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## **A comparative acoustic examination of infant cries: Children at high risk versus low risk for autism spectrum disorder development**

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A Comparative Acoustic Examination of Infant Cries: Children at High Risk versus Low  
Risk for Autism Spectrum Disorder Development

Ildiko Eva Bruz

A report submitted in partial fulfilment of the requirements for the award of the Bachelor of  
Speech Pathology Honours, School of Psychology and Social Science

Edith Cowan University

Submitted November, 2015

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## Abstract

**Background:** Early interventions for individuals with Autism Spectrum Disorders (ASD) have shown positive gains in children who enter therapy at a young age. However, commencement of early intervention is often hindered by challenges with diagnosis. Due to the complex nature of ASD, the age of detection can range from 2 years old into adulthood. This highlights the need for methods of early detection. Previous research has found infants at risk for ASD to present atypical cry characteristics, possibly as a result from damage to the brainstem. In particular, measures of fundamental frequency appear to be the most sensitive to variations between infants at risk for ASD and those at low risk.

**Objectives:** The present study is an examination of cries between infant's at high risk and low risk for ASD, to investigate if there is an acoustic measure which could act as an early risk indicator.

**Methods:** 150 cries from 50 12 month old infants were examined for differences in fundamental frequency, intensity, formant frequencies. A further aim was to investigate perceived levels of distress felt by infants and its relationship to the reason for distress.

**Main Results:** There were no significant differences between the high risk and low risk cohorts on any of the acoustic measures. Distress ratings indicated that fatigue and unpleasant stimulation may impact the level of perceived distress observed by a listener.

**Conclusion:** As it stands, infant cries may hold important diagnostic information, however the variance in methodologies between studies makes it difficult to corroborate findings. Increased reporting on methods of acoustic analysis and taking into account infant position and reason for distress would strengthen the finding sin future studies.

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Dated: 19/11/2015

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## Table of Contents

Abstract.....	ii
Acknowledgements.....	iv
Early Intervention.....	1
Current Diagnosis.....	2
Previous Research into Infant Cry.....	4
Neurological Basis of Infant Cry.....	4
Autism and Infant Cry.....	6
Parental Perceptions.....	8
Key Literature Findings.....	10
Research Aim.....	10
Methods.....	11
Research Design.....	11
Participants.....	11
Collection of Demographic Information.....	11
Collection of Cry Samples.....	12
Acoustic Analysis.....	12
Statistical Analysis.....	14
Ethics Approval.....	15
Results.....	15
Acoustic Parameters.....	17
Distress Rating.....	18
Length of Cry Units.....	20
Discussion.....	21
Fundamental Frequency.....	21
Intensity.....	22
Formant Frequencies.....	22
Distress Ratings.....	23
Position.....	25
Methodological Differences.....	25
Strengths and Limitations.....	29
Summary and Implications.....	30
Future Directions.....	31
References.....	32
Appendix A.....	38
Instruction to Parents.....	38

## **A Comparative Acoustic Examination of Infant Cries: Children at High Risk versus Low Risk for Autism Spectrum Disorder Development.**

Early intervention studies have reported positive gains from children with autism spectrum disorder (ASD) accessing speech, language and joint attention therapy at a young age (Dodici, Draper & Peterson, 2003; Kasari, Gulsrud, Wong, Kwon, & Locke, 2010; Kasari, Paparella, Freeman, & Jahromi, 2008). However, commencement of early intervention is hampered by the complex nature of ASD diagnosis. Promising results from the early intervention studies have spurred researchers to investigate methods of early detection. Given individuals with ASD often exhibit communication difficulties, it may be possible to find early indicators in our earliest form of communication, the infant cry. Studies indicate possible neural abnormalities in the brainstem which may disrupt the coordination of vocal structures during a cry episode resulting in atypical cry characteristics (Rodier, 2002; Rodier, 1996). Detection of atypical cry characteristics may lead to identifying a child is at risk for ASD as early as 12 months of age.

### **Early Intervention**

ASDs are pervasive developmental disorders which are often characterised by difficulties with communication, social interaction and impaired intellectual function (Cassel, Messinger, Ibanez, Haltigan, Acosta & Buchman, 2007). Early speech and language intervention is typically defined as treatment starting before the child is six years old (Glicksman, 2012) and it is widely believed to improve long term outcomes in children with ASD (Charman, 2014). Recent randomised control (RCT) trials, in which intervention was started when participants were between 21 months and five years in age, have shown improvements in post intervention outcomes in the areas of: joint interaction and expressive

language (Kasari et al., 2008), joint engagement (with parent), response to joint attention bids, number of functional play acts (Kasari et al., 2010), parental synchrony and child initiations in interactive play (Green et al., 2010). A two year RCT of the Early Start Denver Model (ESDM) provided intervention focused on interpersonal exchange, shared engagement, adult responsivity, and sensitivity. Participants commenced intervention between 18 and 30 months of age. The study found improved IQ for children in the ESDM group when compared to the control group post intervention. Improvement was attributed to improved language skills developed through the program (Dawson, et al., 2010).

Given the complex nature of ASD, more research is necessary into early intervention and whether more positive gains can be made if therapy is commenced at an even earlier age. With increasing prevalence of ASD and research supporting positive outcomes for early intervention, the need for early diagnosis is paramount.

### **Current Diagnosis**

The current prevalence of ASD is estimated to be one in every 160 children (World Health Organisation, 2013). The ASD diagnostic protocol relies heavily on parental observation of developmental norms. Dover and Le Couteur (2007) report on the approach to diagnosis, stating that parents or carers are often the first to raise concerns regarding a child's development which leads them to seek advice or a referral from a general practitioner. The process of differential diagnosis at this stage can be challenging, given the developmental progression of neurodevelopmental disorders, as well as the difficulties in ruling out global developmental delay, auditory difficulties or specific language disorders. Dover and colleagues (2007) comment that the continuum of behaviours and characteristics noted in ASD adds an additional confounding factor to diagnosis. Not only do the types of symptoms range between individuals, but the degree to which they present and impact an individual is

also highly variable. The diverse nature of ASD means diagnosis requires the involvement of a multidisciplinary team who observe atypical development in communication, social interaction and note the child's repertoire of interests and behaviours (Dover & Le Couteur, 2007). ASD specific observation tools such as the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000) can assist in making a diagnosis, however correct diagnosis can still be time consuming and difficult (Dover & Le Couteur, 2007).

