Auditory Perception of the Foetus

Study Protocol

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1. Background

Music. Music is a human universal (Blacking, 1995; Wallin, 1991). Musical behaviour is not only an integral part in today's societies (Mehr et al., 2019), but it has had an important role in all cultures throughout history (Koelsch, 2011; Särkämö et al., 2013). Although it is debatable whether music had adaptive functions during evolution (Cross, 2001; Pinker & Bloom, 1990), there is consensus about its importance in human ontogenesis (Pearce & Rohmeier, 2012). Music is a form of (non-verbal) communication which increases social bonding, enables emotional expression and (self-) regulation. It supports identity formation as well as the acquisition of various cognitive, motor, and social skills during development (Pearce & Rohmeier, 2012; Särkämö et al., 2013). Humans are born with the ability to detect and differentiate various structural and affective aspects of music such as pitch, rhythm, and their emotional meanings (e. g. Trehub, 2003). Recent studies suggest the onset of these abilities during the foetal period, but the exact age at which foetuses acquire different aspects of music perception is still unclear.

Auditory development. The ability to hear and process sounds is a prerequisite for perceiving and discriminating musical stimuli. The neural basis of human hearing starts developing in early gestation with the maturation of cochlear hair cells between the 20th-35th Week of Gestation (WG; Hepper & Shahidullah, 1994; Pujol et al., 1991; Rubel & Fritzsch, 2002). At 23-25 WG, foetuses can actively listen to auditory stimuli (Whitwell, 1999) and show auditory preferences (Foster & Verny, 2007). The cochlea is functionally mature between the 30th-35th WG (Pujol et al., 1991). At the same time, the neural path length increases in the brainstem (Moore et al., 1996).

The womb is a relatively quiet place where sounds from inside the maternal body predominate (Gerhardt & Abrams, 2000; Lecanuet et al., 2013). External sounds are altered as they pass through maternal tissues and amniotic fluid, with smaller attenuation rates in the low-frequency ranges and increased attenuation rates for higher frequency levels. This results in an auditory environment which is richer in low-frequency sounds (Gerhardt & Abrams, 2000).

Most studies find evidence for the onset of foetal auditory perception between the 23rd-33rd WG (Birnholz & Benacerraf, 1983; Crade & Lovett, 1988; Jardri et al., 2008; Kisilevsky et al., 2000). Foetal responsiveness increases with gestational age (GA) and depends on the frequency as well as on the sound pressure level (SPL) of the stimuli (Hepper & Shahidullah, 1994). First, relatively loud, low-frequency sounds are processed with a latency of up to 400 ms. As development progresses, foetuses begin to respond to higher frequencies, and can detect stimuli with lower SPL and within shorter periods of time. The latency drops from 200-300 ms at 30-35 WG to 100-200 ms at 35-40 WG (Dunn et al., 2015). Furthermore, the

development of the ventral cochlear nucleus, the brainstem, the auditory nerves, and the auditory system in general also contributes to these improvements (Eggermont & Salamy, 1988; Nara et al., 1993). In sum, previous studies suggest that the foetal auditory system is functional after the 28th WG (Welch, 2001).

Foetal music perception. Because the auditory abilities that are necessary to detect and discriminate musical stimuli are not present before the 24th WG (but see Hepper & Shahidullah, 1994), music perception is unlikely to occur beforehand. Only a few studies have investigated intrauterine music perception so far, but they all support this assumption.

Several auditory abilities are necessary to percept and discriminate musical stimuli. These are discussed next.

Pitch perception is the first musical ability to develop which has been studied using mainly habituation and dishabituation paradigms (e. g. Hepper & Shahidullah, 1994), oddball paradigms (e. g. Holst et al., 2005), but also neuroimaging methods like functional magnetic resonance imaging (fMRI; Jardri et al., 2008). At 19-27 WG, foetuses only respond to low-frequency stimuli of 100 Hz, 250 Hz and 500 Hz. At 29-33 WG, foetuses can detect higher frequencies (around 1000 Hz) and by 31-35 WG, they respond to tones as high as 3000 Hz (Hepper & Shahidullah, 1994). As the auditory system matures, foetuses also start to respond to lower intensity levels. At 35-36 WG, first discriminatory abilities are shown between two low-frequency tones (Hepper & Shahidullah, 1994; Lecanuet et al., 2000).

Separate to pitch, rhythm perception has also been studied using habituation and dishabituation paradigms which show improved rhythm perception as development progresses. During gestation, foetuses are stimulated by and can respond to rhythms of the maternal body (e. g. heartbeat, breathing, movements), and those from the external environment, including the human voice, speech, and music (Lecanuet & Schaal, 1996; Porcaro et al., 2006; Provasi et al., 2014).

The perception and discrimination of human voices as vocal instruments have been the main subject of previous intrauterine music perception studies. These studies have reported first foetal responses to the mother's voice around the 32nd-34th WG. The recognition of the maternal voice as well as the discrimination of human voices in general improves as gestation advances (for review, see Carvalho et al., 2019). This enables the unborn child to differentiate the maternal voice from other voices at approximately 36 WG (Kisilevsky et al., 2003; Kisilevsky & Hains, 2011).

In addition to pitch, rhythm, and voice perception, tempo is also an important aspect of intrauterine music perception abilities. Foetuses between the 30th-40th WG show orientation as well as habituation patterns to different tempos in both physiological and behavioural measures (Tristao et al., 2020). Heart rate (HR) usually reflects the tempo of the stimulus with

faster tempos resulting in acceleration while slower tempos lead to deceleration (Martens, 2013). Foetal behavioural activity typically increases when music is played (Reissland et al., 2016; Tristao et al., 2020), but calming effects of low-intensity music on foetal HR and movements have also been reported (Lecanuet, 1996).

Studies with preterm infants suggest a change in the processing of complex musical stimuli at around the 32nd-33rd WG. Then, auditory evoked potentials are measurable in the olivo-cochlear system, whereas younger preterm infants do not show such electrical activity after auditory stimulation (Morlet et al., 1993; Pasman et al., 1991).

Timbre is one of the more complex musical aspects that can be processed prenatally. It is the quality of musical sounds which allows the human ear to discriminate between different instruments or voices even when pitch, volume, tempo, and other aspects of the sound are the same. A previous review by Carvalho et al. (2019) showed that foetuses can discriminate different human voices in late gestation. Furthermore, one-week old infants can recognize timbres they had been exposed to in utero (alto voice, trumpet, clarinet, and violin), and they can discriminate them from ones they have not been exposed to previously which suggests that timbre perception starts during gestation (O'Connel, 2003). Other than O'Connel (2003), no one has examined intrauterine timbre processing so far. Similarly, chord perception has not been subject of previous intrauterine music perception studies, but newborns are already able to recognize and discriminate major vs. minor chords (e. g. Virtala et al., 2013).

