OBSTETRICS

Evaluation of fetal exposure to external loud noise using a sheep model: quantification of in utero acoustic transmission across the human audio range

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BACKGROUND: There is mounting evidence that neural memory traces are formed by auditory learning in utero and that premature newborns are particularly sensitive to the intense, sustained noises or impulses sounds associated with the use of intensive care equipment. One area of critical importance is the determination of sound level exposure in utero associated with maternal occupation. The attenuation factors provided by the abdomen and tissue as well as the routes by which the inner ear receives stimulation need careful consideration and investigation to provide prenatal protection from external sound levels and frequencies that may cause harm.

OBJECTIVE: To measure how sound from external sound sources is transmitted to the fetus inside the uterus of a pregnant sheep in 6 Hz frequency steps between 100 Hz and 20 kHz (ie, across most of the human audio range).

STUDY DESIGN: We measured acoustic transfer characteristics in vivo in 6 time-mated singleton pregnant Romney ewes (gestational age, 103—130 days, weight, 54—74 kg). Under general anesthesia and at hysterotomy, a calibrated hydrophone was attached to the occiput of the fetal head within the amniotic sac. Two calibrated microphones were positioned in the operating theater, close to the head and to the body of each ewe. Initial experiments were carried out on 3 pregnant ewes 3 days after transport recovery to inform the data acquisition protocol. This was followed by detailed data acquisition of 3 pregnant ewes under general anesthesia, using external white noise signals. Voltage signals were acquired with 2 calibrated microphones, located near the head and the body of each ewe and with a calibrated hydrophone located in the amniotic fluid.

RESULTS: Measurement of acoustic transmission through the maternal abdominal and uterine walls indicates that frequency contents above 10 kHz are transmitted into the amniotic sac and that some frequencies are attenuated by as little as 3 dB.

CONCLUSION: This study provides new data about in utero sound transmission of external noise sources beyond physiological noise (cardiovascular, respiratory, and intestinal sounds), which help quantify the potential for fetal physiological damage resulting from exposure to high levels of noise during pregnancy. Fine-frequency acoustic attenuation characteristics are essential to inform standards and clinical recommendations on exposure of pregnant women to noise. Such transfer functions may also inform the design of filters to produce an optimal acoustic setting for maternal occupational noise exposure, use of magnetic resonance imaging during pregnancy, and for neonatal incubators.

Key words: fetal auditory system, fetal ear, fetal sound exposure, hearing damage, in utero acoustics, noise pollution, occupational noise, pregnancy, prenatal sound exposure, teratology

Anatomical studies have shown that the cochlea and peripheral sensory-end auditory organs of the human fetus are fully formed by 24 weeks of gestation.[1] Maturation of the auditory pathways of the central nervous system develop from the beginning of the third trimester of pregnancy, with significant activation to sound seen in the left temporal lobe of the fetal brain, con-firming that sound processing occurs beyond the reflexive subcortical level.[2] Ultrasonographic observations of blink-startle responses to vibroacoustic stimulation are first elicited from 24 weeks of gestation and are consistently present after 28 weeks,[1] whereas maternal voice recognition in utero appears between 33 and 34 weeks of gestation.[3]

There is mounting evidence that neural memory traces are formed by auditory learning in utero, and that auditory change detection is crucial for the development of the auditory system.[4,5] These development changes start from 27 to 28 weeks of gestation and are a prerequisite for language development.[4,5]

Interpretation of acoustic and linguistic information on intrauterine recordings suggests that the prosodic features of speech (pitch contours, rhythm, and stress) are detectable by the fetus. Extensive prenatal exposure to a melody or to specific human speech induces neural representations that last for several months[4,5] and may contribute to language acquisition during the first year after birth. The hearing threshold or intensity at which neonates perceive sound at 27—29 weeks of gestation is approximately 40 dB,[6] decreasing to a nearly adult level of 13.5 dB by 40—42 weeks of gestation, indicating continuing postnatal maturation of acoustic neural pathways.