Currently the age range of diagnosis varies across studies from 18 months to adulthood (Green, 2012; Santos et al., 2013). Variation in age can in part be attributed to the complex nature of ASD and diversity of symptoms presented. Early diagnosis is difficult as there is no singular indicator of ASD and detection relies heavily on parental observation of developmental delays (Lai, Lombardo, Baron-Cohen, 2014). While there is some evidence to support the idea that children at risk for ASD are more likely to display certain characteristics, such as a reduced use of eye contact in interaction, in general it has been difficult to pinpoint robust deficits in social behaviour (Ozonoff et al., 2011). Additionally early signs of ASD may not be the presence of abnormal behaviours, but rather the absence or delay of typically developing behaviours (Camarata, 2014; Charman, 2014). Delayed onset of speech is often one of the first signs a child may be a risk for ASD (Camarata, 2014; Charman, 2014). Even at two years of age however, an accurate diagnosis is difficult as many of the characteristics commonly observed in children with Autism are also typical of other developmental disorders (Camarata, 2014; Charman, 2014).

The prevalence of ASD appears to be increasing in today's society. Changes to the diagnostic criteria in the Diagnostic and Statistical Manual-Fourth, in addition to increased awareness about ASD among health professionals and parents is reported to be partly responsible for the trend of increasing prevalence (Cassel et al., 2007; Green, 2012; Hansen, Schendel & Parner, 2014). However, these two factors combined are unable to account for the

overall increase observed in recent years. This leads to the conclusion that the prevalence of ASD in today's society is on the rise. Increase in prevalence and difficulties with diagnosis due to the complex nature of ASD have prompted research into new methods of detection such as investigation into infant cry.

### **Previous Research into Infant Cry**

Research into the infant cry has been conducted to examine differences in acoustic parameters which may act as early indicators of developmental abnormalities (Fisichelli & Karelitz, 1996; LaGasse, Neal & Lester., 2005; Wasz-Hockert, Lind, Vuorenski, Partanen & Valanne, 1968). These studies have often compared the cries of typically developing (TD) children to the cries of children with developmental delays or a specific medical condition in order to identify measures that may be used for early detection. Results from these studies have found measurable differences in vocal production, in particular measures of fundamental frequency ( $f_0$ ), in children with specific medical conditions when compared to TD children. There is evidence to support atypical infant cry characteristics in children with: brain damage (Fisichelli et al., 1996; LaGasse et al., 2005); Down's syndrome (Wasz-Hockert, et al., 1968); Krabbe's disease (Thoden & Michelsson, 1977); and hypothyroidism (Michelsson & Sirvio, 1976). These findings suggest that children who have experienced damage to their neural system may present with atypical cries when compared to their TD peers. However, little research has investigated the nature of cries in children at risk for ASD.

### **Neurological Basis of Infant Cry**

Crying is an infant's first form of intentional communication. It acts as a biological siren used to ensure survival by alerting caregivers to the infant's basic needs and wants (Esposito & Venuti, 2008). Appropriate reactions by the caregiver to alleviate distress become an important step in building the parent-infant bond which is necessary for future

relationships (LaGasse et al, 2005). Researchers suggest that three profiles of crying are widely observed in infants: i) the basic cry which is used to express hunger and is rhythmic and repetitive; ii) the anger cry which is a louder and more prolonged vocalisation; and iii) the pain cry which is characterised by a sudden onset of an initial long cry followed by extended breath holding (Esposito et al, 2008). These styles of crying are present in children who exhibit typical development; despite differences in culture (Barr, 1991; Esposito et al, 2008). This suggests there are specific domains of the brain responsible for cry production. In particular LaGasse and colleagues, (2005) propose crying is a result of coordination between the limbic system, brainstem and cranial nerves. The limbic system and the hypothalamus participate in the initiation of the cry in response to an internal or external negative stimulus. Vocal production of the cry is the same as for speech; the lower brain stem innervates the muscles of the larynx, pharynx, chest and upper neck through the vagal complex and phrenic thoracic nerves (LaGasse et al, 2005). Increased subglottal pressure then pushes the vocal folds apart causing them to vibrate. The number of glottal openings per second is referred to as the fundamental frequency and is perceived as the pitch of the cry. Faster vibrations or increased glottal openings per second result in a high pitched vocalisation. Fundamental frequency is primarily determined by vocal fold tension which is controlled by the intrinsic muscles of the larynx through innervation of the vagus nerve (LaGasse et al., 2005; Rodier, 2002). Once the vocal folds begin oscillating, they set in motion the vibration of air in the vocal tract. These vibrations are referred to as resonance and can be changed depending on the shape of the cavities in the vocal tract. This can be observed when an individual moves their jaw up and down during vocalisation of a vowel. This changes the shape of the oral cavity and in turn the produced sound as vibrations will be different lengths depending on how much area is in the cavity. Resonance is measured through the formant frequencies (F1 and F2). They reflect the resonance of the fundamental frequency and as such are impacted

by neural control of the upper vocal tract. Therefore damage to the brain stem in the area of the vagus nerve nuclei may reduce control over areas of vocal production and cause atypical patterns of fundamental frequency and formant frequencies (LaGasse et al., 2005).

Research into neurological characteristics of ASD has described abnormalities within the brainstem, possibly due to complications at the time of neural tube closure (Rodier, 2002). Specific areas of abnormalities identified vary between studies including shortening of the midbrain, major reduction of neurons in the facial nucleus (Bailey et al. 1998), and enlarged arcuate nuclei in the medulla which is involved in respiratory regulation (Rodier, 1996). Instability in neural control of the respiratory system can impact the inspiration and expiration capabilities of an individual. In the case of infant cries it can be reflected in the length and amplitude of expiration which has shown to be shorter and louder in some children with ASD (Esposito et al, 2014; Sheinkopf, Iverson, Rinaldi, & Lester et al, 2012). The variability of findings makes it difficult to pinpoint an exact area of deficit in individuals with ASD, however there does appear to be evidence to support brainstem malformation. Analysing the infant cry can therefore be a means of detecting developmental abnormalities by investigating acoustic properties which could reflect incoordination of vocal structures controlled by the vagus nerve and brainstem.

