues (11).

iii. **Utterances preceded switching pauses.** Switching pauses occurred in between utterances either by the mother or child. These utterances were preceded either by a moderate or long pause. Moderate pauses ranged between 0-3000ms and long pauses were greater than 3000ms. For this study, only utterances preceded by long pauses were examined. The ratio of utterances preceded by long pauses to the total conversational turn-taking was calculated and expressed as a percentage for both the mother and child. A percentage of 20% or more on utterances preceded by long pauses was classified as "poor" conversational turn-taking as defined by Heldner and Edlund (42).

2.4 Procedure and ethics

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009), Stellenbosch University (N12/02/0002) and the Western Cape Provincial Health Research committee (2011RP45).

Written informed consent was obtained from each participant prior to administering the video-audio recordings, which were carried out in a safe, confidential and supportive environment. Following consent, the mother and child were settled in a comfortable position facing each other and the video recorder and microphone were placed near them. The assessors requested the mother to engage in free play with the child as they normally would

by using a set of blocks, dolls and additional toys for 10 minutes. In the first 5 minutes, blocks were provided, and in the following 5 minutes dolls and animal toys were provided. These were age appropriate toys for children at the 42-month assessments. Assessors had background experience in paediatric clinical and research environments and were trained by a neuropsychologist.

On completion of the video-audio recordings, the coder and a trained assistant directly transcribed the mother and children's conversational turn-taking. Conversational turn-taking was manually coded using the Computerized Language Analysis (CLAN) program of the Child Language Data Exchange System (43) to derive the frequencies of overlapping utterances and utterances preceded by long pauses.

Inter-coder reliability. Coders blinded to all participant information rated the conversational turn-taking of each available dyadic-interaction. Twenty percent of the transcripts were double coded by two raters. The inter-coder reliability analysis showed high agreement between raters with scores above 0.90 ($p<0.001$).

2.5 Statistical analysis

Descriptive statistics included frequencies and percentages for categorical or count data, while means (SD) were presented for normally distributed data. Medians (IQR) were presented for data that were not normally distributed. For comparisons between alcohol exposed and unexposed children, chi-squared tests were used for categorical variables while t-tests, or, in the case of data that were not normally distributed, the Mann-Whitney U tests were used. Multiple logistic regression was used to explore the associations between PAE, overlapping utterances and utterances preceded by pauses, adjusted for potential sociodemographic and psychosocial confounders (clinic, BMI, smoking exposure, PTSD-depression and SES). Significance was set at 0.05 and 95% CIs were reported for all estimates.

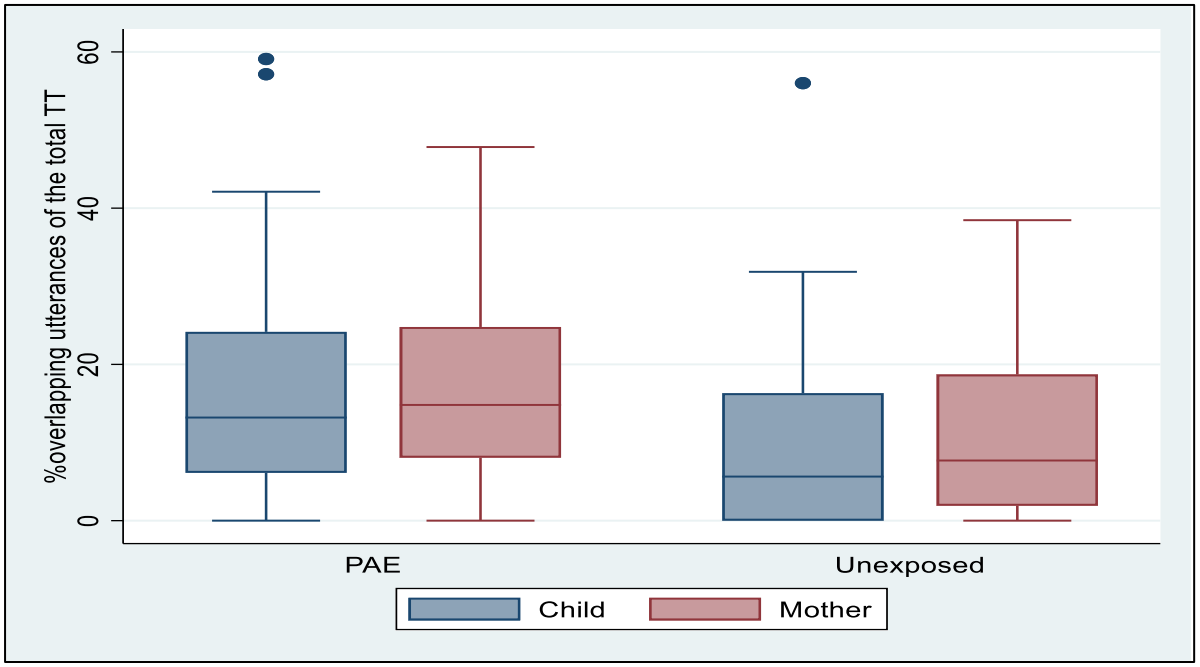
3. Results

Maternal and child characteristics are presented in Table 1. The median maternal age for this sample was 30 years (IQR 27.0-34.0) and the median child age was 3 years and 5 months (IQR 3.4-3.5). In the alcohol group, 10.0% of the mothers were HIV infected, 30.0% had PTSD-

depression risk and 0% completed tertiary education, while in the unexposed group, 10.0 % of the mothers were HIV infected, 29.0% had PTSD-depression risk and 1.7% completed tertiary education. There were no differences across the groups on any sociodemographic and psychosocial variables, except for smoking risk. Mothers who drank alcohol were more likely to smoke than their unexposed counterparts (exposed: 80.0% versus unexposed 45.0% respectively, p=0.002).

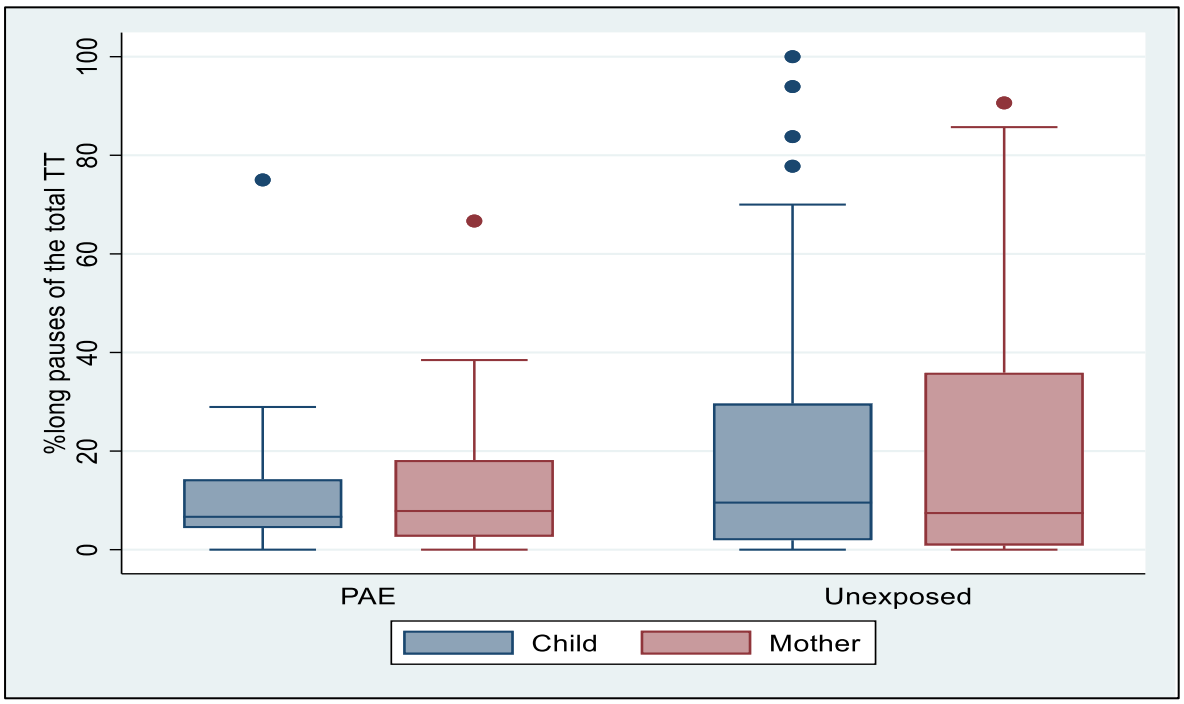
The alcohol group had significantly higher median scores than the unexposed group for percentage child overlapping utterances [median scores- 13.20 (IQR 6.12-24.17) versus; 5.64 (IQR 0-16.32) respectively, p=0.02] (Figure 2). There was a trend toward significance for percentage maternal overlapping utterances [median scores- 14.81 (IQR 8.06-24.79) versus; 7.70 (IQR 1.91-18.71) respectively, p=0.06] (Figure 2). No significant differences occurred between the exposed and unexposed groups for percentage maternal utterances preceded by long pause [median scores- 7.84 (IQR 2.63-18.18) versus 7.42 (IQR 0.86-35.90) respectively, p = 1.00] or child utterances preceded by pauses [median scores- 6.67 (IQR 4.39-14.29) versus 9.55 (IQR 1.91-29.63) respectively, p=0.42] (Figure 3).

Figure 2. Box plot of percentage overlapping utterances grouped by alcohol exposed versus unexposed



Percentages were calculated by dividing number of overlapping utterances by total conversational turn-taking (mother or child) and x 100. Dots indicate outliers.