The fetus is exposed in-utero to physiological auditory stimuli that are both internally derived, such as the maternal heartbeat, voice, and other body sounds, and external sounds crossing the different tissue layers of the maternal abdomen.[7] Sound is transmitted easily into the uterine

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AJOG at a Glance

Why was this study conducted?
There are no prior studies providing fine frequency measurement data of in utero acoustic attenuation characteristics in the spectral width of the audio range, between 100 and 20 kHz.

Key findings
Measurement of sound attenuation in the uterus of pregnant ewes shows that, at specific frequencies and relative to a given microphone location, the external noise source is attenuated by as little as 3 dB through the abdominal wall and uterine cavity.

What does this add to what is known?
Measurement of the attenuation of acoustic sources through the abdominal wall indicates that significant frequency content above 10 kHz is transmitted inside the uterus, suggesting that chronic loud noise exposure could have an impact on fetal development.

environment.[8] In fetal sheep, exposure to intense broadband noise altered the fetal auditory brain stem response and damaged cochlea hair cells.[9] Noise levels over 100 dB, which are capable of damaging cochlea hair cells, have been recorded in adult intensive care units.[10]

Excessive noise is implicated in intensive care psychosis, increased pain sensitivity and high blood pressure, poor recovery, and higher risk of readmission. In 1997, the American Academy of Pediatrics Committee on Environmental Health expressed concern about the effect of high noise levels on the fetus and newborn and recommended further research.[11]

Fetal exposure to artificial environment noise has risen dramatically in the second half of the 20th century, particularly in urban areas. A systematic review using the World Health Organization environmental noise guidelines for the European region found environmental noise, specifically aircraft and road traffic noise to be associated with, and adverse birth outcomes including preterm birth and low birthweight.[12]

The quality of the evidence however, was found to be low, especially in older studies because of limitations with the recording technology. One of the most prevalent types of noise exposure in working women is occupational noise. A Swedish nationwide cohort study has shown an association between occupational noise during pregnancy and hearing dysfunction in children.[13]

A more recent study by the same authors has also shown that full-time exposure to high levels of occupational noise during pregnancy is associated with slightly reduced fetal growth but not with preterm birth.[14] The effect of intermediate occupational noise exposure (75–85 dBA) showed a small but statistically increased risk for all studied birth outcomes, strengthening the evidence that pregnant women should not be exposed to a long-term exposure to levels >85 dBA of occupational noise during pregnancy. Other new sources of high sound levels for fetuses include magnetic resonance imaging (MRI), music concerts, and fetal stimulation musical devices.

Previous experiments in sheep and goats have indicated that intrauterine noise is predominantly low frequency,[15] whereas energy above 0.5 kHz is attenuated by 40–50 dB.[8,16,17] Sheep experiments have also indicated that sounds in the environment of a pregnant woman penetrate the tissues and fluids surrounding the fetal head and stimulate the inner ear through a bone conduction route.[8]

These data are limited by the quality of recording technology and access to computer models enabling the translation of animal data into human data. The aim of our study was to use state-of-the-art data acquisition systems and calibrated instrumentation to measure the in utero acoustic transfer characteristics on pregnant ewes to help quantify the potential for fetal auditory damage resulting from exposure to high levels of noise during pregnancy.

Materials and Methods
Two sets of experiments were carried out, each involving 3 time-mated pregnant Romney ewes (formally called Romney March sheep), weight range of 54–74 kg carrying 103–130 days of gestation singleton fetuses, supplied by the Royal Veterinary College (Hertfordshire, United Kingdom).

All procedures on animals were conducted in accordance with UK Home Office regulations and the Guidance for the Operation of Animals (Scientific Procedures) Act (1986). Ethics approval was provided by the Animal Studies Committees of the Royal Veterinary College and the University College London, United Kingdom (Study reference number 2014-N-0050).