### **Autism and Infant Cry**

Communication impairments are a defining feature of ASD (Dover & Le Couteur, 2007). One aspect of communication breakdown associated with children with ASD is a difficulty in reflecting emotion within the tone of voice and the pragmatically appropriate use of pitch and intonation within interaction (Dover & Le Couteur, 2007). If this characteristic can be observed in earlier forms of communication, such as infant crying, it may provide useful diagnostic information for children at risk for ASD.

Recent investigation into the use of infant cry as an early indicator of ASD has focused on the analysis of the acoustic properties of the cry. Typically studies have focused on the fundamental frequency, formant frequencies, amplitude and duration of cry as the key variables; however some studies have further investigated bandwidth frequencies, dysphonation, jitter and shimmer. Studies have been either retrospective in nature or involved children who have siblings with a prior diagnosis of ASD. Siblings of children with ASD are involved in studies as they are 18% more likely to receive a diagnosis for ASD and have been shown to display some characteristics typical of ASD even without a diagnosis (Lai, Lombardo, Baron-Cohen, 2013; Ozonoff et al., 2011). Data from sibling studies are gathered from either naturalistic home environment settings or in an assessment room. Data has typically been collected through the use of audio recording devices or video recordings or a combination of both. Therefore sibling studies offer the opportunity to gather early life information without the bias of retrospective home videos. Retrospective studies typically analyse video recordings from when a child diagnosed with ASD was an infant. In this manner they can analyse acoustic characteristics of their voice in conjunction with observing their behaviour at the time to understand the reason for distress. However this can introduce bias as videos are often recorded for certain reasons or at certain events without the ability to control for factors such as background noise, length of recording, matched age at time of recording and distance of microphone to the infant.

Previous research analysing acoustic properties of infant cries have reported differences exist between children at risk for ASD and their TD counterparts. In particular, fundamental frequency was found to be higher in children at risk for ASD (Esposito et al, 2010; Esposito et al., 2014; Sheinkopf et al., 2012). Additionally it was observed that children at risk for ASD had a shorter duration of cry utterances (Esposito et al, 2014), an increased amplitude for the cry utterance (Sheinkopf et al, 2012), a shorter length of pauses

between cry episodes and increased dysphonation (Esposito et al., 2010). It is worthy to note Sheinkopf and colleagues, (2012) only observed significant differences in fundamental frequency on pain related cries and not on non-pain related cries. Additionally, few studies have reported on infant position during a cry episode. This is a relatively large oversight given the impact posture has on vocal production. Lin and Green (2007), discuss the effect of posture on new born crying. Their findings concluded that infants in an upright position had shorter and more frequent cry utterances than infants in a supine position. Not only did they find a decrease in duration but also a decrease in variability in those durations during upright cries. Another notable finding by Lin and Green (2007), was an increase in fundamental frequency when infants moved from a supine position to being upright. Thus recording infant position when analysing acoustic features such as fundamental frequency is necessary if there is to be consistency between multiple study findings.

Collectively these studies provide a foundation for the theory that infant cries hold early diagnostic information for ASD. However, difficulties exist when comparing study outcomes as not all studies differentiate between types of cries in their findings. Additionally studies tend to have methodological differences in how the cries are collected and analysed and differences exist in the exclusion criterion applied to the research.

### **Parental Perceptions**

Other areas of infant cry research have investigated parental perceptions of infant cries and how possible acoustic differences impact on parental responses. Gustafson and Harris (1990) proposed that acoustic properties of an infant's cry inform caregivers of the infant's level of distress. The cry then elicits a physiological reaction in adults, such as increased heart rate and endocrine responses to spur the caregiver to eliminate the cause of unease. Typically the caregiver is able to understand the cry signal and respond appropriately;

however responding appropriately becomes difficult when the caregiver is unable to correctly interpret the cry signal. Notably there appears to be a different element to cries of infants with ASD which can be subjectively perceived without the use of a spectrograph. A number of studies have reported parents find cries with a higher fundamental frequency to be more aversive and distressing than cries with lower fundamental frequency scores (Esposito & Venuti, 2010; Esposito, Nakazawa, Venuti & Bornstein, 2012). Additionally caregivers also reported increased unease and distress when listening to cries which were modified to have shorter length of pauses (Zeskind et al., 1992). Overall parents reported feeling more negative states when listening to cries of children with ASD than cries from TD children.

The differences in acoustic characteristics can then impact the parent's perception of the cry and ultimately how they attempt to alleviate the distress (Esposito and Venuti 2008). Differences in mothers' reactions to infant cries were noted by Esposito and Venuti (2009). Mothers with TD children or those with developmental delays were more likely to use tactile or vestibular methods to sooth their child, whereas mothers of children with ASD were more likely to use verbal methods in an attempt to settle their infant. One explanation for this could be linked to sensory characteristics of children with ASD. As reported by Baranek, Boyd, Poe, David and Watson (2007), young children and infants with ASD may overreact to tactile or vestibular stimuli. Therefore mothers may attempt tactile methods of soothing less often as they have not worked in the past and may further upset the infant. Interpreting social requests and responding appropriately is a prerequisite for emerging language and an important step for building the infant-carer relationship (Topping, Dekhineta & Zeedyk, 2012). Failure to respond correctly to the child's needs because of ambiguity in deciphering the cry signal, may impact upon this important relationship (LaGasse et al, 2005).

## **Key Literature Findings**

Given the diverse characteristics of ASD highlighted in the literature, it remains very difficult for health professionals to make a diagnosis at a young age. Owing to this difficulty in interpreting ASD symptoms, the age of diagnosis is highly variable which emphasises the need for earlier means of detection. Analysing key acoustic variables such as fundamental frequency, intensity, formant frequencies, cry duration and parental perception of distress has highlighted possible early indicators of ASD. In particular, measures of fundamental frequency appear to be the most indicative of possible neural abnormalities which may arise from atypical development. Previous studies however have differed in their methodology, for instance, using cries from different mediums, analysing different types of cries (pain vs non-pain), stimulus elicitation, participant sizes and variable reporting on infant position or reason for distress. Analyses which factor in a number of these elements are missing from the literature and could highlight previously overlooked relationships. While ASD is too variable to be diagnosed by infant cries alone, the information gathered from acoustic analysis in conjunction with other risk factors, may indicate a child is vulnerable. Further study is required to identify factors that indicate an infant is at risk of being diagnosed with autism.