2. Aims

To date, it is unclear whether foetuses can percept and discriminate different musical timbres and if so when these abilities develop. The same is true for the perception and differentiation of major vs. minor chords. There is a lack of intrauterine music perception studies in general, and foetal discrimination of musical timbres and chords have not yet been studied. The PhD therefore uses cardiotocography (CTG; studies 1a & 1b) and for the first time, functional near-infrared spectroscopy (fNIRS; studies 2a & 2b) to investigate foetal abilities to percept and differentiate musical timbres as well as major from minor chords. Foetal brain activity during musical stimulation will be measured with fNIRS which will act as a proof of concept for foetal brain imaging in addition to giving a better understanding of the development of music processing during the foetal period.

The PhD has the following research questions:

1. Do foetuses at 35-36 weeks GA habituate to auditory stimuli over the course of multiple trials?

2. Can foetuses at 35-36 weeks GA discriminate between different timbres?
3. Do foetuses show differentiable HR responses between major vs. minor chords at 35-36 weeks GA?
4. Is it possible to investigate foetal brain activity with fNIRS?
5. Are foetal discriminatory abilities for timbres and chords present at a cortical level at 35-36 weeks GA?

3. General

Each of the four studies of the PhD will use an experimental, cross-sectional, within-subjects design. Foetal HR will be collected in studies 1a (timbre) and 1b (chord) as it has proven its reliability in previous foetal music perception studies (e. g. Kisilevsky et al., 2004; Lecanuet et al., 2000; Tristao et al., 2020). In addition, foetal brain activity will be measured using fNIRS in studies 2a and 2b to investigate timbre and chord processing on a cortical level. This will be the first study using this state-of-the-art neuroimaging technique to measure foetal brain activity. Introducing fNIRS into foetal research will not only expand the knowledge of foetal music perception, but it might also be groundbreaking for foetal research in general, as well as possibly offering a non-invasive method for intrauterine clinical assessments.
The data collection for all four studies will take approximately 1-1.5 years.

4. Materials and methods

Ultrasound. Doppler ultrasound assesses foetal behaviour with ultrasonic sound which is emitted by a probe and reflected by tissues while it travels through the amniotic fluid surrounding the foetus. Tissue reflections are then received by a probe and transformed in either a two-, three- or four-dimensional image (Reid & Dunn, 2021). Ultrasound allows direct assessment of foetal behaviour either to explore specific foetal behaviours (observational studies) or to investigate causal relationships between external stimulation and foetal responses (experimental studies). One of the drawbacks of this method is its high attrition rate (> 50 %) as the orientation of the foetus cannot be determined or changed in advance as well as the method itself restricts external stimulation. Nevertheless, the high resolution of ultrasound enables the assessment of foetal behaviour, which is a key strength of this method and one of the reasons why it has been used extensively for intrauterine perception studies (Reid & Dunn, 2021). All studies of this PhD will therefore use two-dimensional ultrasound (2D-ultrasound) in a transverse probe orientation to determine foetal behavioural state and to

ensure the correct positioning of the cardiotocograph in studies 1a and 1b and of the fNIRS probe in studies 2a and 2b.

CTG. CTG is a well-established method to monitor foetal HR during pregnancy and labour (Marzbanrad et al., 2018). It can be applied either internally via a disposable electrode placed onto the foetal skull or breast, or externally using one-dimensional Doppler ultrasound. Both methods then identify foetal heart beats to estimate inter beat intervals, HR accelerations, decelerations, and foetal HR variability (e. g. Grivell et al., 2015). While internal CTG can only be applied after membranes have been ruptured and is not recommended before 32 WG (Ayres-de-Campos et al., 2015), external CTG is a non-invasive, inexpensive alternative which can be used as early as 20 WG (e. g. Marzbanrad et al., 2018). External CTG measures foetal cardiac activity via a one-dimensional ultrasound transducer in a belt positioned above the foetal heart which is attached to the maternal abdomen. A second transducer is placed at the fundus of the foetus to simultaneously measure uterine contractions which is especially relevant during labour. Both signals are then electronically transformed to continuously show foetal HR and uterine muscle activity on a paper trace, the cardiotocograph (Grivell et al., 2015). Although the reliability of this method as well as its effectiveness in correctly diagnosing foetuses at risk has often been criticized (for reviews, see Grivell et al., 2015; Marzbanrad et al., 2018), it is not only commonly used, but was found to be the primary method for antenatal foetal monitoring in clinical settings (e. g. Freeman, 1982) as well as being prominent in foetal HR research (e. g. Kisilevsky et al., 2004; Lecanuet et al., 1992; Morokuma et al., 2008). Therefore, external CTG will be used to measure foetal HR in studies 1a and 1b.

FNIRS. The human brain is continuously provided with oxygen to ensure normal brain functioning. If a brain area is involved in the processing of a stimulus, it will increase its oxygen consumption as the corresponding neurons will have a higher energy metabolism rate (Allaman & Magistretti, 2013). Higher oxygen demand is first followed by a decrease of oxygenated (HbO_2) and an increase of deoxygenated blood (HbR) in the activated area. To ensure normal functioning, more oxygen will be transported to this area via delayed increased cerebral blood flow. Then, the ratio of HbO_2 to HbR will change again (de Roever et al., 2018). This typical haemodynamic response can be measured with fNIRS because HbO_2 and HbR have different absorption characteristics of the near-infrared wavelengths (Chance et al., 1998; Villringer & Chance, 1997). FNIRS is a safe, non-invasive method which uses these different absorption characteristics to localize brain activity. First, near-infrared light is emitted by a diode to pass through the skull, scalp, and meninges until it reaches the grey matter and corresponding blood vessels. Then, HbO_2 and HbR blood cells partly absorb the light, and the reflected wavelengths are finally detected by a photodetector (de Roever et al., 2018). In sum,

the signal provides an indirect measure of cortical activity which is limited by the time of the haemodynamic response. Although fNIRS has poorer spatial resolution than fMRI and is limited to two-dimensional images of up to 5 cm thickness (Araki & Nashimoto, 1992), it is widely used in clinical and scientific settings with adults, children and even neonates (for reviews, see Ferrari & Quaresima, 2012; Peng & Hou, 2021). To date, no one has used fNIRS to measure foetal brain activity, but previous studies with neonates and especially with preterm infants show its value by accessing the haemodynamic response to increased neural activity in the immature brain (for review, see Peng & Hou, 2021). Furthermore, fNIRS has several advantages compared to fMRI which has been used in previous foetal music perception studies (for review, see Chorna et al., 2019). The stimuli presentation is neither limited by the magnetic field nor by the scanner noise and the pregnant women must not lie in the scanner (Bunce et al., 2006). Therefore, changes in foetal brain activity in response to musical stimulation in studies 2a and 2b will be measured with fNIRS.