General anesthesia was induced with thiopental sodium 20 mg/kg[-1] intravenously (Thiovet; Novartis Animal Health UK Ltd, Hertfordshire, United Kingdom) and after intubation, animals were maintained with 2–2.5% isoflurane in oxygen (Isoflurane-Vet; Merial Animal Health Ltd, Essex, United Kingdom) as previously described.[18] The number of fetuses and gestational age were confirmed using ultrasound examination of fetal size according to standard measurements.[19]

The abdomen was sheared, cleaned with povidone iodine, and draped. Laparotomy was performed using a midline incision below the umbilicus and the abdomen was opened in layers. In an area of myometrium away from placentomes, a 5 cm uterine incision was performed over the fetal head, which was then exteriorized through the uterine incision.[20]

Babcock clamps were used to compress the amniotic membrane against the uterine wall to minimize bleeding and reduce amniotic fluid loss. A calibrated hydrophone was sutured to the occiput of the fetal head, and cabling was secured to the posterior aspect of the fetal neck

using 2.0 Prolene (Ethicon, Cincinnati, OH). The distance between the hydrophone and the maternal skin was measured with ultrasound at 5—7 cm in all cases. This hydrophone features a flat frequency response (±1 dB) between 100 Hz and 20 kHz (Appendix 1). The uterine incision was closed in 2 layers as described.[20]

The cabling from the hydrophone was then exteriorized onto the ewe's right flank and secured to the skin. Antibiotics for infection prophylaxis and analgesia were given to the ewe at the end of the procedure as previously described.[20] The abdomen was then closed in layers.

In the first set of experiments, 1 of the ewes was allowed to recover and sound experiments were conducted 3 days after transport in an open stable area. A companion sheep was always placed in the same stable area as the awake experimental animal. In the second experiments, the ewes were maintained under general anaesthesia until the end of the experiment. When sound experiments were completed, animals were killed with an overdose of Pentobarbitone (Thiovet; Novartis Animal Health UK Ltd, Hertfordshire, United Kingdom).

The preliminary experiments investigated whether the study carried out by Gerhardt et al[8] could be replicated using modern hydrophones and digital recording technology. The preliminary experiments (Appendix 2) were needed to overcome the challenges of positioning the hydrophone inside the amniotic sac and were of a more qualitative nature than the main experiments (Appendix 3). The experimental setup is shown in Figure 1.

The waveform audio file format files corresponding to the hydrophone and microphone output voltage signals were imported into MATLAB (MathWorks, Natick, MA) for signal processing. The methodology used to estimate the attenuation resulting from the maternal tissues and fluid is based on a transfer function estimate, whereby the frequency content of the hydrophone signals is compared with that of the microphone signals and frequency domain transfer characteristics derived. This data-processing protocol is described in Appendix 3.

FIGURE 1
Data acquisition setup involving measurement of in utero sound attenuation
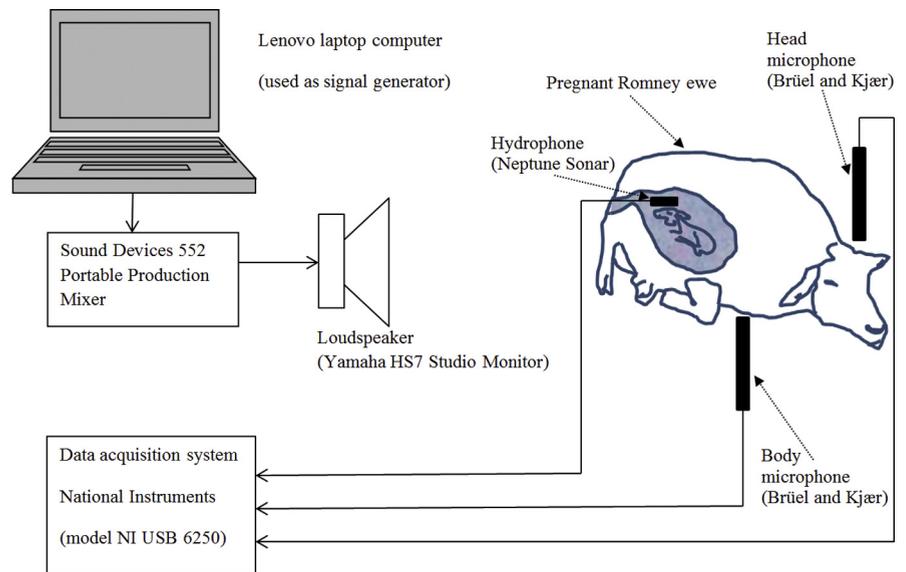
Diagram showing the data acquisition setup involving the measurement of in utero sound attenuation in pregnant Romney ewes. An acoustic source was placed in the room and excited with bursts of white noise, generating spectral content between 100 Hz and 20 kHz. The sound was detected by the 2 microphones and the 1 hydrophone in the amniotic cavity and data transferred via cables to the computer.