Figure 3. Box plot of percentage utterances preceded by long pauses grouped by alcohol exposed versus unexposed



Percentages were calculated by dividing number of utterances preceded by long pauses by total conversational turn-taking (mother or child) and then x 100. Dots indicate outliers.

Table 2 shows the association between PAE and overlapping utterances for both mothers and their children. Using multiple variable logistic regression, there was a significant association between PAE and child overlapping utterances (overlaps comprises 20% or more of the total turn-taking) after adjusting for sociodemographic and psychosocial confounders [OR=3.25, 95%CI 0.98-10.76, p=0.05]. Alcohol exposed children were 3.25 times more likely to display overlapping utterances than their unexposed counterparts after adjusting for potential confounders. There were no significant associations between PAE and maternal overlapping utterances.

Table 3 demonstrates the association between PAE, and utterances preceded by long pauses for both mothers and their children. There was a trend towards a significant association between PAE and child utterances preceded by long pauses (long pauses comprise 20% or more of the total turn-taking) after adjusting for potential confounders [OR=0.30, 95%CI 0.08-1.06, p=0.06]. There were no significant associations between PAE, and maternal utterances preceded by long pauses.

4. Discussion

This novel study comprehensively assessed the association between PAE and conversational turn-taking in a South African sample of mother-child dyads at 42-months testing age. We found that children with alcohol exposure had higher median percentage scores for overlapping utterances compared to unexposed children, and these associations continue to exist after adjusting for potential confounders. Furthermore, PAE was not associated with maternal overlapping utterances, maternal or child utterances preceded by long pauses in this sample.

A strikingly unequal distribution of overlapping utterances was noted in our study across the alcohol and their unexposed counterparts. Alcohol exposed children had higher percentage of overlapping utterances (13%) than their unexposed counterparts (6%). This is contradictory to a previous study by Levinson and Torreira (18) who reported that typically developing children engage in about 5% of overlapping utterances during conversation with their mothers. The high proportion of overlapping utterances in children with alcohol exposure is likely evident, in part, because smooth conversational turn-taking is a task that is dependent on attentional control between speakers (44). Consistent with previous work by Lindinger and colleagues (45) exploring the effects of PAE on Theory of Mind (TOM), young children with a diagnoses foetal alcohol spectrum disorder (FASD) displayed impairments in attention control when responding to social cues. These authors found deficits in children's ability to understand and interpret other people's intentions, feelings and beliefs between the ages 9 to 11 years. Thus, there may be several reasons for the findings in our study. Children may struggle to focus their attention during conversation or lack the knowledge of the timing of turns, or perhaps they do not yet have a sufficiently developed TOM to deal with the combination of speaker and listener interaction. Considering the work by Hilbrink and colleagues (11), object play can be an effective tool to stimulate ideas in young children and further serves as a precursor for developing efficient turn-taking skills. Joint object play may provide an important context to explore conversational turn-taking abilities in young children with PAE and generate insight into their interaction with their mothers or caregivers. Our study adds to the growing body of literature implicating the association between PAE and specific components of conversational turn-taking in mother-child interactions.

There were important differences between our study and previously reported studies on the association between PAE and children's conversational patterns of talk. Previous studies have focused on social communication as a narrative discourse and used parent or teacher behaviour reports to assess the social interaction of school-going children (46, 47). These studies (48, 49) have reported that PAE may be associated with disruptive communication, poor understanding and inappropriate social behaviour in school-going children. In addition to the few studies on the association between PAE and conversational interaction, these studies (46, 47) have mainly focused on children in middle and high-income communities at school age. Our study, however, provided an observational account of conversational turn-taking in preschool children with PAE and their mothers residing in low-income communities.

Some limitations of our study deserve emphasis. Firstly, our study explored the conversational turn-taking of mothers and their children and excluded nonverbal communication such as gestures, eye contact and facial expressions. Secondly, despite assurances of confidentiality, some women may have chosen not to disclose, or minimized reporting alcohol use to the research staff, and the low reported alcohol consumption may therefore represent an element of response bias. The lack of information on father-child conversational turn-taking hampered the generalizability of the results to parent-child interactions. An additional limitation of this study was that sensory information was not explored in the study.

This is first study to report on the association between PAE, overlapping utterances and utterances preceded by long pauses in the mother-child dyad. Our study, reporting the association between PAE and child overlapping utterances, included a sample from two low-income communities as well as adjusted for potential confounders. As child overlapping utterances were affected, future research may be useful in determining strategies of improving conversational turn-taking which may include talk initiation, topic maintenance as well as appropriate use of overlaps during conversation in joint-play interactions.

The final chapter of this thesis will contain a general discussion of the findings presented hitherto, as well as the limitations of the studies, the value of the body of work described in

the thesis, and recommendations for future directions concluding remarks and recommendations for future work in the field.

Table 1. Maternal baseline sociodemographic and psychosocial characteristics

	Alcohol exposed N(%)=30 (33.3)	**Unexposed N(%)=60 (66.7)**	**p-value**
Maternal variables			
Clinic, n(%)			
Mbekweni	4 (13.3)	8 (13.3)	1.00
TC Newman	26 (86.7)	52 (86.7)	
SES, n(%)			
Lower levels of SES	25 (83.3)	43 (71.7)	0.58
Higher levels of SES	5 (16.7)	17 (28.3)	
Education, n(%)			
Primary	2 (7.0)	4 (6.7)	0.64
Secondary	28 (93.0)	55 (91.6)	
Tertiary	0 (0)	1 (1.7)	
Marital status, n(%)			
Married or cohabiting	12 (40.0)	21 (35.0)	0.41
Other	18 (60.0)	39 (65.0)	
HIV status, n(%)			
Infected	3 (10.0)	6 (10.0)	1.00
Uninfected	27 (90.0)	54 (90.0)	
Smoking risk (cigarette and cannibas), n(%)			
No	6 (20.0)	33 (55.0)	0.002*
Yes	24 (80.0)	27 (45.0)	
PTSD-depression risk, n(%)			
Absent	21 (70.0)	43 (71.0)	0.87
Present	9 (30.0)	17 (29.0)	
Parity, Median (IQR)	1 (0-1.0)	0 (0-1.0)	0.61
Maternal age, years, Median (IQR)	29.0 (27.0-34.0)	30.0 (27.0-33.8)	0.70

Child variables			
Gender, n(%)			
Male	16 (53.0)	32 (53.0)	1.00
Female	14 (47.0)	28 (47.0)	
Birth weight, Median (IQR)	2765 (2542.5-3292.5)	2905 (2465.0-3247.5)	0.91
Childs age, Median (IQR)	3 (3.4-3.5)	4 (3.4-3.5)	0.90
BMI z-score, Median (IQR)	-0.2 (-0.7-0.1)	-0.2 (-0.8-0.5)	0.91
Gestational age, weeks Median (IQR)	39 (38.0-39.0)	39 (39.0-40.0)	0.37

* $p<0.05$

*Comparing the sample of mothers whose children had outcome data for the analysis to those who did not have outcome data displayed no differences in terms of sociodemographic and psychosocial characteristics.

Table 2. Logistic regression analysis of predictors for percentage overlapping utterances

	Mother			Child		
	Odds ratio (OR)	**95% CI**	**p-value**	**Odds ratio (OR)**	**95% CI**	**p-value**
PAE	1.33	0.44-4.05	0.62	3.25	0.98-10.76	0.05*
Clinic	2.89	0.29-28.57	0.36	0.69	0.12-3.87	0.66
BMI	0.94	0.55-1.61	0.82	1.05	0.59-1.86	0.88
Smoking risk	1.32	0.40-4.33	0.65	0.77	0.20-2.98	0.70
PTSD-depression risk	2.46	0.83-7.33	0.11	2.25	0.69-7.30	0.18
SES	1.27	0.76-2.13	0.36	1.15	0.65-2.06	0.63

* $p<0.05$

Table 3. Logistic regression analysis of predictors for percentage utterances preceded by long pauses

	Mother			Child		
Variables	**Odds ratio (OR)**	**95% CI**	**p-value**	**Odds ratio (OR)**	**95% CI**	**p-value**
PAE	0.65	0.21-2.08	0.47	0.30	0.08-1.06	0.06
Clinic	1.58	0.27-9.17	0.61	1.13	0.21-6.12	0.88
BMI	1.82	1.09-3.06	0.02	1.79	1.06-3.02	0.03
Smoking risk	0.98	0.30-3.16	0.97	0.94	0.30-3.00	0.92
Psychological variables	2.29	0.76-6.91	0.14	0.78	0.24-2.47	0.67
SES	1.32	0.79-2.21	0.29	1.01	0.61-1.67	0.97

* $p<0.05$

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CHAPTER 7

DISCUSSION

1. Introduction

This chapter comprises a discussion of (a) the key findings of this thesis, in a summarised and integrated form; (b) the significance in light of prior published work; (c) the limitations and strengths of the studies; (d) and recommendations for future directions with a focus on research in low and middle-income countries (LMICs) such as South Africa; and (e) final conclusions.