This will be the first study using this neuroimaging method to investigate foetal musical timbre and chord processing on a cortical level. Prior studies have shown that at 34 WG, the foetal head is within 5 cm of maternal external skin (Reid et al, 2017; Donovan et al., 2020). FNIRS is therefore technically feasible for the acquisition of foetal brain function. Using this state-of-the-art technique to investigate foetal brain activity will not only expand the knowledge of foetal music perception, but it might also be groundbreaking for future foetal perception studies in general, as well as possibly offering a non-invasive method for intrauterine clinical assessments.

5. Study procedure

Participation in one of the studies includes providing demographic information as well as attending one experimental session at the Women's Health Clinic, Level 1 Elizabeth Rothwell Building, Waikato Hospital. The experimental session will take approximately 45-60 minutes. This includes informed consent, foetal check-ups via 2D-ultrasound, explanations of the procedure, time for questions, the experimental parts (either 1a & 1b or 2a & 2b), a short break in between them as well as a debriefing at the end of the session.

Prior to participation. Every participant will be sent the informed consent sheet (appendix B) and the demographic questionnaire (appendix C) in advance to the session at Waikato Hospital. This allows the mothers-to-be to review the information, to discuss the studies with their family and friends, to take notes regarding any questions or concerns, and to contact the research team if necessary. The criteria for taking part in the studies and their rationales are

clearly outlined in the information sheet and will be discussed with each woman prior to participation.

Every woman will be given a detailed map with instructions where to park and how to get to the entry of the Waikato Hospital Emergency Department where one member of the research team will wait to bring the mother-to-be to the study room. Every woman will get a call or a text the day before her scan to remind her of her appointment on the next day. Contact details of the research team will be provided on the informed consent sheet in case there are any questions or concerns about participation, if the woman cannot make it to the session, or has troubles finding her way to the meeting point.

Experimental session. One member of the research team will greet the mother-to-be and her whānau or friends at the meeting point in front of the hospital and will bring her to the sonography unit of the Women's Health Clinic. They will then go through the informed consent sheet in a room next to the one where the studies will take place. The informed consent includes an explanation of the purpose of the studies, explains the safety of the stimuli and methods used, has a brief description of the experimental procedure, and informs about what will be done to ensure the anonymity and the safety of the collected data. All explanations will be done in a clear, easy to understand language, and every participant will be asked for questions or concerns. Afterwards, the mothers-to-be will be asked to sign two copies of the informed consent sheet of which one of them will be given to the participant to keep for her records and one of them will be kept by the researchers.

After written informed consent, the participant will be brought to the sonography room where every member of the research team present will be introduced and her or his role in the session will be explained. Next, the woman will lie down in a comfortable position on her back and routine foetal check-ups will be performed by a qualified and registered diagnostic medical sonographer with a current annual practicing certificate from the New Zealand Medical Radiation Technologists Board in Wellington. These check-ups will include measurements of the foetal bi-parietal diameter, head circumference, abdominal circumference, and femur length to estimate foetal weight and GA. Furthermore, foetal HR as well as single deepest pocket of amniotic fluid will be recorded. Following New Zealand Obstetric Ultrasound Guidelines of the Ministry of Health (2019), development will be considered normal if abdominal circumference is > 5^{th} percentile, estimated foetal weight is ≥ 10^{th} percentile, and single deepest pocket is equal or above 2 cm. Furthermore, foetal HR will be considered normal in the range of 120-160 beats per minute (bpm; Von Steinburg et al., 2013). The mothers-to-be are welcomed to always watch the ultrasound screen and the measurements will be explained to every woman regarding the development of her baby. They are also

welcomed to ask any questions. Afterwards, the experimental procedure will be explained in more detail and exemplary stimuli will be played to give the mothers-to-be an idea of what their babies will hear during the experiments. Each participant will be explicitly asked if she has any questions or concerns, and each will be reminded that the procedure can be stopped in case she feels uncomfortable or wants to withdraw. Neither of them must be justified. Afterwards, the mother-to-be will be asked if she agrees to proceed with the procedure. If she does, she will be reminded to remain silent during the experiments and to lie as still as possible to ensure a high quality of the collected data throughout the experimental parts. Next, the research team will identify foetal position as well as foetal behavioural state (after Nijhuis et al., 1982) using 2D-ultrasound. For studies 1a and 1b, the cardiotocograph (Huntleigh Sonicaid Team3) and the loudspeaker (Marshall Acton III Bluetooth Speaker) will be set-up afterwards and the participant and her whānau or friends will be given headphones before the experiments will start. To access fronto-temporal brain activity in studies 2a and 2b a particular position of the foetus will be required. As previously described, the near-infrared light of fNIRS penetrates up to 5 cm into the maternal abdomen (Araki & Nashimoto, 1992) and if the foetal head is not within this range, the participant will be asked to be taken for a short walk or to drink a glass of water to stimulate foetal repositioning. Otherwise, the experimental procedure cannot be continued, and the women will be asked to return a few days later if she is still interested in participating. If the foetus is in an appropriate position, the fNIRS probe and the loudspeaker will be put into place, headphones will be given to the mother-to-be and her company and the experiments will start.

Each session will include one timbre (studies 1a & 2a) and one chord experiment (studies 1b & 2b) which are separated by a short break in between. The specific structure of each experiment is outlined further below (paragraphs 6 & 7).
After the completion of both experiments, the mothers-to-be will be asked if they have heard any of the stimuli and if they felt any foetal movement during the session. Everyone will be thanked for participating, debriefed, and given the opportunity to ask further questions or to comment about the studies. Every participant will be given $ 30 vouchers in recognition of travel costs and to show appreciation of taking part in the studies. In total, each session will take about 45-60 minutes.

After participation. Following delivery, health notes of the mothers and their babies will be accessed. These include the delivery method, complications during pregnancy or birth, gestational age of the baby at birth, sex, birth weight, as well as the results of the first prenatal hearing test. The collection of these information and the reasons why they are accessed are clearly outlined in the informed consent sheet and will be brought to every participant's

attention as part of giving written informed consent. The mothers will not be contacted about this again.

The informed consent sheet further gives every participant the opportunity to sign up for getting a brief overview of the main results of all studies. These will be summed up after the data has been analysed and will be send to all women who had requested a copy during participation.