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Results
Microphone and hydrophone signals

Figure 2 (A and B) shows the microphone and hydrophone white noise excitation acoustic pressure signals obtained on ewe 2 for the sixth repeat of the main experiments, respectively. Calibration correction factors were applied. The signals were de-trended and then filtered with a bandpass filter with upper and lower —3 dB cutoff points at 100 Hz and 25 kHz, respectively.

From the acoustic pressure data displayed in Figure 2A, the sound pressure level (SPL) for each white noise burst was calculated using the root mean square pressure value. On this particular repeat, a logarithmic average of the 20 SPL values was found to be 106 dB at the head microphone location and 107 dB at the body microphone location.

The ambient SPL inside the operating theater, calculated from a logarithmic average of 4 repeat measurements each of 98 seconds duration, with the loudspeaker switched off, was found to be 72

dB at the head microphone location and 77 dB at the body microphone position. The background noise recorded by the amniotic sac hydrophone for corresponding measurements was found to be 96 dB.

Because physiological noise (breathing, digestive noises, etc) was reported by Gerhardt et al[8] to be of the order of 50 dB, it is likely that this noise is a result of electromagnetic interference. This was indeed confirmed by looking at the spectral content of the signal. A logarithmic average of the SPL during the white noise excitation intervals was obtained as 102 dB. Assuming that the radio frequency noise is not coherent with the white noise excitation signal, the SPL inside the amniotic sac is therefore 101 dB (ie, only 5—6 dB lower than at the 2 operating theater microphone locations).

Sound transmission inside the uterus

For each repeat measurement, 20 transfer functions estimates were obtained (ie, one for each white noise burst [see