## **Research Aim**

The aim of the present study was to compare the acoustic characteristics of infant cries between infant siblings of children with ASD and infants with no family history of ASD. A further aim was to investigate perceived levels of distress felt by infants and the relationship to the reason for distress. Based on the existing scientific literature, it is hypothesised that infant siblings of children with ASD will have increased fundamental frequency, increased cry amplitude and variation between first and second formants

compared to the typically developing control group. Furthermore, it was expected that infants at risk for ASD would have cries with higher distress ratings than their low risk counterparts.

## **Method**

### **Research Design**

This observational study involved a between groups comparison of infant cries between children at risk for ASD and children at a low risk, in an effort to identify early markers for diagnosis.

### **Participants**

This study used a subset of data from the PRenancy Investigation of Siblings and Mothers (PRISM) of children with Autism cohort study in Perth, Western Australia. The PRISM study is a longitudinal investigation into the early indicators of ASD with participants recruited from 2011-2013. Two groups of pregnant woman were recruited to the PRISM study: i) a high risk (HR) group which was comprised of 25 women who had previously given birth to a child later diagnosed with either Autistic Disorder or Pervasive Developmental Disorder- Not Otherwise Specified according to the DSM-IV; ii) a low risk (LR) group of 33 women who had previously given birth to a child who was at least three years of age, with no diagnosis of a developmental disorder. Women were preferentially recruited prior to 18 week pregnancy; however women were enrolled if they agreed to participate at any time throughout their pregnancy.

### **Collection of Demographic Information**

At the first appointment (during the mother's pregnancy), families were invited to a face-to-face behavioural assessment at the Telethon Kids Institute. At this time, mothers were asked to complete a comprehensive case history questionnaire regarding their pregnancy, family and medical history, and development of the proband child. All proband children in

the HR group were administered the Autism Diagnostic Observation Schedule-Generic to confirm their diagnosis as part of the study protocol (ADOS-G; Lord et al., 2000). The control siblings from the LR cohort were administered the Mullen Scale of Early Learning to control for possible developmental delays (Mullen, 1995).

### **Collection of Cry Samples**

Caregivers were provided with an audio recording device once their child was 12 months old (+/- 3 weeks). They were instructed to record their child once during the day, and once during the night as minimum. Cries were to be as naturalistic as possible with recorded cries occurring spontaneously rather than being elicited by the carer, additionally infant positioning was not manipulated. At the end of each recording caregivers were requested to report the date and approximate time of recording, describe the child's posture and provide a reason as to why they believed their child was crying. Caregivers were encouraged to hold the recorder as close as possible to the child and minimise surrounding sounds in an effort to reduce interference during analysis. A copy of the instructions given to parents is attached as Appendix A.

### **Acoustic Analysis**

All data were de-identified and acoustic analysis of each cry was then conducted by an individual blinded to group allocation to avoid bias. For each infant cry episode the aim was to extract and analyse three cry units. A cry unit, is defined as the expiratory phase of respiration during a cry which lasts a minimum of 0.5 seconds (Sheinkopf et al., 2012). As recordings were collected in the home environment, recordings with less than 30dB difference between the mean intensity of the cry episode and the mean intensity of the nearest pause were excluded from the study. Where the difference in intensity was less than 30dB, cry units were extracted from the next recording and reanalysed for differences in intensity

until each child had 3 acceptable cry units. If three units of acceptable quality could not be extracted from the same recording, units were extracted from multiple recordings.

To prevent particular cry characteristics, such as coughs and pauses during expiration, from interfering with analysis only voiced segments were used for analysis. Extracting the voiced segments required identifying the voice boundaries within the cry unit. Boundaries were distinguished through spectrographic analysis focusing on waveform contours, pitch lines and onset and conclusion of pulses. Segments void of pulses and pitch lines indicate the absence of voice and were therefore excluded from analysis. Voiced segments were extracted and then concatenated to produce an exclusively voiced sample for calculating acoustic measures. For each cry, the following acoustic measures were obtained:

- *Fundamental Frequency (F0)*: This measure refers to the number of glottal openings per second and reflects the stability of the lower vocal tract (LaGasse et al., 2005). The mean fundamental frequency of the voiced segment was calculated with the parameters set to 100 Hz as the pitch floor and 1000 Hz as the pitch ceiling. The settings were selected as spontaneous infant cries range from 200Hz to 600Hz and have been set as such in similar studies (Etz, Reetz, Wegener, Bahlmann 2014). The minimum and maximum fundamental frequency was also obtained to calculate the variance in F0.
- *Intensity*: Intensity scores can reflect neural control over the respiratory system and as well as the capacity of the respiratory system (LaGasse et al., 2005). The minimum and maximum intensity values were obtained as was the range of intensity.
- *Formant Frequencies*: The first and second formants (F1 and F2) were calculated as they relate to the f0 and reflect vocal tract control (Santos et al., 2013). Formant

celling was set to 8000 Hz which is in line with prior research methods (Etz et al., 2014).

- *Cry duration*: The length of cry units was documented after being extracted from the original recording. This measure was documented before excluding non-voiced segments and therefore includes coughs and minor pauses.

### **Coding of Cry Episodes**

Additional coding was performed to detail infant positioning, cause of distress and perceived level of distress felt by the infant.

To obtain a distress rating, one researcher who was blind to group assignment, listened to one recording for each infant and rated the infant's distress using an adapted version of the Perceived Level of Distress measure (Esposito et al., 2015). The tool is used to rate the perceived level of an infant's distress on a 7- point Likert scale (1= lowest level of distress and 7= the highest level of distress). For the purpose of this study and due to time constraints the tool was rated by one individual who was not a parent, rather than a mix of five parents and 5 non parents. Additionally, this study only recorded the perceived level of distress felt by the infant and not distress felt by the individual scoring.

### **Statistical Analysis**

For statistical analyses, the median value of all three cry units was calculated per child on each measure. The use of median values was chosen to give a representative score of the combined three cries while avoiding the impact of outlying data on a measure such as the 'mean'. These results were then subject to independent samples *t*-test to compare the HR and LR groups on acoustic variables. Additional ANCOVA analyses were computed to control for gestational age.

## **Materials**

The audio recording device provided to caregivers was a Sony Intelligence Noise Cut recorder. Data segmentation and acoustic analysis was performed using the voice analysis software Praat (Boersma and Weenink, 2005) with results recorded in Excel spread sheets for descriptive coding. Statistical analysis was computed using the Software SPSS Statistics (2013, version 22.0).