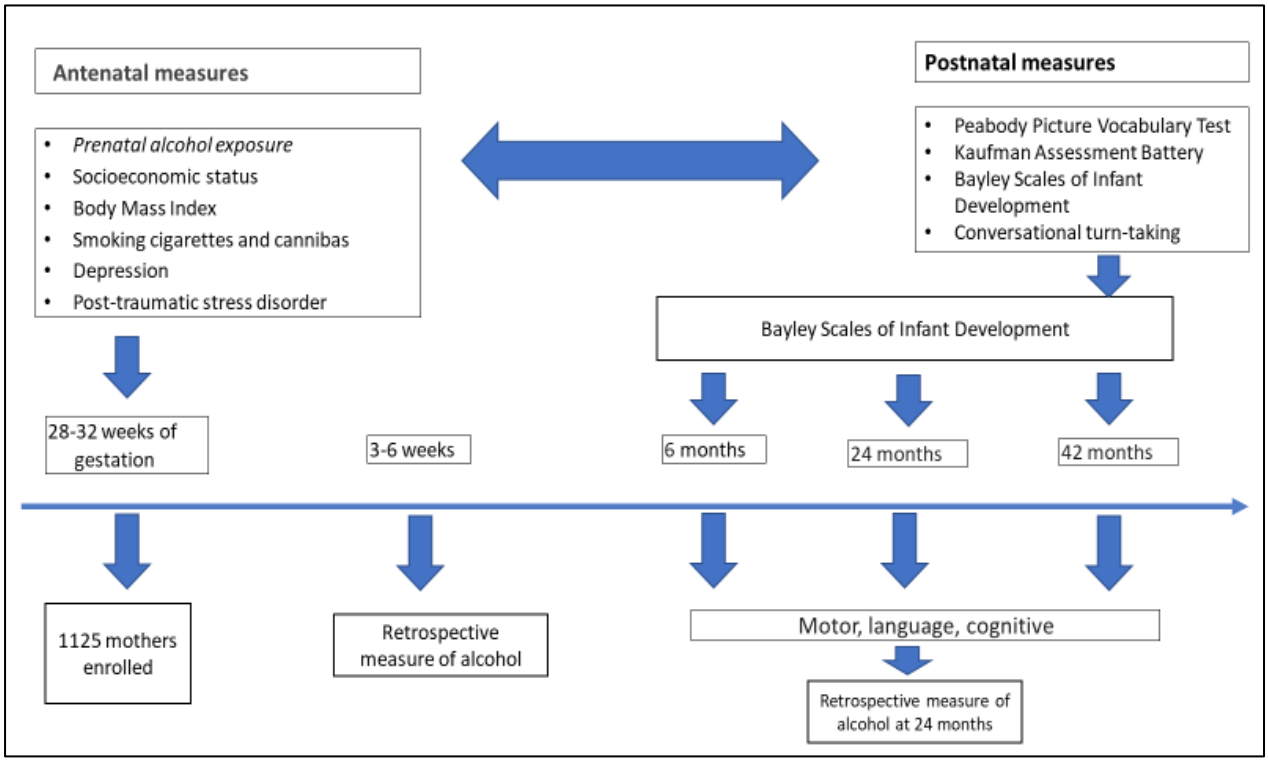
2. Summary of findings

Prenatal alcohol exposure (PAE) produces central nervous system impairments, which is a significant threat to early and later developmental outcomes (1-4). Uncertainty remains regarding the association between moderate-to-severe levels of PAE, as well as the developmental trajectories of infants and young children, especially in the context of high sociodemographic and psychosocial risk factors (5). Prior cross-sectional studies examining the association of PAE and early neurodevelopmental outcomes have demonstrated impairments in motor, language and cognitive outcomes. However, through the early years, the potential association of PAE and early neurodevelopmental trajectories have not been well described in longitudinal studies, especially in low- and middle-income countries (LMCs). Previous work has also highlighted that PAE depends on several contextual factors, including but not only limited quantity, frequency, and timing of alcohol consumption during pregnancy (4), maternal risk factors, and genetic predispositions (4, 5). In addition, postnatal socioeconomic status (SES) and environmental factors may impact the outcomes related to PAE (5). Considering the large number of children globally with PAE as well as important contextual risk factors to which these children are frequently co-exposed, it is particularly important to examine early neurodevelopment in both the broader environmental context as well as across time. This also highlights the complexities in identifying the effects of PAE over the developmental trajectory and the need for early intervention that mitigates the negative enduring effects of PAE in addition to other contextual factors. Given that the bulk of longitudinal investigation have been conducted in North American and European contexts, relatively less is known on the longitudinal motor, language and cognitive outcomes associated with prenatal alcohol exposure in LMCs such as South Africa.

The overall aim of this thesis was to assess the association between PAE and early neurodevelopmental outcomes within the same group of children across 6, 24 and 42 months of age as part of the Drakenstein Child Health Study (DCHS), a South African birth cohort. The specific objectives of this thesis included: understanding the existing literature reporting longitudinal studies exploring the impact of PAE on language, speech and communication development through a systematic review; exploration of the association between PAE and early neurodevelopment through two years of age; investigation of the association between PAE, including comorbid smoking exposure, and the neurodevelopmental trajectories of children in the first 4 years of life and a comparison of conversational turn-taking between mothers and their alcohol exposed children, to those between mothers and their unexposed children at the age of 42 months.

This study incorporated exposure data through a self-report measure of alcohol use in pregnancy as well as other antenatal sociodemographic and psychosocial measures. It also included child neurodevelopmental measures postnatally in the same group of children (Figure 1).

Figure 1. Summary of maternal characteristics and child developmental assessments



The key findings of this thesis were as follows: (1) PAE was significantly associated with early fine and gross motor outcomes at 6 months only [(when controlling for body mass index (BMI), SES, smoking exposure, post-traumatic disorder (PTSD) and depression)]. These findings are somewhat in line with Hypotheses (7.1) of this thesis (see Chapter 1, Section 7, p 28), i.e. We expected to find lower neurodevelopmental outcomes than their unexposed counterparts after adjusting for confounders at both 6 and 24 months; (2) PAE, and comorbid tobacco smoking exposure, were found to be significantly associated with lower fine motor functioning at 6 and 24 months but these associations improved by 42 months, while the interaction between prenatal alcohol and tobacco smoking exposure resulted in lower receptive vocabulary and cognitive functioning at 24 months only (when controlling for SES, smoking, PTSD and depression). These results are somewhat in keeping with Hypothesis (7.2) of this thesis (see Chapter 1, Section 7, p 28), i.e. We expected to find notable associations between PAE, in interaction with comorbid tobacco smoking exposure, and lower neurodevelopmental outcomes on trajectories of 6, 24 and 42 months of age; (3) PAE, after controlling for clinic site, BMI, smoking exposure, PTSD, depression and SES, were found to be significantly associated with poor conversational turn-taking particularly child overlapping utterances at 42 months of age. These findings are also somewhat in line with Hypothesis (7.3) of this thesis (see Chapter 1, Section 7, p 28) i.e. We expected to find significant differences between the conversational turn-taking of mothers and their alcohol exposed children with that between mothers and their unexposed children at 42 months testing age.

Throughout all studies, PAE, was notably associated with lower early neurodevelopmental outcomes similarly to prior studies. However, some findings were also not consistent with the initial hypotheses; nor with prior work in the field. These discrepancies may have been attributable to multiple factors, including limited power to detect smaller effect sizes; heterogenous assessment of PAE or unmeasured (residual) confounding factors.

The four studies (or papers) presented below directly addresses the objectives and the significance of the findings in light of prior published work.

2.1 Effects of prenatal alcohol exposure on language, speech and communication outcomes: A review of longitudinal studies

PAE has been found to be associated with a continuum of physical, cognitive and behavioural impairments in young children. However, few studies exist on exploring the longitudinal association of PAE and early neurodevelopmental outcomes, especially language development (6-8). Most previous studies report on cross-sectional associations; however, restrictions of these studies include the inability to infer strong causal associations on neurodevelopmental outcomes over time. Longitudinal designs provide the opportunity to explore cause and effect associations, including the consideration for potential confounding factors which may exert important additional influences on child developmental outcomes over multiple timepoints. Studies exploring the impact of PAE on the progression of language, speech or communication which explores the impact of environmental confounders remain less common (7, 8). While most previous review studies mainly explored cross-sectional associations, it is crucial to conduct a systematic review focussing on longitudinal studies exploring the association between PAE and the early language outcomes across multiple timepoints (Chapter 3 Publication 1). This study therefore aimed to assess previous longitudinal literature on the reported associations between PAE and language, speech and communication development in the first few years of life. Additionally, it included previously reported data from more than one timepoint in the same (or at least overlapping) group of children.

The systematic review reported six publications exploring the association of PAE and language development over time, presenting varied results across the first few years of life. Three studies highlighted associations between PAE and expressive communication and receptive vocabulary in the first three years of life (7-9). Follow-up studies, however, demonstrated attenuation of PAE effects with no differences between the alcohol exposed and unexposed children beyond three years of age (8-10). The above-mentioned studies, though controlling for some potential sociodemographic confounders, were based in the context of well-resourced countries (9, 10). However, contextual risk factors such as low levels of income, alcohol use disorders and trauma-related exposures were found to be particularly relevant to LMICs settings (5, 6). Others have reported low levels of maternal education in addition to

dysfunctional home environments contributing to increased risk for early language impairments (11-12). While there has been a wide range of studies exploring sociodemographic factors, there is a scarcity of published studies investigating the mental health risk factors associated with PAE in LMICs settings.