6. Experiments 1a and 1b – HR

Background. So far, the discrimination of musical timbres and chords have not been investigated during gestation, but several studies have shown that foetal HR is a valid measure of foetal auditory and music perception abilities. For example, 70 % of the foetuses (36-39 weeks GA) who participated in the study by Lecanuet et al. (1999) showed a HR deceleration after the presentation of a note (D4/ C5), and 90 % of these foetuses responded with another HR deceleration after a second note (C5/ D4) was presented. Furthermore, Kisilevsky et al. (2004) reported an initial HR decrease of foetuses at 35-36 weeks GA in response to a lullaby played at 95 dB. This change in HR peaked approximately 5 seconds past stimulus onset. When the same lullaby was played at higher SPL (100-110 dB), foetuses did not show an initial HR decrease, but responded with a continuous HR acceleration while the lullaby was played for 5 minutes. This finding is in line with other studies which reported a significant HR deceleration to sounds played at lower SPL (e. g. Lecanuet et al., 1988; 1992; 1993), while an increase of the SPL to 100 dB and above typically leads to the occurrence of cardiac accelerations (for review, see Lecanuet & Schaal, 1996). Following the initial HR decrease, foetuses show a continuous HR acceleration during auditory stimulation which slowly returns to baseline after stimulus offset (e. g. Kisilevsky & Hains, 2011; Lee, 2010).

These last studies have investigated foetal voice perception which is especially interesting for the timbre experiments as every voice has a unique timbre. Near-term foetuses (≥ 36 weeks GA) showed discriminatory abilities between a male and a female voice uttering the same sentence which was reflected by an initial HR deceleration that peaked 10-20 seconds after stimulus onset (Lecanuet et al., 1992). Furthermore, the initial HR decrease was followed by a prolonged HR increase in term foetuses (≥ 38 weeks GA) with low vagal tone (Smith et al., 2007).

At the same time, there was no difference in foetal behavioural responses to the maternal voice at 36-38 weeks GA, neither compared to another female's (Hepper et al., 1993; Kisilevsky et al., 2003), nor to the father's voice (Lee, 2010). It was therefore decided to investigate only foetal cardiac responses, and not foetal movements.

Aim. The aim for studies 1a and 1b is to measure foetal HR to see if foetuses at 35-36 weeks GA can differentiate musical timbres (study 1a) and major from minor chords (study 1b).

Hypotheses.

1. Foetuses will show an initial HR deceleration as part of an orienting response after stimulus onset in the first block of experiment 1a and 1b.
2. The amplitude of the initial HR deceleration will decrease over the blocks of both experiments which will reflect habituation to the presented stimuli.
3. The initial HR decrease after stimulus onset will be followed by a slower HR acceleration.
4. The HR acceleration will decrease over the course of each stimulus.
5. If foetuses can discriminate a clarinet, a trumpet, and a violin timbre, a new HR decelerative response will be expected after the first presentation of the second and third timbre in experiment 1a. This response is expected to have an increased amplitude compared to the last block of the previous timbre.
6. If foetuses can discriminate major from minor chords, a new HR decelerative response will be expected after the change to the second chord category in experiment 1b. This response is expected to have an increased amplitude compared to the last block of the previous chord category.
7. Foetal HR responses within the chord categories might differ (C- vs. G- vs. D-major; a- vs. e- vs. d-minor), but this difference would be smaller than the one between the two categories (major vs. minor).

Independent variables. Study 1a: Timbre – clarinet vs. trumpet vs. violin.
Study 1b: Chord – major vs. minor.

Dependent variables. Foetal HR variables, e. g. amplitude, absolute value.

Materials and methods - stimuli. Study 1a will investigate foetal perception and differentiation of a clarinet, a trumpet, and a violin timbre. These timbres were selected not only because they have been used in previous studies (e. g. O'Connel, 2003), but also because they represent three of the primary instrument categories, namely woodwind, brass, and (bowed) string instruments which have distinctive, category-specific temporal and spectral characteristics (e. g. Donnelly & Sheppard, 2014). All audio files have the same note (C4; pitch = 261.63 Hz), a total length of 3.492 seconds and are played at the same volume. The SPL is within the range of the ones used in previous studies and is safe for the foetal auditory system (e. g. Kisilevsky et al., 2004; Lecanuet et al., 2000; Shahidullah & Hepper, 1994). Additionally,

a fourth file with a piano timbre was generated to match the other files in pitch, amplitude, and length which will be played to the mothers-to-be as an example for the stimuli in study 1a.
In study 1b, C-, G-, and D-major as well as a-, e-, and d-minor chords played by a piano will be used to investigate foetal perception and discrimination of major and minor chords. The chords were selected based on the results of Burgoyne et al. (2011) who reported them to be the most common chords in western popular music. Each audio file is 1.327 seconds long and the stimuli are matched for the amplitude of the sound.
The clarinet sample was provided by the University of New South Wales (Australia), the other three timbre files were given by the International Audio Laboratories Erlangen (Germany), and the chord stimuli were downloaded from auralwiz.com. All audio files were used and altered with permission of the copyright holders.

Materials and methods - image acquisition. A qualified and experienced sonographer will use a Philips Epiq7 Elite Ultrasound System with either a 5C1 or 9C2 transducer (thermal index for bones < 0.3) to perform foetal check-ups, determine foetal position, foetal behavioural state, and to ensure the correct placement of the CTG. A Huntleigh Sonicaid Team3 CTG will be used to measure foetal HR four times per second using a directional pulsed Doppler Ultrasound transducer.
Each session will be recorded and saved on two external hard drives as well as stored in a secure folder on the university server for later analyses.

Procedure. After written informed consent and foetal check-ups, the experimental procedure of the two studies will be explained to the participant. Two exemplary trials of each of the two experiments will then be presented. These are a C4 played by a piano for study 1a and one trial each with a F-major and a f-minor chord for study 1b. Both are also played by a piano. After the participant confirms that she has no further questions and agrees to move on to the actual experiments foetal position and behavioural state will then be identified. Baseline 1 will follow and experiment 1a will start thereafter. Each timbre will be presented in three separate blocks of 30 seconds which are separated by 30 seconds of silence to allow the foetal HR to return to (near) baseline levels in between sound blocks (e. g. Morokuma et al., 2008). The sound blocks include six sounds of 3.492 seconds alternated with 1.508 seconds of silence. This design was chosen based on previous studies which showed successful HR responses to auditory stimuli using blocks of sounds with silence and blocks of no sound in between them (Kisilevksy et al., 1989; Lecanuet et al., 2000; Lee, 2010). The timbre experiment will take 9 minutes. Afterwards, a brief break will follow. Next, baseline 2 will start and the chord stimuli will then be presented in three separate blocks for each chord category. Each of the blocks is also 30 seconds, but the sounds are only 1.327 seconds long and separated by 1.673 seconds

of silence which results in 10 sound presentations per block. The shorter sound length is due to the specific temporal characteristic of the instrument used to play these stimuli as tones played by the piano have a very quick attack and a very short release phase (Müller, 2015). The chord experiment is 6 minutes long.
The order of the stimuli in both experiments as well as the order of the experiments themselves will be counterbalanced across participants to control for possible order effects.