## **Ethical Approval**

All procedures involving human participants were in accordance with the ethical standards of the institutional and/or national research committee. Ethics approval for data collection and use was obtained by Telethon Kids Institute.

## **Results**

An initial group of 58 infants, 30 female and 28 male, met the eligibility criteria to participate within the study. Of these, 11 females and 14 males had a sibling diagnosed with ASD (HR group) and 19 females and 14 males had no family history of ASD (LR group).

From this group of infants an initial 247 mp3 formatted infant cry recordings were reviewed. On average, there were four recordings per child; however total number of recordings per individual ranged from 1 to 18. From the initial group of 58 infants, 8 participants were excluded from the study. Of the 8 excluded infants, 2 male infants and 0 female infants were excluded from the HR group and 0 male and 6 female infants were excluded from the LR group. Overall, six infants were excluded as the difference between the mean intensity of the cry episode and the mean intensity of the nearest pause was less than 30dB for all cry samples. One infant was excluded as the recordings were formatted incorrectly and one infant had only two acceptable cry units. For five infants, the three cry

units were extracted from two different recordings. These infants were not excluded as a median was still calculable. The final sample size comprised 150 cry units from 50 children. Characteristics for the infants are described in Table 1 and 2.

Parental compliance for reporting infant position was 56% whereas compliance reporting on perceived reason for distress was 88%. Of the 56% who reported on position, 15 infants were sitting, 6 were lying down (1 on their back, 1 on their front and 4 unspecified), 6 infants were standing up, 2 changed between multiple positions and 1 was being held. There did not appear to be any correlation between infant position and acoustic properties.

Infants crying because they had just woken up and because they were tired were the highest reported reasons for distress given by carers with 9 reports each. This was followed by cries of request with 8 parental reports, 7 reports for frustration, 5 parents reported hunger cries and 3 reports for parents who were unsure.

Table 1

*Baseline Characteristics of HR Infants vs LR Infants after Exclusion Criteria*

Risk of ASD	Male	Female	M (SD) GA* (Weeks)	M (SD) Age at Recording (Months)	Total
High Risk	13	11	38.09 (1.10)	12.04 (0.46)	14
Low Risk	13	13	38.65 (1.66)	11.92 (0.41)	26

\*GA- Gestational Age

Table 2

*Proband and Control Sibling Gender Distribution after Exclusion*

Risk of ASD	High Risk		Low risk	
	Male Proband	Female Proband	Male Control	Female Control
Frequency	19	5	12	14

Independent *t*-tests were computed to analyse the difference in key family variables between the HR group and LR group. No significant difference was found on infant gender, age at recording or gestational age. Comparison of Proband and control sibling gender reported a higher number of males ( $N = 31$ ) than females ( $N = 19$ ); however this is likely due to the increased prevalence of ASD in males (Dover et al., 2007).

Analyses of covariance were computed, controlling for gestational age as there is evidence to suggest that the risk of receiving a diagnosis of ASD increases with lower gestational age (Atladóttir, Schendel, Henriksen & Parner, 2015). No significant differences were observed between high- and low-risk groups on gestational age.

### **Acoustic Parameters**

Separate independent-samples *t*-tests were conducted on each of the acoustic measures procured from the cry samples (see Table 3). There were no significant between-group differences for any of the acoustic measures.

Table 3

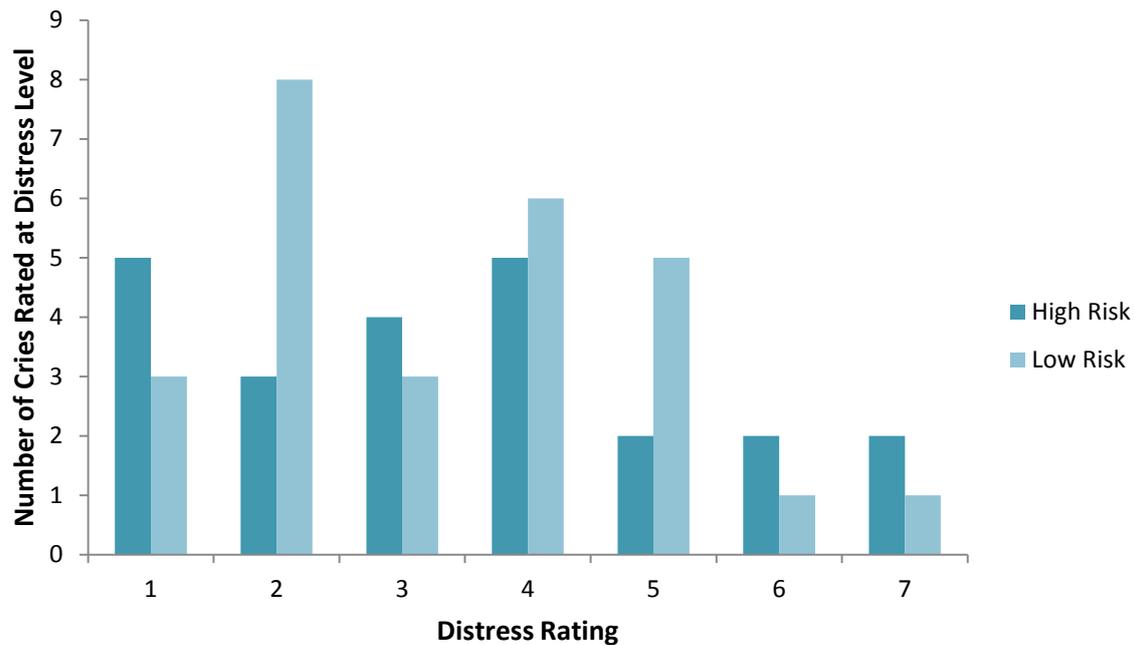
Mean and Standard Deviation of Acoustic Properties between Groups

Acoustic Parameters	High Risk N = 24		Low Risk N = 26		P value
	M	SD	M	SD	
F0	440.40	115.76	443.55	86.27	0.91
Max F0	687.13	184.20	687.73	155.27	0.99
Min F0	267.96	128.31	257.48	81.62	0.73
F0 Variance	417.79	203.10	430.80	187.10	0.81
Max Intensity	87.04	0.86	86.89	1.35	0.63
Min Intensity	70.06	5.40	68.34	6.16	0.31
Intensity Variance	16.90	4.74	18.19	6.49	0.81
F1	1136.06	299.51	1051.98	245.50	0.28
F2	2376.08	215.82	2370.38	233.11	0.92

*Note:* F0, Fundamental frequency; F1, First formant frequency; F2, Second formant frequency.  
\*all *p-values* < 0.05

### **Distress Rating**

Figure 1 presents a comparison of distress ratings between HR and LR groups. The HR group scored marginally higher than low risk infants on high distress level ratings (scores of 6 or 7). High risk infants also scored higher on level 1 and 3 distress ratings while low risk infants scored higher on ratings 2, 4 and 5.