This review identified significant gaps in the current literature regarding the investigation of PAE and early language development (Chapter 3 Publication 1). First, similar to previous work (17), this review confirmed that few studies have reported on the association between PAE and child developmental outcomes over time in the same cohorts of children. Second, the lack of research on psychosocial factors (e.g. trauma related exposures) associated with PAE is particularly relevant for research in LMICs such as South Africa. Our review further demonstrated that the lack of control groups in some of the studies ultimately meant that contextual factors could not be adequately considered. This review also indicated that many previous studies measured alcohol exposure using diagnostic instruments. The criteria for PAE in these studies included women who meet the criteria for alcohol abuse and dependence, with a lack of information on the alcohol exposure effects in young children. While most of the included maternal data were self-reported measures, the inclusion of objective and quantitative measures of PAE (hair, nails or meconium) have been limited. These objective PAE measures have become increasingly available and may assist in countering self-report bias and underreporting. This approach, where it can be afforded, should be included in future study designs in order to supplement maternal recall of alcohol exposure. Additional research on child neurodevelopment in children with PAE, as well as a consideration for contextual risk factors in alcohol exposed children are needed which may be important for preventive interventions for young children.

2.2 Prenatal alcohol exposure impacts early infant motor, but not language development in a South African birth cohort.

Many contextual factors appear to exacerbate the impact of PAE on the developing, exposed child. This is particularly problematic and presents a concern for women of reproductive age and their children in many communities in South Africa (13-17). In a large prospective birth-cohort study (N=9912), namely the Safe Passage Study, the maternal characteristics

associated with different patterns of exposure of both American and South African pregnant women were explored (15). In this study (15), it was found that mothers with high-to-low levels of alcohol consumption were more likely to smoke moderate-to-high levels of tobacco, compared to unexposed mothers or quitters. This study (15) reflected the high prevalence of PAE and additional risk factors, and the scarcity of published studies investigating the interaction of these maternal exposures on child developmental outcomes. Not many previous studies have explored additional psychosocial confounders (15), such as maternal depression and/or trauma exposure, which frequently co-occur with prenatal alcohol consumption and which may have a detrimental impact on young children's outcomes. Our study includes particularly an at-risk population, with high prevalence of alcohol or substance use and other maternal exposures (13). In addition to substance exposures, other factors include low SES, exposure to violent behaviours and employment instability may place additional stress on individuals (13-15). While these risk factors may place children at increased risk for later impairments, very few studies included developmental data at different timepoints in the first two years of life (Chapter 4 Publication 2).

This study aimed to investigate the association between PAE and early neurodevelopment through two years of age, adjusting for sociodemographic and psychosocial factors in the DCHS, a South African birth cohort study. We found that PAE was significantly associated with both poor gross and fine motor functioning at 6 months of age, after adjusting for potential sociodemographic and psychosocial confounders (Chapter 4 Publication 2). This is consistent with a previous longitudinal study in Western Ukraine, where Coles and colleagues (12) explored the associations of PAE with preterm delivery and child developmental outcomes. These authors reported a significant mediating effect of preterm birth on the association between PAE, motor and cognitive development at 6 months of age. Overall, our findings demonstrated the need to focus on alcohol exposed infants in LMICs such as South Africa. While PAE has been a great burden in this country, a seminal review of development in low-resource settings emphasized that more than 200 million children under the age of 5 years do not reach their developmental potential due a wide range of maternal risks factors (14). These risk factors include poor health and inadequate nutrition, living in a culture where binge or heavy drinking is common and accepted, limited knowledge and awareness of alcohol

consumption during pregnancy, and not receiving adequate prenatal care. This is a crucial concern especially when overall motor functioning is closely associated with other developmental domains (18-20). In a previous Millennium Cohort Study (21), it was found that impaired motor development at 9 months was significantly associated with cognitive and language development at 5 years of age, and this may have detrimental consequences for overall development, especially if there are additional contextual factors that may contribute child developmental impairments. Although our findings are consistent with other studies on the effects of PAE on fine and gross motor functioning in the early years (18), evaluating these measures in later age cohorts will give us a clearer understanding of their long-term significance.

On investigation of other developmental domain outcomes, our study demonstrated no PAE-associated effects for language and cognitive outcomes at 6 and 24 months. Previous studies have reported that other factors may explain the lack of an association between PAE and the neurodevelopmental outcomes of young children (6, 7, 24-25). Gabor and colleagues (22), for example, showed that the lack of positive reinforcement in the home environment was associated with delayed expressive communication among infants. This association was explained in part by the back-and-forth verbal responses of mothers and the influence it has on children's language development (22). Our research highlights the importance of taking contextual factors (home environment, parent-child relationship, other substance exposures) that may be important for early neurodevelopmental outcomes into consideration.

2.3. Prenatal alcohol exposure is associated with early neurodevelopmental trajectories in a South African birth cohort: the first 4 years.

Numerous studies have investigated the associations of comorbid PAE and smoking exposure with neurodevelopment; however, very few studies investigated the association of PAE, including tobacco smoking exposure, across trajectories of developmental outcomes. While these studies have reported significant cross-sectional associations (12-17), inconsistent findings demonstrate the need for data on comorbid prenatal substance exposures on longitudinal neurodevelopmental trajectories. Longitudinal analyses have unique potential to improve understanding of the dynamic processes that shape child development, including

trends and trajectories. Longitudinal designs usually provide high statistical power to detect small effect sizes (23), and also provide analytical benefits such as the ability to examine developmental change over time (e.g., Chapter 5 Publication 3). Thus, understanding the effects of both prenatal alcohol and tobacco smoking exposure is critical to explore characteristics that are uniquely associated with child neurodevelopmental trajectories across multiple timepoints.

The findings in our study showed that both PAE and tobacco smoking exposure were significantly associated with fine motor performance through 24 months, and these effects dissipated by 42 months (Chapter 5 Publication 3). Similar to previous studies, the severe effects of both PAE and tobacco smoking exposure have demonstrated worse developmental outcomes in comparison to children exposed to alcohol or to tobacco alone in the early years (23, 28, 29). Hamulka and colleagues (25), for example, demonstrated that concurrent prenatal alcohol and tobacco exposure contributed to increased risk for adverse outcomes in the health status of infants, such as low birth weight, reduced Apgar scores and increased risk for infectious diseases (25). Our findings further suggests that tobacco smoking exposure are especially important in the context of recent reports suggesting that 'high-risk' mothers are exposed to a wide variety of interrelated risk factors during pregnancy (e.g. alcohol, smoking and/or substance exposures, depression, intimate partner violence), and these factors continue to persist between prenatal and postnatal periods (24-26). Our study differs from other studies in that it described the interaction of PAE and tobacco smoking exposure on early neurodevelopmental trajectories, and in addition, it followed children in the same cohort across three timepoints. The high prevalence of maternal smoking in our cohort further highlighted the need to explore a pattern of co-occurring psychosocial risk factors associated with PAE on early neurodevelopmental outcomes, especially in high-risk environments.

Our study demonstrated that PAE, including comorbid tobacco smoking exposure, were significantly associated with lower receptive language and cognitive vocabulary at 24 months only (Chapter 5 Publication 3). Similarly, previous work (18) has demonstrated that both alcohol and tobacco risky behaviours were associated with poor cognitive and language outcomes among infants with significant differences between exposed and control groups in

the early years. Other studies have demonstrated that it is vital to consider the complex interplay of factors (alcohol and other exposures) that may influence early cognitive development in high risk environments (24-27). For example, previous studies have investigated a wide range of developmental outcomes associated with PAE which may be identifiable in the first months of life before higher order cognitive, motor or language functions emerge, and postnatal environmental factors confound developmental functioning (24-27). In a previous publication from the DCHS, Donald and colleagues (13) have found that young children in South Africa are exposed to multiple risk factors, and therefore, advocate for the importance of assessing child developmental outcomes using a multidimensional approach (13). This is in relation to current evidence which suggests that PAE may comprise one of multiple factors which may interact across physical, environmental and psychosocial domains during critical windows, thus resulting in greater risk for impaired developmental outcomes in high-risk environments. Postnatal factors, including body mass index (BMI) and lifelong and current nutrition deficiencies, may have a substantial impact on the alcohol exposed child and place them at greater risk for developmental impairments (27). The value of this longitudinal study in predicting, motor, language and cognitive developmental outcomes later in childhood, support the crucial need for the use of a standardized screening protocol to help clinicians identify infants or toddlers who may have been exposed to alcohol prenatally and will thus benefit from closer monitoring of their development.

2.4. Prenatal alcohol exposure impacts conversational turn-taking between young children and their mothers in a South African birth cohort study.

PAE may be associated with a wide variety of neurodevelopmental effects, including cognitive, language and sociocommunicative impairments. Previous work has reported that PAE was associated with impaired language outcomes, including receptive or expressive communication, throughout the preschool years (27, 28). To date, the associations between PAE and conversational pattern of talk among young children have not been widely investigated. Previous reports have primarily assessed the association between PAE and structural components of language (receptive and expressive) (7-10). Very few studies have explored the association between PAE and conversational patterns of talk in young children (27, 28). While previous studies have shown that PAE can adversely impact the way children

engage in conversation (27), the mother-child dyad can provide an important context for advanced vocabulary development, open-ended engagement and nonverbal exchanges. This inevitably aid long-term language development (28). To our knowledge, no studies have explored the association between PAE and conversational turn-taking, which is important for examining the language development of young children. This study represents one of the first cohorts at preschool age investigating the effects of PAE on the conversational turn-taking of mother-child dyads. Further empirical research was therefore needed to explore the conversational patterns of talk amongst alcohol exposed mother-child dyads. The aim of this sub-study was to compare conversational turn-taking between mothers and PAE children, to those between mothers and unexposed children (Chapter 6 Publication 4).