Data analyses. Foetal HR will be analysed in two separate two-way repeated analyses of variances (ANOVA) to compare foetal responses to the three different timbres (study 1a) as well as between major and minor chords (study 1b).
Degrees of freedom will be adjusted if the assumption of sphericity is violated in either of the analyses. Post hoc comparisons will be done with Tukey tests ($p < .05$) and Bonferroni-correction will be used for α-adjustment to take multiple comparisons into account.
Furthermore, sex, foetal HR variability (HRV) at baseline, and foetal state will be analysed exploratory in a multiple linear regression analysis to test for moderating effects. These variables were chosen based on the previous literature which has found moderating effects of foetal HRV at baseline (Lecanuet et al., 1986; 1992; Smith et al., 2007) and foetal behavioural state on foetal responses to external auditory stimuli (Devoe et al., 1990; Schmidt et al., 1985). To date, only a few studies have analysed a possible influence of sex on foetal responses. These studies have found non-significant effects (e. g. Groome et al., 1996; López-Teijón et al., 2015), but there is also evidence of sex-specific responses. Female foetuses showed a more mature response compared to male foetuses (Buss et al., 2009) as well as general foetal HR dynamics depend on foetal sex (Bernardes et al., 2007). Foetal sex will thus also be analysed exploratory as a moderating variable which is especially valuable considering the previous neglect of many studies in not analysing if female and male foetuses responded differently to the presented stimuli.

7. Experiments 2a and 2b – brain activity

Background. As summarised above, previous research on foetal auditory development and the results of foetal studies investigating cardiac responses to musical stimuli suggest that foetuses at 35-36 weeks GA have discriminatory abilities for musical stimuli. The perception and discrimination of musical stimuli can also be measured with neuroimaging techniques, such as fMRI and foetal magnetoencephalography (for review, see Dunn et al., 2015). These methods have shown mainly frontal and temporal activity after musical stimulation of the foetus (for review, see Chorna et al., 2019). For example, at 33 weeks GA, the presentation of pure

tones led to increased cortical activity in the foetal temporal lobe (Jardri et al., 2008) which is in line with studies at neonatal intensive care units showing that auditory evoked potentials are measurable in the olivo-cochlear system of preterm infants after 33 weeks GA (Morlet et al., 1993; Pasman et al., 1991). Furthermore, the location of foetal temporal lobe activity following musical stimuli is consistent with the position of the primary auditory cortex (Jardri et al., 2008; Moore et al., 2001). Thus, neuroimaging methods can be used to measure foetal brain activity as well as they extend our understanding of musical and general auditory development during gestation.

Foetal brain activity has not been studied with fNIRS although this state-of-the-art neuroimaging method is widely used with adults, children and even neonates, both for clinical and research purposes (for reviews, see Ferrari & Quaresima, 2012; Peng & Hou, 2021). Several fNIRS studies have investigated newborns' responses to auditory stimulation with significant results for the activity in fronto-temporal areas (for review, see de Roever et al., 2018). When tonal sounds or music were used for auditory stimulation, a positive change in HbO_2 could be observed, sometimes accompanied by a change in HbR which was less consistent as it could either drop, increase, or not change significantly in either way (for review, see de Roever et al., 2018).

In sum, previous neuroimaging studies with foetuses show that fronto-temporal brain activity can be successfully measured during gestation. Furthermore, fNIRS studies with newborns, infants, and children demonstrate that fNIRS can be successfully used to measure cortical responses in the developing brain (for reviews, see de Roever et al., 2018; Wilcox & Biondi, 2015). Although fNIRS has poorer spatial resolution than fMRI and is limited to two-dimensional images of up to 5 cm thickness (Araki & Nashimoto, 1992), previous studies have shown that at 34 weeks GA, the foetal head is within 5 cm of maternal external skin (Donovan et al., 2020; Reid et al, 2017). FNIRS is therefore technically feasible for the acquisition of foetal brain function.

Aims. Studies 2a and 2b use fNIRS to investigate foetal brain responses in fronto-temporal areas following the presentation of different musical timbres and chords. One aim is to find out if it is possible to measure foetal brain activity with fNIRS in general. The second aim is to see if foetuses at 35-36 weeks GA can differentiate musical timbres (study 2a) and major from minor chords (study 2b) on a cortical level.

Hypotheses.

1. It is possible to measure foetal brain activity with fNIRS.
2. Differentiating brain responses will be expected if foetuses can discriminate a clarinet, a trumpet, and a violin timbre.

3. If foetuses can discriminate major from minor chords, this will be reflected in differentiating brain activity patterns after the presentation of major and minor chords.
4. Foetal brain activity responses within the chord categories might differ (C- vs. G- vs. D-major; a- vs. e- vs. d-minor), but this difference would be smaller than the one between the two categories (major vs. minor).

Independent variables. Study 2a: Timbre – clarinet vs. trumpet vs. violin.
Study 2b: Chord – major vs. minor.

Dependent variables. Foetal brain activity, e. g. HbO_2 and HbR.

Materials and methods - stimuli. Same as for studies 1a and 1b (see above).

Materials and methods - image acquisition. A qualified and experienced sonographer will use a Philips Epiq7 Elite Ultrasound System with either a 5C1 or 9C2 transducer (thermal index for bones < 0.3) to perform foetal check-ups, determine foetal position, foetal behavioural state, and to estimate the correct placement of the fNIRS probe. The later one will be a custom-made device consisting of one light emitter and one light detector which will be placed directly on the maternal skin. The fNIRS probe will be using two wavelengths, namely 690 nm and 830 nm, which are known to be optimal for measuring deoxy- and oxygenated blood in early developmental populations (for reviews, see Lloyd-Fox et al., 2010; Wilcox & Biondi, 2015). These wavelengths also possess the physical characteristics to enable penetration of human tissue (León-Carrión & León-Domínguez, 2012; Strangman et al., 2002). The strength of the near-infrared light will be adjusted based on the individual thickness of the maternal tissue. This will enable a consistent sensitivity to measure foetal brain activity across participants and ensure that light levels are well within boundaries for safe use.
Each session will be recorded and saved on two external hard drives as well as stored in a secure folder on the university server for later analyses.