*Figure 1.* Comparison of perceived cry distress ratings between children at risk for ASD and children at low risk. Level of perceived distress was rated on a 7 point Likert scale where 1 = the lowest level of distress and 7 = the highest level of distress.

A comparison of distress ratings against reason for distress, indicated cries during nappy changing were consistently perceived as more distressing. Of the six infants in this group, four were from the HR cohort. Cries for hunger and request were both rated on the low end of distress ratings while cries relating to frustration, waking up and unknown causes were all rated closely together between levels 2 and 4. Out of the three parents who were unsure why their child was distressed, two had infants in the HR group. Infants who were tired had the most varied responses, ranging from 1 to 7. See Figure 2 for the distribution of distress ratings over reason for distress.

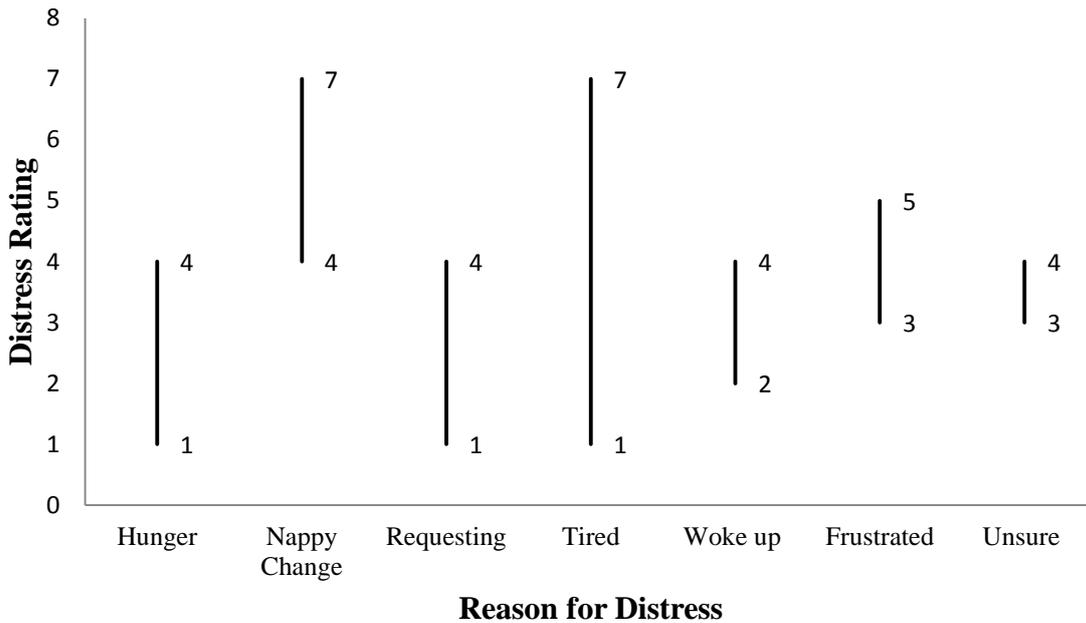


Figure 2. Distribution of distress ratings over parent reported reason for distress.

### Length of Cry Units

There was no significant difference between groups on length of cry unit. Visual inspection of the data showed a higher proportion of high risk infants were found to have produced shorter length of cries while the low risk infants tended to have longer cries. However, this difference did not reach significance (See Table 3). The average length of cry unit across all infants was 1.98 seconds.

Table 3.

Risk of ASD	M Length of Cry Unit	SD
High Risk	1.75	0.83
Low Risk	2.04	0.82

## **Discussion**

This study explored the acoustic properties of infants at risk for ASD in pursuit of identifying a possible early risk marker. Guided by previous research, it was expected that significant differences would be found on key acoustic measures between HR and LR infants. Specifically, measures of fundamental frequency, intensity, formant frequencies and perception of infant distress were the key focus of this investigation. The findings of each measure will be discussed individually followed by a comparison of methodological differences in the literature which may account for the results.

### **Fundamental Frequency**

The fundamental frequency was examined as it relates to neural control of the vocal tract via vagus nerve innervation. In particular, findings by Rodier (2002), which detected abnormalities in the medulla oblongata, from where the vagus nerve exits, was theorised to possibly impact control of the larynx and in turn impact the fundamental frequency. As such, it was expected that the fundamental frequency of HR infants would be higher than LR infants. However, results from this study concluded no significant differences between the HR cohort and the LR cohort. Interestingly, the scores for two groups were closely matched which has not been observed in prior studies of infants this age. These results do not corroborate the majority of findings from previous research which have found significant differences in the fundamental frequencies of HR infants. They do however support Sheinkopf and colleagues (2012), who also reported no significant differences in fundamental frequency in non-pain related cries. They did however report differences in pain related cries. Sheinkopf and colleagues suggested that it may be necessary to stress the neurobiological system of an infant in order to reliably observe differences in control of vocal production. As a result, pain related cries may be a more sensitive measure of detecting ASD than non-pain related cries. The present study was unable to investigate pain related cries, as examination of

data revealed few parents reported cries relating to pain between the two groups. The finding Sheinkopf and colleagues, (2012) does highlight the potential confound that indicators for ASD may only be detected under specific conditions.

### **Intensity**

Investigation into the intensity of infant cries was explored as it relates to control of respiratory function. Difficulty regulating respiration may result in incoordination during expiration (LaGasse et al., 2005). This may present as increased or decreased amplitude during vocal output. This study anticipated a difference in either maximum or minimum intensity values between infant cries from the two cohorts. There was also an expectation that the range of intensity would be smaller in the HR group when compared to the LR group. The results found no significant difference between groups on either maximum or minimum intensity variables. Notably, the two groups were once again very similar in their results indicating little difference in the control of their vocal structures. Variance in intensity also failed to indicate any significant difference. This finding does not corroborate those of Sheinkopf and colleagues (2012), who reported infants at risk for ASD produced cries with a shorter range of amplitude. However, the cries in Sheinkopf's study were pain related which could account for this disparity. These results suggest that the infants with ASD in this study were able to produce vocalisations with the same strength and control as the LR infants.