The main finding of our study was the association between PAE and altered patterns of talk amongst preschool children (Chapter 6 Publication 4). In particular, PAE was significantly associated with child overlapping utterances, and even after adjusting for potential confounders [(clinic, BMI, smoking exposure, SES, symptom severity of post-traumatic stress disorder (PTSD) and depression)], this specific association remained. This research resonates with a previous study done by Coggins and colleagues (29), who undertook a retrospective examination of environmental risk, language performance and narrative discourse in school-aged children with FASDs located in Washington, USA. They reported that children with FASDs were at an increased risk for disruptive communication, poor receptive vocabulary and inappropriate social behaviour, thus impairing their social communication (29). They also suggested that it is likely that overall environmental risk factors, (e.g. home environment) are important markers for conversational and language development (29). Furthermore, while conversational disruptions occurred in the preschool years in our cohort, this may adversely impact their expressive and receptive communication skills at a later stage. Therefore, it is crucial to consider the relational aspects of language development especially when considering the intervention design for this specific age group.

3. Limitations and strengths

Relative to older infants and children, relatively little research has investigated the early neurobehavioral effects of PAE, and several research gaps remain. The majority of studies

investigating outcomes in neonates drew samples from North American populations mostly consisting of middle-income participants. Further research drawing from a wider population base including a greater range of socio-economic and cultural environments is required to clarify the generalisability of the existing findings. Similarly, evidence on how outcomes identifiable during the neonatal period may predict functioning later in infancy in relation to alcohol exposure patterns remains limited (40). Furthermore, the actual mechanisms by which the effects translate into real life developmental and neurobehavioral outcomes are poorly understood and are compounded and confounded by the complexity of the central nervous system (CNS), rapid body change with time, and other factors.

The limitations of this thesis should be taken into consideration. Firstly, the PAE data considered in this study was dependent on self-report measures. This may have resulted in the risk of socially desirable responses regarding alcohol consumption during pregnancy, thus resulting in biased responses. Despite this limitation of the self-report measures, the DCHS birth cohort provided a population-based platform to prospectively explore associations over time. Secondly, this thesis was administered in one sub-district in South Africa comprising predominantly two low-income, peri-urban communities. While these communities are representative of informal or sub-standard housing and high prevalence of environmental risk factors [(exposures of smoking, HIV, trauma and depression)] including a broad investigation of sociodemographic and psychosocial risk factors, it is not necessarily generalisable to the larger Southern African population.

Specific limitations and strengths for the sub-studies are discussed below:

While two of the studies (Chapter 4 Publication 2; Chapter 6 Publication 4) utilised cross-sectional designs where infants and toddlers were strictly matched to their healthy counterparts, longitudinal data in this thesis was key in establishing trajectories of development across three timepoints (Chapter 5 Publication 3). A limitation of this study (Chapter 5 Publication 3) was the lack of repeated measures for receptive, expressive and cognitive outcomes at 42 months testing age. The rate of change was only assessed for fine motor functioning using repeated measures across the different timepoints, whereas for the other domains, the pattern of association at the different timepoints was observed only, and

this may have had an impact on inferences drawn from the findings of this study (Chapter 5 Publication 3). While longitudinal data requires repeated measures, few single measures are available to adequately assess language and cognitive outcomes in very young children between 6 and 42 months, and this is a period where rapid language and cognitive development occurs. As a result, we attempted to minimise this limitation by internally transforming scores to create z-scores at all three timepoints for both language and cognitive measures.

The relatively small sample sizes reduced statistical power to detect possible associations between PAE and the neurodevelopmental outcomes in this cohort. Despite the small sample sizes, the inclusion of two sub-populations namely the black African and mixed-race populations from poor, peri-urban communities are representative of much of South Africa's sociodemographic composition (30).

4. Recommendations

This thesis provided important evidence for the effects of PAE on early neurodevelopmental outcomes in peri-urban, low SES communities. Consideration of different sub-types of PAE (exposed versus unexposed); as well as important potential confounders (e.g. SES, tobacco smoking, PTSD and depression risk), provided a good basis for drawing inferences about the associations of PAE with early motor, language and cognitive outcomes in this high-risk context.

It is well documented that the development of motor outcomes in infancy and early childhood are influenced by both physical growth and maturity characteristics (physiological and neurodevelopment). During the first few years of life, children gradually develop posture, locomotor and prehensile functioning (31). As children mature into the preschool or higher-grade school years, their finer motor skills increase (arts and craft tasks), which becomes integral for later preschool and later academic development. Evaluating motor performance at school age using our cohort may further provide additional insight into children's fine motor performance in an academic or real-world setting.

While assessing motor development at school age would crucial for understanding children's patterns of development over time, one would expect that not all children would display the same pattern of development. Thus, more research on resilience factors are needed to better understand how some children can cope and thrive despite the challenging and adverse environments they reside in. Parenting styles can be considered as one these examples.

5. Concluding remarks

This thesis investigated the effects of PAE on neurodevelopmental outcomes (motor, language, cognitive and conversational patterns of talk) during early childhood. We discussed summary findings from the literature on the general developmental outcomes and domain-specific effects of PAE on infants and preschool aged children with the aim to highlight the gaps in our understanding and directions for future research. In sum, studies within this thesis highlight the complex association between PAE and various neurodevelopmental outcomes. This thesis shows that the motor, language (including conversational talk) and cognitive effects of PAE are apparent during the early childhood period across multiple timepoints. The findings of our study provide a foundation for further research examining the effects of PAE on the neurodevelopmental trajectories in these crucial years. In addition, the comorbidity of prenatal alcohol and tobacco smoking exposure and how this is associated with negative developmental outcomes will need to be taken into account when considering the long-term clinical impact. This thesis presents a step towards prioritising the implementation of programmes for mothers and their children in communities where alcohol disorders are highly prevalent. It is recommended that primary health care workers monitor the detection of prenatal alcohol consumption, which may allow for the opportunity of prevention and reduction in the severity of developmental impairments in young children.

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Appendix A: Ethics approval HREC

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences

Human Research Ethics Committee

Room E53-46 Old Main Bullding Groote Schuur Hospital Observatory 7925 Telephone [021] 406 6492

21 August 2017 HREC REF: 338/2017

A/Prof K Donald

Division of Developmental Paediatrics Department of Paediatrics & Child Health Rondebosch

Dear A/Prof Donaid

PROJECT TITLE: PRENATAL ALCOHOL EXPOSURE AND LANGUAGE OUTCOMES: A SOUTH AFRICAN PERSPECTIVE-LINKED TO 401/2009 (PhD candidate- G Hendricks)

Thank you for your response letter, addressing the issues raised by the Human Research Ethics Committee (HREC),

It is a pleasure to Inform you that the HREC has formally approved the above-mentioned study. Approval is granted for one year until the 30 August 2018. Please submit a progress form, using the standardised Annual Report Form if the study continues

beyond the approval perlod. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: :h ct. fh arch ethi Ss)

We acknowledge that the student, G Hendricks will also be Involved in this study. Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal Investigator must obtain appropriate Institutional approval before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRBO00001938

HREC 338/2017 This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.

The Human Research Ethics Committee granting thls approval Is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC 338/201

Appendix B: Timeline for maternal sociodemographic and psychosocial factors as part of the Drakenstein Child Health Study (DCHS)

Maternal															
Domain	**Measure**	**Sample size**	**ANC2**	**6-10 wks**	**6m**	**9m**	**1y**	**1.5 y**	**2y**	**2.5y**	**3y**	**3.5y**	**4y**	**4.5y**	**5y**
Social aspects															
Socio-economic status (SES)	- Questions validated from SASH *(incorporated into core maternal battery)*	Total	X	X	X		X	X	X	X	X	X	X	X	X
Social capital/support	- Multidimensional Scale of Perceived Social Support (MSPSS)	Total								X		X		X	
	- Planning of birth and partner support	Total	X												
Participant experience	- Participant experience questionnaire	Total					X				X				
Psychological aspects															
Trauma, resilience & PTSD	- Modified Life Events Questionnaire (LEQ)	Total	X	X	X		X	X	X		X		X		X

	- Childhood Trauma Questionnaire (CTQ)	Total	X												
	- Peri traumatic distress inventory (PDI)	Sub-set	X												
	- Clinician administered PTSD scale (CAPS)	Sub-set	X					X			X				
	- Modified PTSD symptom scale (MPSS)	Total	X	X	X			X	X		X		X		X
	- Intimate partner violence (IPV)	Total	X	X	X		X	X	X		X		X		X
	- Survey of Exposure to Community - Violence(SECV)	Total								X					
	- *Connor-Davidson resilience scale (CD-Risc)	Total								X				X	
Psychological Distress	- Self Report Questionnaire (SRQ-20)	Total	X	X	X		X	X	X		X		X		X
	Measure	**Sample size**	**ANC2**	**6-10 wks**	**6m**	**9m**	**1y**	**1.5 y**	**2y**	**2.5y**	**3y**	**3.5y**	**4y**	**4.5y**	**5y**
	- MINI International Neuropsychiatric Interview (MINI)	Total	X					X			X				

Depression (& suicidality)	- Edinburgh Postnatal depression rating scale (EPDS)	Total	X	X	X		X	X							
	- Beck Depression Inventory (BDI)	Total	X				X	X	X		X		X		X
	- Suicide and self harm scale (SSH)	Total	X				X	X	X						
Maternal coping style	- Brief COPE	Total								X					
Emotion regulation, cognitive, social abilities	- Difficulty in Emotions Regulation scale (DERS)	Sub set +/- 200									X				
	- *Affect Regulation Checklist (ARC)	Total												X	
	- Eye tracking assessment	Sub set +/- 200									X				X
	- Questionnaire of Cognitive and Affective Empathy – Mother (QCAE)	Total										X		X	
	- Emotion Regulation Questionnaire (ERQ)	Sub set +/- 200									X				
Substance use/abuse	- Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)	Total	X	X	X		X	X	X		X		X		X
	- Retrospective measure of alcohol use during	Total – together with 24					X								

	pregnancy- devised for purpose of study (Retro Alc)	month Bailey													
Aggression/hostility in relationship	- *NIH TB Emotion Anger Questionnaire (EAQ)	Total												X	