FIGURE 2
External microphone and intrauterine amniotic sac hydrophone output signals
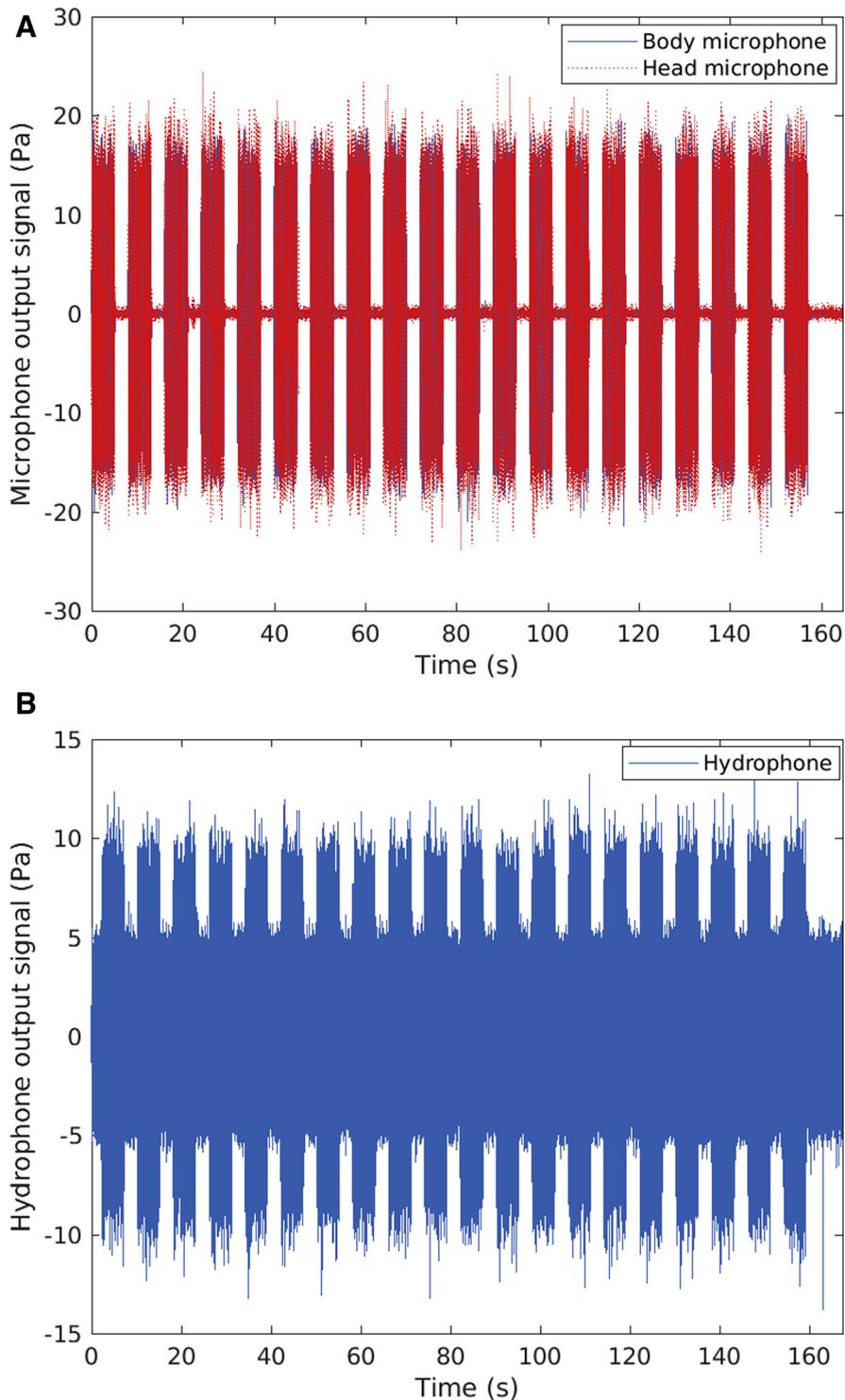
A, Microphone output signals resulting from white noise burst excitation signal for ewe 2 (main experiment). B, Amniotic sac hydrophone output signal resulting from white noise burst excitation signal for ewe 2. All signals are corrected for calibration factors.

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Appendix 3]). Figure 3 (A and B) shows the microphone to hydrophone transfer function estimates as a function of frequency between 100 Hz and 20 kHz. The mean together with the mean μ plus or minus the SD σ is plotted, based on the data set comprising 80 transfer function estimates from the 4 repeat measurements. The procedure previously described was repeated to derive the corresponding microphone and hydrophone output signals for ewe 3.

From the data in Figure 4A, the logarithmic average of the SPL for the 20 white noise bursts was found to be 107 dB for the body microphone and 109 dB for the head microphone. The background SPL in absence of any loudspeaker excitation was found to be 72 dB at the body microphone locations and 77 dB at the head microphone location. These figures are comparable with those recorded with ewe 2 inside the operating theater.

The hydrophone inside the amniotic sac recorded a logarithmic average SPL of 103 dB over the 20 white noise bursts. The background noise recoded by the amniotic sac hydrophone for was found to be 98 dB. Hence, the SPL recorded by the hydrophone is 102 dB when removing contribution from the noise, assuming that the latter is not coherent with the loudspeaker signal. This indicates that the SPL inside the amniotic sac is therefore only 5−7 dB lower than at the 2 operating theater microphone locations. Again, this result is comparable with that obtained for ewe 2. Following the same procedure as for ewe 2, the transfer function estimates are displayed in Figures 5A and 4B, for both microphone locations.