### **Formant Frequencies**

The first and second formants were examined in this study as they correspond to the formant frequencies and upper vocal tract control. Therefore atypical fundamental frequencies or incoordination of the upper vocal tract may present as irregular productions of the formant frequencies (LaGasse et al., 2005). It was anticipated that there would be a variance between the HR and LR risk infant scores on first and second formants, however no

significant differences were highlighted in the results. These results support findings by Sheinkopf and others (2012), who likewise found no significant difference between HR and LR infants on measures of formant frequencies. This may in part be due to the difficulties in calculating formant frequencies in infant vocalizations. While these findings were not significant, these measures along with Sheinkopf and others (2012), provide a basis for comparison in future research.

### **Distress Ratings**

Prior investigations into parental perception of infant cries have reported that cries with higher fundamental frequencies are perceived as more aversive and distressing to the listener. Consequently caregivers report increased difficulty decoding the reason for distress and subsequently face challenges soothing the infant (Esposito et al., 2008; 2009). After visual inspection of the data comparing distress ratings and previously mentioned acoustic measures, there was no obvious relationship among the key variables. While examination of distress and fundamental frequency values did not indicate a strong interaction, there was a slight trend to find the lowest quarter of frequency values associated almost exclusively with low distress ratings. Higher distress ratings were also more commonly observed in the highest quarter of fundamental frequency scores, however low distress ratings were also not uncommon in the upper quarter. While the observed relationship is less prominent in this study in comparison to findings by Esposito and colleagues (2015), it does suggest there could be a trend which finds low fundamental frequencies to be less distressing. There did not appear to be any trend between infants at HR producing more distressing cries as proposed by Esposito and others (2010). This may be a result of similar fundamental frequency scores between the two cohorts.

Comparison of infant distress and interpretation of distress highlighted some interesting findings. The most notable was that cries recorded during a nappy change were consistently rated as highly distressing. Of the six infants recorded during a nappy change, four were HR infants. Given that individuals with ASD are often sensitive to sensory stimulation, it can be surmised that some infants with ASD may find the cold, wet and uncomfortable sensations of a nappy change to be distressing. This may account for the consistently high distress ratings in HR infants having their nappy changed.

Another interesting finding was the variance of distress heard in tired infant cries. Distress ratings for tired infants showed the most variability with ratings on every level of distress, while in comparison other reasons ranged a maximum of four distress points. This may be a result of tired cries being one of the most commonly reported reasons for distress in this study. Thus there is more opportunity for cries to be perceived differently. However, cries due to waking up were also reported the same number of times and had a small variance of three points. Therefore it seems unlikely the dispersed range of scores for tired cries is a result of increased number of infants allocated to this group. One explanation could be attributed to the level of tiredness felt by the infant. Fatigue may result in less control over vocal structures which in turn changes the acoustic features of the cry. These changes in acoustic features, as a result of incoordination, could be perceived as more aversive. This could also account for why wake up cries have a small variance between ratings as the infant had more control over vocal structures and thus similarly perceived distress rating.

It has been stated before by Esposito and Venuti (2008), that parents of infants with ASD often have difficulty interpreting the cry signal and are unsure how to alleviate the distress. This study also found that of the three parents who were unsure why their child was crying, two had infants in the HR cohort. While this is a small sample of infants and not enough to be significant, it is interesting to note the trend is still observable. However overall,

most parents were able to provide a reason for distress regardless of whether their child was in the HR or LR group. Cries relating to hunger, requests or frustration, were found to be less distressing than tired or nappy change cries. This may possibly be a result of less aversive stimulation in comparison to stimuli which is present during nappy changing.

### **Position**

Examination of infant positioning did not appear to influence any of the acoustic measures; however low parent compliance when reporting on infant position impacted results. Correlations may have been revealed with higher compliance in reporting this factor. With the results collected, it was not possible to determine any compelling relationships.

### **Methodological Differences**

Several methodological differences have been noted between this study and the literature such as setting, methods of elicitation, exclusion criteria, method of recording and the number and age of participants. These differences and the associated implications are discussed in the following sections.

**Setting and Elicitation.** These two elements have been merged together as they have been shown to influence each other. For example, naturalistic settings avoid manipulation of the environment and infant and therefore avoid attempts to elicit specific cries. Such was the case in this study and the study by Sheinkopf and colleagues (2012). This may partly account for the similar results found between the two investigations on non-pain related cries. Other studies which have attempted to manipulate the environment or type of cry elicited have tended to find significant differences in their results. For example, Esposito and colleagues (2014) who analysed cries elicited during a Strange Situation Procedure in an assessment room foreign to the infant. This method manipulated the elicitation of a cry by inducing

distress caused by separation of the infant from its mother. The investigation by Santos and colleagues (2013) used retrospective videos of assessments which again removed the naturalistic element of sample collection. The study reported in this paper prioritised using samples from naturalistic settings without manipulation to gain a realistic representation of everyday infant cries. This decision was made to investigate if differences between HR and LR infants were still observable in all types of cries. Additionally, maintaining a naturalistic setting reduces the possibility of manipulating results when attempting to control the environment and onset of cry vocalisations. Furthermore this method allowed for a greater variance in types of cries which enabled further exploration into comparisons between distress rating and parental reports. As this study and the similar study by Sheinkopf and others found no significant differences, it can be surmised that cries in a naturalistic setting vary too much to detect possible anomalies. It may be necessary to elicit a particular cry before detection of atypical acoustic features can be observed.

**Exclusion Criteria.** As the recordings used in this study were made in a naturalistic environment, controlling for background noise was important. However neither studies by Esposito and Venuti (2009), Esposito and colleagues (2014), or Sheinkopf and others (2012) reported the method they used to establish acceptable sound quality. This makes it difficult to compare the quality of recording's used between studies. The method employed in this study ensured minimal influence from background noise or other interference thus reducing the impact on analysis. Hence it can be assumed that results obtained were an accurate representation of the infant's vocal production and not confounded by poor quality management.