Appendix C: Timeline for child psychosocial factors as part of the Drakenstein Child Health Study (DCHS)

Child Domain	Measure	6-10 wks	6m	9m	1y	1.5y	2y	2.5y	3y	3.5y	4y	4.5y	5y
Trauma exposure and symptoms	- Child exposure to community violence checklist									X		X	
Aggression and personality style	- Child behaviour checklist						X			X			X
	- Paediatric emotional distress scale									X		X	
Empathy	- Chicago Empathy task												X
	- Questionnaire of Cognitive and Affective Empathy-Parent report on child												X
Moral decision making	- Chicago Moral Judgment task (moral dev tasks)												X

Emotion regulation, self regulation, cognitive, social abilities, prosocial behavior	- Delay tasks ○ Kochanska Snack Delay task									X			
	○ Kochanska Gift Delay task ○ Kochanska Whisper task												X

Appendix D: Alcohol Smoking and Substance Involvement Screening Test

These are some questions about your experience of using substances across your lifetime and in the past three months. These substances can be smoked, swallowed, snorted, inhaled, injected or taken in the form of pills. Some of the substances listed may be prescribed by a doctor (like amphetamines, sedatives, pain medications). For these questions, do not record medications that are used as prescribed by your doctor. However, if you have taken such medications for reasons other than prescription or taken them more frequently or at higher doses than prescribed, please record these. While we are also interested in knowing about your use of various illicit (illegal) drugs, please be assured that information on such use will be treated as confidential.

1	In your life, which of the following substances have you ever used? (NON-MEDICAL USE ONLY)	NO	YES
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)		
B	Alcoholic beverages (beer, wine, spirits, etc.)		
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)		
D	Cocaine (coke, crack, etc.)		
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)		
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)		
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)		
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)		
I	Opioids (heroin, morphine, methadone, codeine, etc.)		
J	Other – specify:		
	If all answers are negative: “Not even when you were in school?” If “No” to all items, go to Question 6. If “Yes” to any of these items, answer Question 2 for each substance ever used.		

2	In the past three months, how often have you used the substances you mentioned (FIRST DRUG, SECOND DRUG, ETC)?	Never	Once or twice	Monthly	Weekly	Daily or Almost Daily
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)					
B	Alcoholic beverages (beer, wine, spirits, etc.)					
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)					
D	Cocaine (coke, crack, etc.)					
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)					
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)					
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)					
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)					
I	Opioids (heroin, morphine, methadone, codeine, etc.)					
J	Other – specify:					
	If all answers are negative:					
3	In the past three months, how often have you used the substances you mentioned (FIRST DRUG, SECOND DRUG, ETC)?	Never	Once or twice	Monthly	Weekly	Daily or Almost Daily
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)					
B	Alcoholic beverages (beer, wine, spirits, etc.)					

C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)					
D	Cocaine (coke, crack, etc.)					
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)					
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)					
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)					
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)					
I	Opioids (heroin, morphine, methadone, codeine, etc.)					
J	Other – specify:					
	If all answers are negative:					

4	During the past three months, how often has your use of (FIRST DRUG, SECOND DRUG, ETC) led to health, social, legal or financial problems?	Never	Once or twice	Monthly	Weekly	Daily or Almost Daily
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)					
B	Alcoholic beverages (beer, wine, spirits, etc.)					
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)					
D	Cocaine (coke, crack, etc.)					
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)					

F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)					
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)					
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)					
I	Opioids (heroin, morphine, methadone, codeine, etc.)					
J	Other – specify:					
	If all answers are negative:					

5	During the past three months, how often have you failed to do what was normally expected of you because of your use of (FIRST DRUG, SECOND DRUG, ETC)?	Never	Once or twice	Monthly	Weekly	Daily or Almost Daily
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)					
B	Alcoholic beverages (beer, wine, spirits, etc.)					
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)					
D	Cocaine (coke, crack, etc.)					
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)					
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)					
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)					

H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)					
I	Opioids (heroin, morphine, methadone, codeine, etc.)					
J	Other – specify:					
	If all answers are negative:					

6	Has a friend or relative or anyone else ever expressed concern about your use of (FIRST DRUG, SECOND DRUG, ETC)?	Never	Once or twice	Monthly
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)			
B	Alcoholic beverages (beer, wine, spirits, etc.)			
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)			
D	Cocaine (coke, crack, etc.)			
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)			
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)			
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)			
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)			
I	Opioids (heroin, morphine, methadone, codeine, etc.)			
J	Other – specify:			
	If all answers are negative:			

7	Have you ever tried and failed to control, cut down or stop using (FIRST DRUG, SECOND DRUG, ETC)?	Never	Once or twice	Monthly
A	Tobacco products (cigarettes, chewing tobacco, cigars, etc.)			
B	Alcoholic beverages (beer, wine, spirits, etc.)			
C	Cannabis (marijuana, pot, grass, hash, dagga, etc.)			
D	Cocaine (coke, crack, etc.)			
E	Amphetamine-type stimulants (speed, diet pills, ecstasy, Tik, etc.)			
F	Inhalants (nitrous, glue, petrol, paint thinner, etc.)			
G	Sedatives or Sleeping Pills (Valium, Serepax, Rohypnol, etc.)			
H	Hallucinogens (LSD, acid, mushrooms, PCP, Special K, etc.)			
I	Opioids (heroin, morphine, methadone, codeine, etc.)			
J	Other – specify:			
	If all answers are negative:			

8	Have you ever used any drug by injection? (NON-MEDICAL USE ONLY)	No, never Yes, in the past 3 months Yes, but not in the past 3 months

Appendix E: Drakenstein Child Health Study - Consent and Information sheet for mothers

You and your child are invited to take continue to take part in a study that is being done in the Drakenstein sub-district, in collaboration with the Universities of Cape Town and Stellenbosch. We would like to thank you and your child for taking part in this study, we hope to impact child health and your participation will help us achieve that. The following information describes the study and you and your child's role for the next year. Please read this carefully and feel free to ask any questions.

Why is this study being done?

Lung infections and chest problems are common in young children. This study is being done to find out the effect of chest infections in the early years of life on the development of lung disease in children. The study will also look at a number of other factors that may affect your child's health.

You and your child will continue to attend occasional scheduled visits at your primary health care clinic and at Paarl Hospital. During these visits, we will assess the health of you and your child by using questionnaires and doing tests. Should your child get sick with a chest infection, then he/ she will be carefully investigated to try and find out the cause of this infection. This study will help us to better understand why children get chest illness and may help to improve child health.

Cognition and social emotional development are important parts of a child's overall health. By cognition we mean the way a child thinks, learns, plans and pays attention. Social emotional and social cognitive development refer to how a child learns to understand their own and others' emotions, and to reason about what other people think, believe and feel. We want to see how these factors develop over time. This study will help us understand how various other factors we look at in the main study impact on this development, and also how this development affects overall health.

What must I do if I agree to continue in the study?

If you agree to continue in this study, we will follow you and your child regularly to assess his/her health. We will see you and your child at Paarl hospital when your child is about 3 years of age and again at about 4 years of age. We will also schedule a visit at 3 years of age and at 42 months at the primary health care clinic. We will ask you some questions about your child's health, nutrition, growth and development, and any chest illnesses. We will do regular tests to watch these.

At study visits in the next year, you will be asked some questions about you and your child's health. Your child will be examined. Tests will be done on you and your child to assess whether there is any chest problem. The tests that may be done on your child are:

1. Blood tests - these will be to test for allergies or blood problems.
2. A test of the mucus from the nose (nasopharyngeal swab) to test for infection.
3. Saliva will be collected to check for germs which may cause pneumonia
4. A skin test for tuberculosis infection.
5. A urine test for smoke exposure.
6. A stool test to check what germs are in the stool.
7. At 3 years and 4 years of age a breathing test will be done, to measure the air moving in and out of his/her lungs.
8. A skin test if your child has a rash
9. An eye tracking test at 3 and 5 years of age where your child will look at a computer screen showing cartoons and pictures to see how his/her attention and development occurs.
10. A hearing test to see if your child may have any hearing problems

The tests YOU will be asked to complete are:

1. Questionnaires about your socioeconomic status and your levels of emotional distress, stress, life events, social support, exposure to community violence, assessment of maternal parenting styles, your and your child's emotional style, bonding and empathy and drug and alcohol use. If a mental health condition or abuse is suspected, you will be referred to the appropriate local services.
2. An eye-tracking test where you will look at a computer screen showing different baby facial expressions.

3.Neurocognitive and socioemotional assessment at 3.5 years. We will take a short video of you playing with your child, and of your child playing. We will then ask you to answer some questions about your child's feelings and behaviors, and about your own feelings and behaviors as well as any trauma or violence that you or your child may be exposed to. While you are doing this, we will be doing some tasks, games and puzzles with your child.

4. A test of the mucus from the nose (nasal swab) to test for the germs you may carry in your nose when your child is 3 or 4 years of age.
5. A blowing test to check how healthy your lungs are, when your child is 3 or 4 years of age.

We will only share your test results with primary health care staff if it indicates that you or your child require treatment or further follow up. For some assessments, study staff may follow up with you and provide you with information on where you can seek help, if necessary.