Procedure. The experimental procedure of the two studies will be explained to the participant after giving written informed consent and after foetal check-ups have been performed. Two exemplary trials of each of the two experiments will then be presented. These are a C4 played by a piano for study 2a and one trial each with a F-major and a f-minor chord for study 2b. Both are also played by a piano. After the participant confirms that she has no further questions and agrees to move on to the actual experiments foetal position and behavioural state will be identified. Then, baseline 1 will follow and experiment 2a will start thereafter. The three different timbres will be presented 24 times each in a semi-randomised order. Thus, unlike to

experiment 1a no block-design will be used which is also true for experiment 2b. A semi-randomised presentation of the stimuli was chosen because fNIRS is an event related measure of brain activity. This means that brain activity can be measured every time a change in the environment takes place. Every trial of study 2a includes 3.492 seconds of sound and is 11 seconds long (ratio 1:2.15). The whole experiment will take 13 minutes and 12 seconds. Afterwards, a brief break will follow. Then, baseline 2 will start and the chord stimuli will be presented thereafter. Both major and minor chords will be presented 24 times which means that each individual chord will be played eight times, also in a semi-randomised order. All trials are 4.180 seconds long and include 1.327 seconds of sound plus 2.853 seconds ISI (ratio 1:2.15). Experiment 2b is 3 minutes and 20.640 seconds long.
The order of the stimuli will be counterbalanced across participants in both experiments.

Data analyses. Foetal brain activity will be analysed in two separate one-way repeated ANOVA to compare foetal responses to the three different timbres (study 2a) as well as between major and minor chords (study 2b).
Degrees of freedom will be adjusted if the assumption of sphericity is violated in either of the analyses. Post hoc comparisons will be done with Tukey tests ($p < .05$) and Bonferroni-correction will be used for α-adjustment to take multiple comparisons into account.
Furthermore, sex and foetal state will be analysed exploratory in a multiple linear regression analysis to test for moderating effects.

8. Safety

General. The safety and wellbeing of the participating women and their foetuses is our primary concern. All studies are in accordance with the current version of the Declaration of Helsinki (World Medical Association, 2013) and we have a clear disclosure policy about any concerns regarding this issue.
Only healthy women with a normal, low-risk singleton pregnancy will be considered for participation, and age-appropriate foetal development will be ensured through foetal check-ups before the beginning of any experiments. Furthermore, the purpose of the present research, the safety of the stimuli and methods used, a description of the experimental procedure, and the handling of the collected data will be explained to every participant as part of giving written informed consent. All explanations will be done in a clear, easy to understand language, and every participant will be asked for any questions or concerns. In addition, it will be brought to every participant's attention that the procedure can be stopped in case she feels

uncomfortable, or if she decides to withdraw. No justification would be necessary in either of these cases.

The experiments themselves are not associated with a particular risk. Participating in the studies, however, do include the risk of getting adverse information about foetal development or of a maternal abnormality which could be incidentally discovered during the ultrasound scan at the beginning or during the experimental parts of the session. This is clearly outlined in the informed consent sheet and will be explained to all participants prior to giving written informed consent. In case of an unexpected incidental finding, the experiments will be stopped immediately, and the sonographer will follow Waikato District Health Board protocols as well as New Zealand Obstetric Ultrasound Guidelines of the Ministry of Health (2019) to ensure appropriate help for the mother-to-be and her unborn child. This includes consultation of a radiologist, a report of the finding, and the notification of the lead maternity carer of the participant by telephone and fax. This process takes about 30 minutes for urgent findings.

2D-Ultrasound will be used to ensure age-appropriate foetal development, determine foetal position and foetal behavioural state. External CTG and fNIRS will be used to measure foetal HR and foetal brain activity, respectively. The safety of these methods as well as the safety of our auditory stimuli will be explained next.

Safety of ultrasound. Ultrasound is a safe method which is routinely used to assess the developmental progress during pregnancy (Houston et al., 2009; Patey & Corcoran, 2020). Furthermore, all procedures will follow the present standards of practice published by the Australasian Society for Ultrasound in Medicine (2014). Ultrasound will only be used for the present research purpose and not for clinical assessment or personal entertainment. This is explained in the informed consent sheet and will also be discussed with every participant before giving written informed consent. A Philips Epiq7 Elite Ultrasound System with either a 5C1 or 9C2 transducer will be used for all scans, keeping thermal index for bones below .3. All scans will take place in a sonography room at Waikato Hospital and will be performed by a qualified and registered diagnostic medical sonographer who has a current annual practicing certificate from the New Zealand Medical Radiation Technologists Board in Wellington.

Safety of external CTG. External CTG is a well-established, non-invasive method to monitor foetal HR during pregnancy (Marzbanrad et al., 2018). Furthermore, it is not only commonly used, but was found to be the primary method for antenatal foetal monitoring in clinical settings (e. g. Freeman, 1982) as well as being prominent in foetal HR research (e. g. Kisilevsky et al.,

2004; Lecanuet et al., 1992; Morokuma et al., 2008). It is considered safe for the assessment of foetal cardiac activity and will be used to measure foetal HR during the sessions.

Safety of fNIRS. To date, fNIRS has never been used for foetal examinations, but it is also a non-invasive and further nonionizing method which has proven its safety even over prolonged and repeated sessions (e. g. Strangman et al., 2002; Wilcox & Biondi, 2015). This state-of-the-art neuroimaging method has been widely used with adults, children and even neonates in both clinical and scientific settings (for reviews, see Ferrari & Quaresima, 2012; Peng & Hou, 2021). FNIRS uses near-infrared wavelengths of 650-950 nm which is also described as an optical window into human tissue based on the low absorption of these wavelengths by biological tissue (León-Carrión & León-Domínguez, 2012; Strangman et al., 2002). The intensity of the light will be much weaker than the light foetuses are exposed to on a daily basis and are therefore considered to be safe for the foetuses and their mothers.

Safety of auditory stimulation. The womb is a relatively quiet place where sounds from inside the maternal body predominate (Gerhardt & Abrams, 2000; Lecanuet et al., 2013). External sounds are altered as they pass through maternal tissues and amniotic fluid, with smaller attenuation rates in the low-frequency ranges and increased attenuation rates for higher frequency levels. This results in an auditory environment which is richer in low-frequency sounds, especially below 500 Hz (Gerhardt & Abrams, 2000). Therefore, low-frequency musical stimuli were selected. These will be presented at a similar SPL as has been used in previous studies which investigated foetal responses to auditory stimulation at similar developmental stages (e. g. Kisilevsky et al., 2004; Lecanuet et al., 1992). To ensure a safe SPL and the general safety of the auditory stimuli, recommendations for foetal auditory stimulation (e. g. Graven, 2000) will always be followed. Additionally, the loudspeaker will be placed above the maternal abdomen based on recommendations by Krueger et al. (2012).