Comment
Principal findings
Acoustic transfer characteristics measured on 2 pregnant Romney ewes between 100 Hz and 20 kHz at 6 Hz intervals reveal that significant transmission of external noise sources occurs across much of the audio range. In 1 ewe, a hydrophone inserted inside the amniotic sac recorded a 4 dB attenuation between 200 Hz and 400 Hz,

FIGURE 3
Body and head microphones to amniotic sac hydrophone transfer characteristics
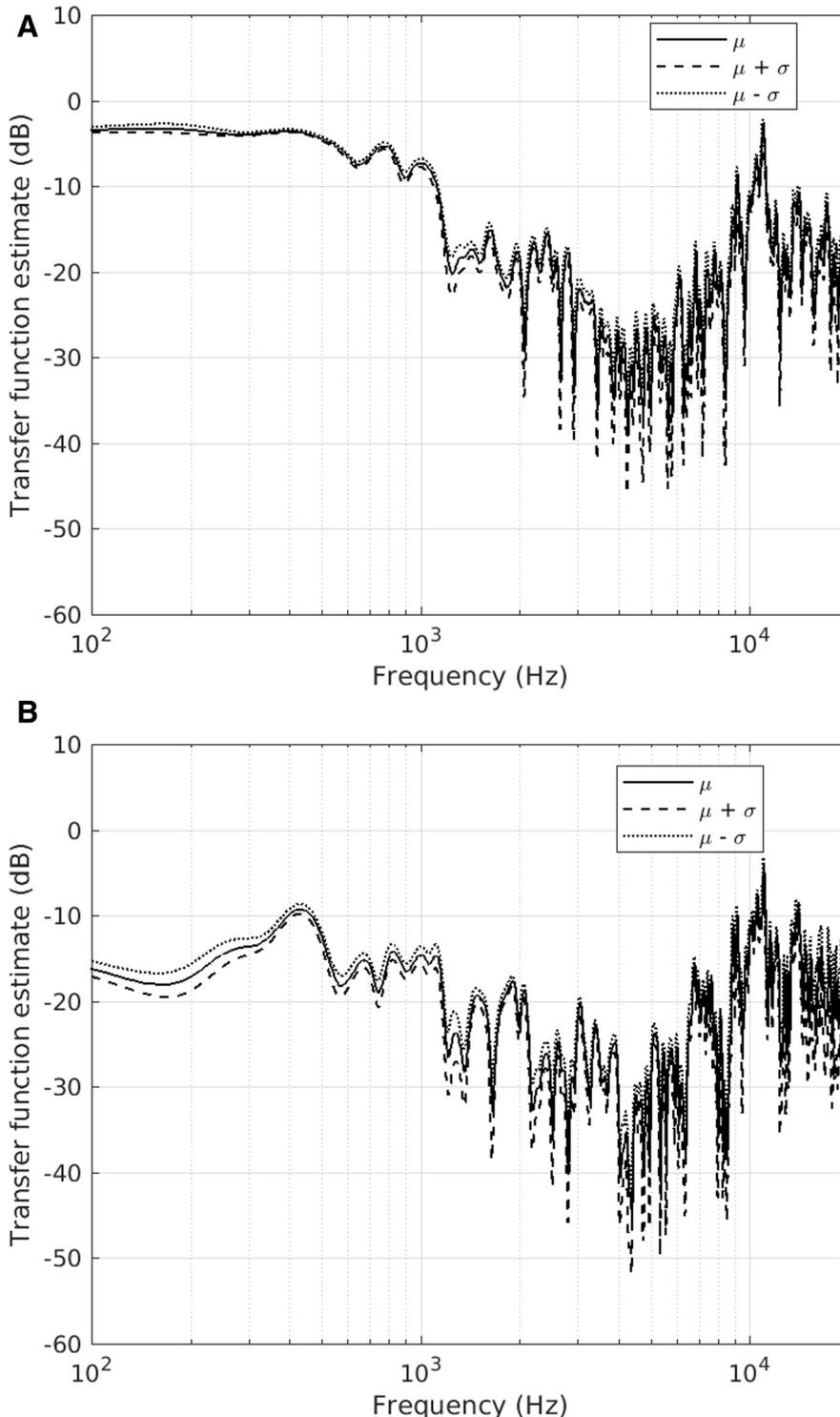
Recording traces showing **A,** Body microphone to amniotic sac hydrophone transfer characteristics for ewe 2 (main experiments). **B,** Head microphone to amniotic sac hydrophone transfer characteristics for ewe 2 (main experiment).