Should your child get sick with a chest infection, then additional tests will be done to try and find out the cause of your child's illness. The tests that will be done will depend on how sick your child is and what the illness is. These tests may include:

1. Blood tests to test for infections, at the time of the illness, and again 4-6 weeks afterwards
2. A test of the mucus from the nose (nasopharyngeal swab) to test for infection
3 A skin test for tuberculosis infection.
4 A test of the mucus from the lungs (induced sputum test) for chest infection.
5 A urine test for smoke exposure
6 Chest X-ray
7 Breathing test
8 A ultrasound test of the lungs
9 A stool test to check what germs are in the stool.

If your child is enrolled in the study and is admitted to hospital, he/she will be followed up in hospital by a member of the study team. The study member will ask you questions about your child's illness, and some tests may be done, including a nose swab and an induced sputum. All of these tests are usual for investigating the cause of pneumonia.

Use of medical data and specimen data for future research

Some of the specimens we have taken from you and your child and will continue to take may be used to study something called “genes”. These “genes” are present in all of us and are what make people in families look like each other but different from others. Once collected, we will store your and your child’s samples for potential future analysis. The current study and investigators from all over the world can ask for the samples and you information for their research. If this happens it will be used without any identifying information and will be used for research to improve knowledge relating to health and illness.

What are the benefits of my child being in the study?

You and your child will be closely followed for the first few years of your child’s life. Any medical illness or problem should be found soon after it develops. Your child’s growth and development will be carefully followed. If an illness or problem is found, then your child will be promptly referred for treatment. If your child gets sick you will be able to take him/ her to your usual health facility, where additional tests to find out the cause of your child’s illness may be done, depending on how sick your child is. If your child requires hospitalisation, then he/ she will be hospitalised at Paarl hospital as is usually done. If your child is hospitalised, then one of the study staff will see your child in hospital and additional investigations may be done to try and find out the cause of the illness. Therefore, the study offers an opportunity for your child to receive appropriate medical care. The study will also help us to better understand the causes of illness in children, and identify the things that may harm their health. We hope that this will lead to improvements in child health. When the study is finished or if you choose to withdraw from the study, you and your child will continue to go to your usual health facility for care and study staff will no longer be involved with you or your child.

What are the risks to my child?

There are no major risks to your child. Your child may also become tired during the tasks, games and puzzles. To minimize this risk, we will take breaks whenever it seems your child is getting tired. There may also be some discomfort associated with some of the tests we will do. These tests are listed below:

(1) Blood tests

Your child may feel sore when blood samples are taken with a needle. Where possible an anaesthetic cream will be used to dull the pain from the needle. Some bruising may occur, but this is not harmful and will disappear. Only a small amount of blood (not more than 3 teaspoons) will be taken from your child at any time

(2) Nasopharyngeal swab

A sample of mucus will be taken from your child's nose, to test for germs that can cause chest infections and to monitor which germs are usually in your child's nose. Your child may experience minor discomfort when the nasal swab is done. Occasionally it can cause bleeding from the nose, but this is not serious, and usually stops by itself.

(3) TB skin test

A small injection is made on your child's arm. This is to test whether your child has TB or not, and will be done at regular visits. Your child will experience minor discomfort due to the needle, with the skin test. There may also be irritation of the skin if the test is positive (reactive). This test will need to be checked 2-3 days after the injection is given.

(4) Induced sputum

Your child will be given salt-water through a nebulizer to loosen the mucous in the lungs. Then a sample of that mucus will be suctioned, or your child will be asked to cough up the mucus. Your child may experience a little discomfort while the sputum test is done. He/ she may develop some coughing or have a small amount of bleeding from the nose after this. These are not serious. Occasionally this test can cause the airways of the lungs to close. If this occurs your child will be given medicine through an inhaler/nebulizer to open the airways.

(5) Breathing test

This test is done after a child recovers from pneumonia, and at the 3 year and 4-year visits at Paarl Hospital and should not cause any discomfort. A mask will be put on his/ her face and the air going in and out of his/ her lungs while breathing will be recorded.

(6) Stool test

This test may be done monthly on your child and then every 6 months after 1 year. Study staff will collect stool from your child's nappy if passed during a study visit. If there is no stool available, a small tube will be inserted into your child's bottom and some stool will be sucked out with a syringe. The tube is thin and bendable and is only put in 1-2 centimeters to reach stool. There is a very small chance of bleeding at the rectum right where the tube goes is.

(7) Ultrasound test of the lungs

This test will be done if your child develops pneumonia so as to better see how the infection is affecting your childs lungs. This is a very safe procedure and there are no side effects.

(8) Eye tracking

This test will involve your child watching a series of images to better understand their attention and development. This is a very safe test and there are no risks or side effects.

(9) Neurocognitive and socioemotional assessment at 3.5 years.

We will take a short video of you playing with your child, and of your child playing. This will take 5 – 10 minutes. We will then ask you to answer some questions about your child's feelings and behaviours, and about your own feelings and behaviours as well as any trauma or violence that you or your child may be exposed to. This will be very safe, though you or your child may become tired. We will take lots of breaks so your child can relax and play in between these tasks. We will provide you both with refreshments. In total, this visit will probably last about 2 hours.

(10) Hearing test

This test will assess whether your child may have hearing problems. This will be very short and there will be no pain or risk.

What are the risks to you?

There are no major risks to you. Some of the questionnaires ask for sensitive information relating to mental health and this may cause some emotional distress or discomfort. Where significant issues are identified, and if you agree, study staff will offer referral to mental health support. You may also choose not to answer certain questions and still remain in the study. You will be able to take breaks, if you need to, and you will be free to terminate or reschedule the interview should the need arise.

(1) Nasal swab

A sample of mucus will be taken from your nose, to test for germs that can cause chest infections and to monitor which germs are usually in your nose. You child may experience minor discomfort when the nasal swab is done. This is a very safe procedure and there are no side effects

(2) Lung function test

This test will be done at each visit to Paarl Hospital. You will be asked to blow into a machine that tests how healthy your lungs are. You will then be given an inhaler with medicine that opens up the chest and asked to blow into the machine again. You should not experience any discomfort during this procedure. You may feel shaky after the medicine, and your heartbeat may be faster, but this will only last for a short while.

What happens if I get hurt taking part in this study?

This research study is covered by an insurance policy taken out by the University of Cape Town. If you suffer a bodily injury because you are taking part in the study, the insurer will pay for all reasonable medical costs required to treat your bodily injury, according to the SA Good Clinical Practice Guidelines 2006. The insurer will pay without you having to prove that the research was responsible for your bodily injury. You may ask the study doctor for a copy of these guidelines.

The insurer will *not* pay for harm if, during the study, you:

- Do not take reasonable care of yourself

If you are harmed and the insurer pays for the necessary medical costs, usually you will be asked to accept that insurance payment as full settlement of the claim for medical costs. However, accepting this offer of insurance cover does not mean you give up your right to make a separate claim for other losses based on negligence, in a South African court.

Will I be paid to participate in the study?

No, you will not be paid to participate in this study. If you agree to take part, we will reimburse your transport costs for visits that are not part of your well child clinic visits.

Will there be any cost to participate in the study?

No, there will be no cost to you

How long will my child be in the study?

This consent form is for permission for you and your child to participate in the study from 3 to 4 years of age. We hope to continue the study for many years; each year we will ask you again to sign permission for you and your child to continue in the study for another year.

Will my child's participation in the study be confidential?

All information that you provide will be considered confidential, and no mention of you or your child's name will appear on the stored samples or in any publication in connection with this study. No persons other than the health care workers overseeing your child's care and the study nurses and doctors will have access to any information that identifies your child personally. All your test results will not be disclosed to anyone other than for the purpose of treating you if there is a problem.

The video material will be securely stored, and only researchers directly involved in this part of the study will have access to it. This material will also be treated as very strictly confidential. Your names will not be attached to the video. The video will give us information about how you and your child interact, and about your child's behaviour.

Mandatory reporting of abuse and/or deliberate neglect

The researcher(s) may not be able to keep confidential, information about known or reasonably suspected incidents of deliberate neglect or physical, sexual or emotional abuse of an adult or a child. If a researcher is given such information, he or she may report it to the authorities such as child welfare.

Does my child have to be in the study?

You can choose not to take part in the study. This will not affect the quality of care your child receives. You will be able to decline to participate at any time should any part of the study be unacceptable to you, you may still take part in the rest of the study.

What do I do if I have any questions?

If you have any questions about this study, you can ask study staff, the Principal Investigator, Professor Heather Zar, or the lung study doctor, Dr. Attie Stadler, at: 021 860 2802. The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study

Informed Consent

I, _____ understand the information contained in this consent form, as explained to me in a language that I understand. I am prepared to participate in this study and give consent for my child to participate in this study. I am prepared to share my information and my child's information collected as part of the Drakenstein Child Health Study and consent to use of specimen data for future research, including genetic data approved by a Human Research Ethics Committee.

I agree to allow study staff to access my medical and hospital records as well as those of my child during the course of the study.

I give consent for the filming interaction between me and my child and consent to its use for the duration of the study.

☐ YES ☐ NO

2. To be completed by mother:

Child's Name:_____

Mother's Name:_____

Mother's Signature: _____

Date: _____

3. Study staff providing information: **Study staff confirming consent:**

Name: _____ Name: _____

Role in Study:_____ Role in Study:_____

Signature: _____ Signature: _____

Date: _____ Date: _____

4. If the mother is unable to read or write the entire counselling process must be observed by an independent witness who can then confirm the procedure once the mother has given consent.