**Acoustic Analysis.** Another methodological difference between this study and others is the detail to which data was scrutinised before analysis. While there appeared to be a consensus between studies agreeing on the definition of a cry, there was no mention of how

other studies controlled for coughs and minor pauses on expiration. These behaviours, if not excluded or accounted for, could impact the analysis by introducing outlying data which may not correspond to the infant's vocal production. To the author's knowledge, this is the first study to excluded these behavioural confounds and use only the voiced segments of a cry sample. Additionally the method in which this was done has been recorded for future replication. The combination of strict exclusion criteria along with rigorous acoustic analysis prior to data analysis is highlighted as a strength of this study.

Furthermore, rather than selecting one cry per infant for acoustic analysis, the present study used the median score of three cries to ensure our sample was representative of the infant's entire cry episode. This exacting process allowed for the exclusion of outlying data which may account for the closely matched results between HR and LR groups which has not been seen in previous investigations.

**Recording Method.** Recording methods differed across all studies. Santos and colleagues, (2013), used retrospective video recordings of assessment sessions while Esposito and Venuti (2009), used retrospective home videos. Esposito and colleagues (2014), used audio recordings from an overhead microphone and Sheinkopf and others (2012) employed the use of both video and audio recordings. The audio recorder in Sheinkopf's study was sewn into a vest which the infant wore. As a result, they were able to somewhat control for distance of recorder to mouth which is a limitation of this current study. The variation in devices and difficulty controlling the distance of the microphone to the infant may lead to differences in recording quality and thus impact results

Furthermore as this study relied on caregivers to commence recording, it is unlikely the first cry utterance was captured for analysis. This may account for why no significant variances were observed as the first cry unit is the most sensitive to control of vocal

production (LaGasse et al., 2005). The onset of a cry is believed to be under direct neural control, specifically the brain stem and limbic system, after which the remaining cry becomes more rhythmic and homogeneous. Thus the initial cry unit may hold the most important information regarding neural control. It is possible this study only captured cries after the initial onset once the infant was in a rhythmic crying pattern. This could be a contributing factor as to why the scores between groups were so similar. Furthermore, it may account for why studies which did include the first cry utterance found different results (Esposito et al., 2014).

**Age of Participants.** A further contributing factor which may account for disparity between findings in this study and those of previous research is the age of participants. To the author's knowledge, this is the only study which has focused on infants at 12 months of age specifically. Sheinkopf and others (2012) conducted their investigation on six month old infants and found no significant differences on acoustic measures in non-pain related cries. In contrast, studies which did observe differences between HR and LR cohorts conducted their investigation with older children ranging between 15-20 months of age (Esposito et al., 2009; Esposito et al., 2014; Santos, 2013).

Interestingly the study by Esposito and others (2009), reported that typically developing infants displayed a decrease in fundamental frequency scores between testing at five and 20 months. This trajectory was not observed in children with ASD over the same period of time. At five months, both TD and ASD groups were recorded having almost identical fundamental frequency scores. However at 20 months, the TD infants had significantly lower scores while ASD infant scores remained constant. Esposito and Venuti (2009), attributed this difference to possible anatomical growth and physiological maturation of the infant larynx during the second year of life. It is challenging to identify when this

difference becomes significant, however this may account for disparity between studies with age differences.

### **Strengths and Limitations**

Acoustic analysis of infant cries in ASD has not been widely researched up to this point. Therefore it was challenging to anticipate the methodological confounds which arose during the length of the study. A strength of the current investigation was the ability to capture cries in a naturalistic setting with no manipulation of the environment or infant. However, as a result it was not possible to capture the first cry unit in a cry episode. The first cry unit is said to be the most sensitive to control of vocal production (LaGasse et al., 2005) and would have been a valuable measure. Furthermore, as the study did not manipulate the elicitation of cry episodes it was unable to gather pain related cries for analysis. This would have allowed further investigation of acoustic properties when the control of vocal production was under increased stress. Finally, the study would have been strengthened with higher rates of compliance on reporting infant position. While the data that was collected from half of the participants did not reveal any significance, it is the author's belief that higher compliance rates would have benefited the study. As would having increased participants for rating distress levels.

Nonetheless, the present study had a number of strengths including a tight age range, closely matched sample of HR and LR infants, large sample size in comparison to previous studies, strict inclusion and exclusion criteria allowing only high quality samples to be included and detailed acoustic analysis. Additionally this study incorporated information regarding infant position, level of perceived distress and reason for distress. A combination of these factors in one investigation is missing from the literature.

## Summary and Implications

While the present study did not observe any significant differences between HR and LR cohort on acoustic measures, it did highlight a number of important factors. Firstly the age of participants should be carefully considered. This study in conjunction with Sheinkopf and others (2012) suggests that variations in acoustic properties of infant cries may not be noticeable between six to 12 months. Differences may only become observed after possible maturation of vocal structures in the second year of life. Second, the type of cry being analysed should be carefully considered. This study investigated non-pain related cries with no observable differences between HR and LR infants, a finding which was also observed by Sheinkopf and colleagues (2012). Greater stress may need to be placed on the control of vocal production to observe differences, particularly at a younger age. Thirdly, care should be taken when selecting cry units for analysis. The present study found very minimal variance between HR and LR cohorts on acoustic measures. This indicates both cohorts have control over vocal production during this phase of the cry and have possibly entered a rhythmic pattern of crying as suggested by LaGasse and colleagues (2005).

While the present study did not observe a trend between increased fundamental frequency and higher distress ratings, there were interesting relationships between distress levels and reason for distress. The results suggest that infants at risk for ASD may have more distressing cries when in stimulatingly unpleasant scenarios. Furthermore, tired cries which showed the most variability in distress ratings may indicate difficulty controlling vocal production once fatigued. This has implications for future research as time of day and reason for distress may influence the results.

## **Future Directions**

Further research in this area is required before using infant cries as an early risk indicator. Follow up investigations of the infants in this study later diagnosed with ASD could provide additional information on possible risk factors which were over looked. For future research, ideally methods of acoustic analysis should be more standardised across studies to ensure comparability of results. Additionally, future studies should include information pertaining to infant position and reason for distress as this data can be useful in the interpretation of results. Furthermore, longitudinal studies which conduct acoustic analysis on a cohort of HR and LR children every few months would provide more information as to when differences in acoustic properties can be observed. This would improve accuracy and narrow the time frame for early risk detection tools which may come to utilise acoustic properties of infant cries.

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