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with respect to a microphone inside the operating theater, placed near the body of the ewe. A common increase in the transfer characteristics above 8 kHz was observed in both ewes.

Strengths and limitations
A key strength of our study is that the excitation protocol uses a broadband loudspeaker to reproduce segments of white noise (ie, signal of equal intensity at different frequencies). This enabled us to evaluate with fine frequency resolution the transfer of sound functions throughout most of the human audio range, from microphones located inside the operating theater to a hydrophone located inside the amniotic cavity next to the fetal head. Furthermore, the use of the H_1 estimator (see Appendix 3) helps remove contamination from uncorrelated output noise, helping to overcome the poor signal-to-noise ratio of the hydrophone signals.

Differences in attenuation response trends are reported, between animals, which are likely to be due to both variations in anatomy and in acoustic tissue properties.

It is possible that such behaviour results from internal acoustic modes that are specific to the animal. We also found that the microphone location may have an impact on the overall transfer function estimate (Figures 3 and 5). This is likely to be due to the complex acoustic environment inside the operating theater.

Reflections on surfaces within the operating room together with acoustic resonances will have an impact on the transfer function estimate. Ideally, such experiments would be better carried out in an anechoic room, but this an undertaking would be technically very challenging. The acoustic transfer characteristics between the loudspeaker and the microphone vary depending on the position of the microphone inside the room and the animal position on the operating table.

The existence of antiresonances in the transfer functions in some cases may be due to room modes that may be picked up more prominently at 1 microphone