Fingerprint of mother:

Witness: I confirm that I am independent of the study and that I witnessed the entire enrolment counselling process in the home language of the mother.

Name:_____

Signature: _____

Date: _____

Appendix F: Retrospective questions for mothers in first, second and third trimester

Q1. First trimester	Did you drink any alcohol?	No	Yes	
Q2.	If yes, how many times did you drink per week?	Once per week or less	Two to three times per week	
Q3.1.	How may drinks did you have per episode?	>2	2 to 3	4 or more
Q3.2.	if >4, please specify average number:			
Q4. Second trimester	Did you drink any alcohol?	No	Yes	
Q5.	If yes, how many times did you drink per week?	Once per week or less	Two to three times per week	Four to six times per week
Q6.1.	How may drinks did you have per episode?	>2	2 to 3	4 or more
Q6.2.	if >4, please specify average number:			
Q7. Third trimester	Did you drink any alcohol?	No	Yes	
Q.8	If yes, how many times did you drink per week?	Once per week or less	Two to three times per week	Four to six times per week
Q9.1.	How may drinks did you have per episode?	>2	2 to 3	4 or more
Q9.2.	If >4, please specify average number:			

Appendix G. Mean scores (SDs) of motor, language and cognitive development in the alcohol and unexposed group at 6, 24 and 42 months of age

	6 months			24 months			42 months		
	Alcohol exposed Mean (SD)	Unexposed Mean (SD)	p-value	Alcohol exposed Mean (SD)	Unexposed Mean (SD)	p-value	Alcohol exposed Mean (SD)	Unexposed Mean (SD)	p-value
Fine motor (n = 144)	20.00 (2.78)	22.40 (3.30)	0.001**	36.88 (3.57)	37.87 (2.76)	0.10	50.47 (3.91)	51.24 (4.18)	0.35
Receptive vocabulary (n=129)	9.23 (1.76)	9.66 (2.12)	0.33	20.40 (3.92)	21.66 (3.62)	0.11	20.77 (7.63)	21.94 (9.28)	0.53
Expressive vocabulary (n= 112)	8.55 (2.67)	9.23 (3.42)	0.37	22.95 (6.34)	24.69 (4.73)	0.24	9.59 (3.14)	10.53 (2.70)	0.16
Nonverbal index (n= 134)	26.45 (2.68)	27.58 (3.15)	0.08	55.28 (5.04)	56.70 (3.62)	0.16	75.21 (15.35)	76.14 (15.74)	0.78

** $p<0.05$

Appendix H: Multiple comparisons between low, moderate and high alcohol exposure for developmental outcomes

Dependent Variable	Alcohol categories	Alcohol categories	Mean Difference	Std. Error	p-value	95% Confidence Interval	
						Lower Bound	Upper Bound
Receptive vocabulary at 6 months	Low risk	Moderate risk	1.459*	0.577	0.033	0.10	2.82
		High risk	-0.567	0.753	0.732	-2.34	1.21
	Moderate risk	Low risk	-1.459*	0.577	0.033	-2.82	-0.10
		High risk	-2.026	0.909	0.068	-4.17	0.12
	High risk	Low risk	0.567	0.753	0.732	-1.21	2.34
		Moderate risk	2.026	0.909	0.068	-0.12	4.17
Expressive language at 6 months	Low risk	Moderate risk	1.108	0.720	0.275	-0.59	2.81
		High risk	-.455	0.939	0.879	-2.67	1.76
	Moderate risk	Low risk	-1.108	0.720	0.275	-2.81	0.59
		High risk	-1.563	1.134	0.354	-4.24	1.11
	High risk	Low risk	0.455	0.939	0.879	-1.76	2.67
		Moderate risk	1.563	1.134	0.354	-1.11	4.24
Fine motor at 6 months	Low risk	Moderate risk	2.561*	0.624	0.000	1.09	4.03
		High risk	0.804	0.814	0.585	-1.11	2.72
	Moderate risk	Low risk	-2.561*	0.624	0.000	-4.03	-1.09
		High risk	-1.757	0.983	0.176	-4.08	0.56
	High risk	Low risk	-.804	0.814	0.585	-2.72	1.11
		Moderate risk	1.757	0.983	0.176	-0.56	4.08
Gross motor at 6 months	Low risk	Moderate risk	2.020*	0.582	0.002	0.65	3.39
		High risk	1.909*	0.758	0.033	0.12	3.70
	Moderate risk	Low risk	-2.020*	0.582	0.002	-3.39	-0.65
		High risk	-0.111	0.916	0.992	-2.27	2.05
	High risk	Low risk	-1.909*	0.758	0.033	-3.70	-0.12
		Moderate risk	0.111	0.916	0.992	-2.05	2.27

Cognitive functioning at 6 months	Low risk	Moderate risk	0.842	0.554	0.283	-0.46	2.15
		High risk	0.193	0.708	0.960	-1.48	1.86
	Moderate risk	Low risk	-0.842	0.554	0.283	-2.15	0.46
		High risk	-0.649	0.862	0.732	-2.68	1.38
	High risk	Low risk	-0.193	0.708	0.960	-1.86	1.48
		Moderate risk	0.649	0.862	0.732	-1.38	2.68
Receptive vocabulary at 24 months	Low risk	Moderate risk	-0.057	0.277	0.977	-0.71	0.59
		High risk	0.550	0.440	0.423	-0.48	1.58
	Moderate risk	Low risk	0.057	0.277	0.977	-0.59	0.71
		High risk	0.607	0.506	0.454	-0.58	1.80
	High risk	Low risk	-0.550	0.440	0.423	-1.58	0.48
		Moderate risk	-0.607	0.506	0.454	-1.80	0.58
Expressive language at 24 months	Low risk	Moderate risk	-0.011	0.334	0.999	-0.80	0.77
		High risk	0.473	0.529	0.645	-0.77	1.72
	Moderate risk	Low risk	0.011	0.334	0.999	-0.77	0.80
		High risk	0.484	0.610	0.707	-0.95	1.92
	High risk	Low risk	-0.473	0.529	0.645	-1.72	0.77
		Moderate risk	-0.484	0.610	0.707	-1.92	0.95
Fine motor at 24 months	Low risk	Moderate risk	0.053	0.352	0.988	-0.77	0.88
		High risk	0.407	0.535	0.726	-0.85	1.66
	Moderate risk	Low risk	-0.053	0.352	0.988	-0.88	0.77
		High risk	0.355	0.623	0.836	-1.11	1.82
	High risk	Low risk	-0.407	0.535	0.726	-1.66	0.85
		Moderate risk	-0.355	0.623	0.836	-1.82	1.11
Gross motor at 24 months	Low risk	Moderate risk	0.064	0.343	0.981	-0.74	0.87
		High risk	0.319	0.527	0.818	-0.92	1.56
	Moderate risk	Low risk	-0.064	0.343	0.981	-0.87	0.74
		High risk	0.255	0.611	0.909	-1.18	1.69
	High risk	Low risk	-0.319	0.527	0.818	-1.56	0.92
		Moderate risk	-0.255	0.611	0.909	-1.69	1.18

Cognitive outcomes at 24 months	Low risk	Moderate risk	0.173	0.262	0.786	-0.44	0.79
		High risk	0.084	0.397	0.976	-0.85	1.02
	Moderate risk	Low risk	-0.173	0.262	0.786	-0.79	0.44
		High risk	-0.089	0.463	0.980	-1.18	1.00
	High risk	Low risk	-0.084	0.397	0.976	-1.02	0.85
		Moderate risk	0.089	0.463	0.980	-1.00	1.18
Receptive vocabulary at 42 months	Low risk	Moderate risk	-0.647	1.282	0.869	-3.66	2.37
		High risk	4.167	2.152	0.129	-0.89	9.22
	Moderate risk	Low risk	0.647	1.282	0.869	-2.37	3.66
		High risk	4.815	2.444	0.121	-0.93	10.56
	High risk	Low risk	-4.167	2.152	0.129	-9.22	0.89
		Moderate risk	-4.815	2.444	0.121	-10.56	0.93
Expressive language at 42 months	Low risk	Moderate risk	-.0149	0.452	0.942	-1.21	0.91
		High risk	1.415	0.710	0.115	-0.25	3.08
	Moderate risk	Low risk	0.149	0.452	0.942	-0.91	1.21
		High risk	1.564	0.821	0.138	-0.37	3.49
	High risk	Low risk	-1.415	0.710	0.115	-3.08	0.25
		Moderate risk	-1.564	0.821	0.138	-3.49	0.37
Fine motor at 42 months	Low risk	Moderate risk	0.116	0.249	0.888	-0.47	0.70
		High risk	0.882	0.422	0.093	-0.11	1.87
	Moderate risk	Low risk	-0.116	0.249	0.888	-0.70	0.47
		High risk	0.767	0.479	0.246	-0.36	1.89
	High risk	Low risk	-0.882	0.422	0.093	-1.87	0.11
		Moderate risk	-0.767	0.479	0.246	-1.89	0.36
Cognitive outcomes at 42 months	Low risk	Moderate risk	1.253	2.129	0.826	-3.75	6.26
		High risk	11.254*	3.748	0.008	2.45	20.06
	Moderate risk	Low risk	-1.253	2.129	0.826	-6.26	3.75
		High risk	10.001*	4.213	0.**040****	0.10	19.90
	High risk	Low risk	-11.254*	3.748	0.008	-20.06	-2.45
		Moderate risk	-10.001*	4.213	0.047	-19.90	-0.10

**The mean difference is significant at the 0.05 level.