9. Participant selection

Inclusion criteria. All participants will be volunteers which will be selected based on the following inclusion criteria:

- singleton pregnancy
- gestational age is between 35 weeks + 0 days – 36 weeks +6 days
- no pregnancy complications
- healthy mother and healthy foetus

- age-appropriate foetal development: abdominal circumference > 5th percentile, estimated foetal weight ≥ 10th percentile, and single deepest pocket ≥ 2 cm (Ministry of Health, 2019)
- foetal HR is within the normal range: 120-160 bpm (Von Steinburg et al., 2013)

Exclusion criteria. Consequently, women will be excluded if they meet one or more of the following exclusion criteria:

- multifetal pregnancy
- gestational age is < 35 weeks or >37 weeks
- pregnancy complications which affect foetal HR, auditory, neuronal, or general foetal development
- maternal health issues which put either the mother-to-be or the foetus at risk or which affect foetal cardiac, auditory, neuronal, or its general development
- foetal abnormalities or health issues which affect foetal HR, auditory, neuronal, or its general development (e. g. Down syndrome)
- the foetus is small for gestational age: abdominal circumference < 5th percentile, estimated foetal weight < 10th percentile, and single deepest pocket < 2 cm (Ministry of Health, 2019)
- the foetal HR is not within the normal range: <120/ >160 bpm (Von Steinburg et al., 2013)

Because the near-infrared light only penetrates up to 5 cm into the maternal abdomen (Araki & Nashimoto, 1992), one further exclusion criteria will be applied for the fNIRS studies:

- Women will be excluded if maternal tissue thickness is > 5 cm.

Women of all ethnicities are welcome to take part in the study, ethnicity is not a criterion for participant selection.

10. Participant recruiting

We will actively cooperate with maternal health care providers and maternal health researchers to promote the present research project and to encourage pregnant women to participate in our studies. This includes, but not limits to, Hāpu Wānanga and Te Aukume a Hineteiwaiwa. Furthermore, already established contacts with midwives and birth centres will be used for participant recruitment as well as there will be a media campaign on social media

which was very successful in a previous study (Leov et al., 2023). Finally, all participants will be asked to promote the project in their whānau and to pregnant friends.

11. Remuneration/ koha

To show our appreciation for participation and to account for travel costs, all mothers-to-be will be given $ 30 vouchers as a small koha. Knowledge is taonga, therefore, all participants will be given information about our studies and related prenatal research. Furthermore, they will be given a copy of the informed consent and debrief form (appendix D) to take home with them to their whānau and friends. If requested, they will also be sent a short summary of the main research findings after the data have been analysed and interpreted. All forms and the summary will be in a clear, easy to understand language.

12. Data management plan

Data collection. Data collection will take part at Waikato Hospital and will be conducted by the PhD candidate Ms Leonie Loehn, the research assistant Ms Madeline Cosgrove, as well as qualified and registered diagnostic medical sonographers with a current annual practicing certificate from the New Zealand Medical Radiation Technologists Board in Wellington.
The sonographers will perform the ultrasound scans while Ms Loehn and Ms Cosgrove will be collecting demographic data, presenting exemplary stimuli to the mothers-to-be and running the experiments which include auditory stimulation of the participating foetuses. Each experimental session will be recorded for later analyses.
After informed consent, all participants will be given a unique code which will be used from then on to correctly match the ultrasound recordings to the demographic data collected. This code cannot be used to trace back the identity of the participant thus, all data will be de-identified**,** and results will only be reported in an anonymised way.
The de-identification of the data will be explained to all participants in both written (information sheet) and spoken ways (informed consent briefing) using clear, easy to understand language.

Incidental findings during the studies. Ultrasound will only be used for the presented research purpose and not for clinical assessment or personal entertainment. This will be explained to all participants as part of giving written informed consent.
Routine foetal check-ups will be performed prior to the start of the experiments. In case of an unexpected incidental finding of a foetal or maternal abnormality during these check-ups or later during the session, the experiments will be stopped immediately, and the sonographer

will follow Waikato District Health Board protocols and clinical guidelines (Ministry of Health, 2019) to ensure the mother-to-be and her unborn child get the help they need. This includes consultation of a radiologist, a report of the finding, and the notification of the lead maternity carer of the participant by telephone and fax. This process takes about 30 minutes for urgent findings.

Post experimental information. After birth, the research team will access maternal and baby patient notes for health information, such as delivery method, gestational age at birth, birth weight, sex, and the results of the first postnatal hearing test. The access of these health information is outlined in the informed consent sheet and will also be explained verbally to all mothers-to-be prior to giving written informed consent. Health information will be directly accessed through the hospital system therefore, the mothers will not be personally contacted about this information again.

Data Storage. All recordings of the studies will be saved at three different locations, namely two external hard drives as well as the university server as a backup. All copies of the data will be password protected and only members of the research team will have access to them. The external hard drives as well as all hard copies of the informed consent sheets and participant questionnaires will be stored in locked file cabinets within Te Kura Whatu Oho Mauri, School of Psychology on Hamilton campus of the University of Waikato. As for the recordings, only members of the research team will have access to these file cabinets.
In line with the New Zealand Ethical Standards for Health Data, 12.13, all data will be stored securely for ten years as described above and will be destroyed afterwards. All participants will be informed about this.

Data analyses. The recordings of all individual experimental sessions will be de-identified through the individual code allocated to the participant after giving written informed consent. Only members of the research team will analyse the data which will be saved on a password protected server belonging to the University of Waikato. All results will be published in a way that ensures the anonymity of all participants.

Results dissemination. During informed consent, the participants will be asked if they would like to receive a one-page summary of the main study findings. This summary will explain the main results in a clear, easy to understand language and will be sent to all participants who had requested it during data collection.

Participant data access. Participants will be informed that they can request a copy of their personal data (questionnaires, health information) and/ or the ultrasound recording. If this inquiry was communicated verbally, the participant will be asked for a written request and upon receiving a written request, the participant will be only given a copy of their personal information.

Participant data withdrawal. Each of the participating mothers-to-be can withdraw their data within 14 days after participation by handing in a written request to the research team. The relevant contact details will be provided on the participant's copy of the informed consent sheet. If data withdrawal was requested within the relevant time, the participant's data, including all personal information and the ultrasound recording, will be destroyed. The request of data withdrawal and the removal of the individual data will be confirmed in writing and will be sent to the participant after the data has been deleted.

13. Statistical plan

All statistical procedures for adequate data analyses are within the scope of the research team. Therefore, statistical consultation is not necessary.

14. Estimation of sample size

As there have not been any previous studies investigating the perception and discrimination of musical timbres and chords in the foetus, there were no previous effect sizes for the estimation of sample size. Therefore, to maximize the probability to find an effect, a conservative, small within factor effect of $f = .1$ was chosen. Based on a probability of $1 – \beta = .95$, an α-value of $\alpha = .05$ and a theoretical correlation of $\rho = .50$ between repeated measurements, a priori calculation using G.Power 3.1.3 (Faul et al., 2009) yielded in a sample size of $N = 84$ for 18 trials per condition for the HR timbre experiment (study 1a). Using the same psychometric input values ($f = .1$, $1 – \beta = .95$, $\alpha = .05$, and $\rho = .50$), a sample size of $N = 60$ was calculated for the HR chord experiment (study 2b) which includes 30 trials per chord category (major vs. minor). Given an attrition rate of approximately 20 % in foetal HR studies (e. g. Lecanuet et al., 1992; Lecanuet et al., 2000) data of 101 participants will be collected for the HR studies.

Using the same psychometric input values ($f = .1$, $\alpha = .05$, $1 – \beta = .95$, and $\rho = .50$), a sample size of $N = 69$ for 24 trials per timbre and $N = 70$ for 24 trials per chord category was calculated for the studies 2a and 2b, respectively. Previous fNIRS studies with newborns reported an

attrition of approximately 40 % (for review, see Lloyd-Fox et al., 2010) while foetal neuroimaging studies sometimes had to exclude 50 % of their participants (e. g. Jardri et al., 2008). Because no one has used fNIRS for foetal research before, sample size estimation will be based on a conservative attrition rate of 50 % and 105 women will be collected for the fNIRS studies.

Please note that the final estimated sample size for both set of studies was oriented on the estimations for the experiment where a larger number of participants was needed to find a small effect of f = .1. This was the timbre experiment for the HR studies and the chord experiment for the fNIRS studies.

15. Māori consultation

Relevance of the research for Māori. Following the Perinatal and Maternal Mortality Review Committee (PMMRC, 2022), Māori wāhine show higher maternal risk factors and poorer maternal health outcomes in Aotearoa. These lead to a higher risk for their pēpi to be born premature and is shown in an increased likelihood for Māori compared to Pākehā pēpi to receive neonatal intensive care (Craig et al., 2004; Ministry of Health, 2017).

This PhD acknowledges the historical lack of investigating Māori related issues as well as the limited involvement of Māori in the development and implementation of previous research activities in the field of experimental psychology. Therefore, the present research project uses an equity-based approach to manākitanga and mātauranga Māori which will be actively encouraged to take part in this project. For example, Miss Madeline Cosgrave (Ngāti Pūkenga) is a research assistant who will help to maintain and grow existing as well as help to establish new connections with Māori practitioners of allied disciplines and Māori participants in the Waikato area.

The research project follows the principles of Te Hā o Whānau: A culturally responsive framework for maternity care (Stevenson et al., 2020) and The Guide to He Korowai Oranga: Māori Health Strategy (Ministry of Health, 2014) to ensure mātauranga Māori and culturally responsive standards. It was developed with the involvement of local Māori researchers and Māori practitioners of allied disciplines, such as midwifery, to comply with Kaupapa Māori research.

Furthermore, to ensure appropriate and respectful interaction with Māori participants and their whānau, the PhD candidate is taking a paper in Te Reo managed by Te Pua Wānanga ki te Ao.

Aroha ki te tangata. We will manākitanga all participants by taking care to correctly pronounce their names and manākitanga their tikanga. Tikanga will not be breached as we will not touch the head of any participants and we will not collect tissue or body fluids from them. During the experiments, the pregnant women will be lying in a for them comfortable position on their back on an ultrasound bed. They are welcome to bring whānau and friends for support.

He kanohi kitea. Establishing good relationships between the research team and practitioners in the midwifery and in local birth clinics has been an important part of the present research project to allow the recruitment of pregnant wāhine and adequate data acquisition for our studies. Everyone who is involved in data collection will introduce themselves to the participant and their whānau or hoa as well as building rapport. In addition, the research team will answer any questions related to the studies before the beginning of the experiments.

Manāki ki te tangata. To show our appreciation for participation and to account for travel costs, all wāhine will be given $ 30 vouchers as a small koha. Knowledge is taonga, therefore, all participants will be given information about our studies and related prenatal research. Furthermore, they will be given a copy of the informed consent sheet and debrief form to take home with them to their whānau and friends. If requested, they will also be sent a short summary of the main research findings after the data has been analysed and interpreted. All forms and the summary will be in a clear, easy to understand language.

Kia tupato. The safety and wellbeing of the participating women and their foetuses is our primary concern. We have a clear disclosure policy about any concerns regarding this issue.

Māori consultation. Māori consultation is an integral part of the present research to ensure that Māori values and tikanga are correctly followed therefore, Māori consultation has been sought from The Māori Health Service - Te Puna Oranga of the Waikato DHB. The research committee was provided with a summary of the research outline as well as with copies of the informed consent sheet, consent from, the demographic questionnaire, and the debrief form. They endorsed the project and had only a minor correction for Māori wording (01.11.2023) which has been implemented in the present protocol.

16. Pacific consultation

All interactions with people from the Pacific Islands will be following the pacific cultural competency principles from the Pacific Health Research Guidelines (Health Research Council of New Zealand, 2014).

Relevance of the research for Pacific people. In Aotearoa, women with Pacific origin show higher maternal risk factors and poorer maternal health outcomes than women with European origin. Furthermore, they show more complications during pregnancy, and have worse maternal and neonatal health outcomes compared to any other ethnicity. In addition, neonates of Pacific women are at a significantly higher risk to die shortly after birth than babies with European origin (PMMRC, 2022).

The present studies investigate foetal timbre and chord processing to better understand foetal auditory and music development. The results will not only increase our knowledge of normal foetal development, but they will also have the potential to identify and quantify atypical development of foetal hearing which can be used for clinical purposes. Furthermore, by using fNIRS, a state-of-the-art neuroimaging technique which has never been used before to access foetal brain activity, the PhD has the potential to introduce this technique into foetal research and clinical applications. This could not only lead to improved prenatal assessment of foetuses from Pacific women, but also increase postnatal outcomes for Pacific mothers and their babies by identifying atypical development during pregnancy.

17. Funding

The present research is funded by the University of Waikato.

18. Indemnity

The University of Waikato sponsors the presented studies and will be accountable for indemnity.

19. Ethical approval

Ethical approval is being sought from the national Health and Disability Ethics Committee as well as from the local District Health Board in Waikato.

20. Publication plan

The results of the presented studies might be published as posters, conference presentations, articles in peer reviewed journals, and/ or as part of a PhD thesis.