FIGURE 4
Microphone output and intrauterine amniotic sac hydrophone signals obtained for ewe 3
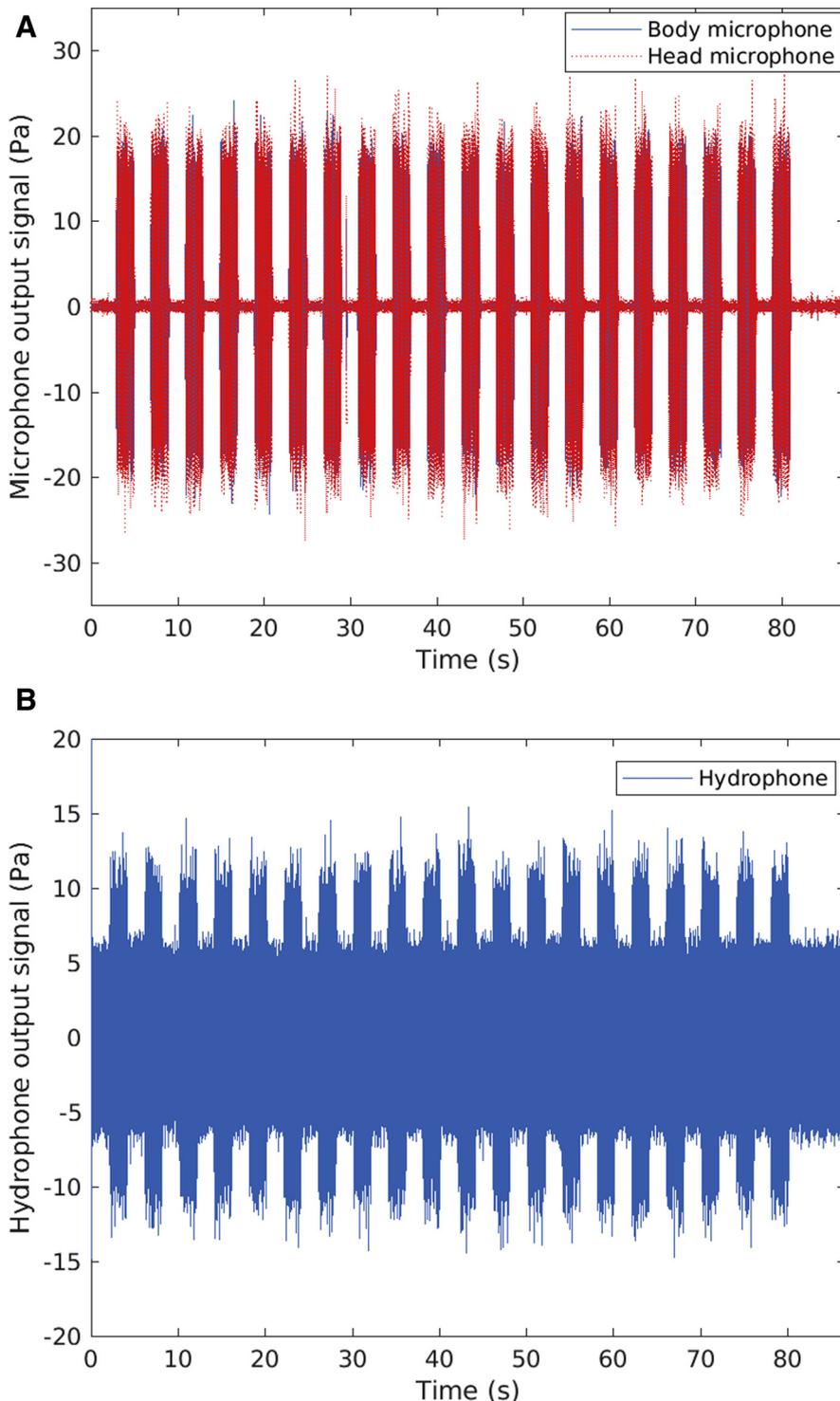
Recording traces showing **A,** Microphone output signals resulting from white noise burst excitation signal for ewe 3 (main experiment). **B,** Amniotic sac hydrophone output signal resulting from white noise burst excitation signal for ewe 3 (main experiment). All signals are corrected for calibration factors.

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position rather than another. For example, it is likely that the trough at around 750 Hz in Figure 3B results from a room resonance that is more prominent at the location of the maternal head microphone than that of the body microphone.

Results in context

Like previous authors, we have used the sheep model because of the similarities between the sheep and human fetus in size and uterus at term. Previous in vivo experiments in the near-term ewe have indicated that of the external airborne sounds, only the low-frequency (below 0.3 kHz) components reach the uterine cavity, and that frequencies above 0.5 kHz are attenuated by 40—50 dB.[7,8] Overall, previous studies show that low-frequency sound energy easily penetrates to the fetal head, with less than 5 dB attenuation for frequencies below 0.5 kHz, whereas higher frequencies are attenuated by up to 20—30 dB.

The sound energy in amniotic fluid stimulates fetal hearing through a bone conduction route rather than through the external and middle ear systems.[21] Intrauterine sound recording in humans during labor after membrane rupture showed that low-frequency sounds (0.125 kHz) generated outside the mother were enhanced by an average of 3.7 dB.[22] There is a gradual increase in attenuation for increasing frequencies, with a maximum attenuation of 10.0 dB at 4.0 kHz, but the interpretation of these data is difficult in the absence of amniotic fluid around the fetus.

The results of the present study indicate that at specific frequencies, the abdominal wall together with overlying tissue and the amniotic fluid provide as little as 2—3 dB acoustic attenuation of external airborne sound inside the uterine environment at frequencies below 1 kHz, confirming the results of previous studies.[7,8] Above 1 kHz, the attenuation increases to 20—40 dB, depending on the animal and position of the external microphone. Above 10 kHz, the improved measurement protocol used in this study reveals that significant external

FIGURE 5
Microphone to intrauterine amniotic sac hydrophone transfer characteristics obtained for ewe 3
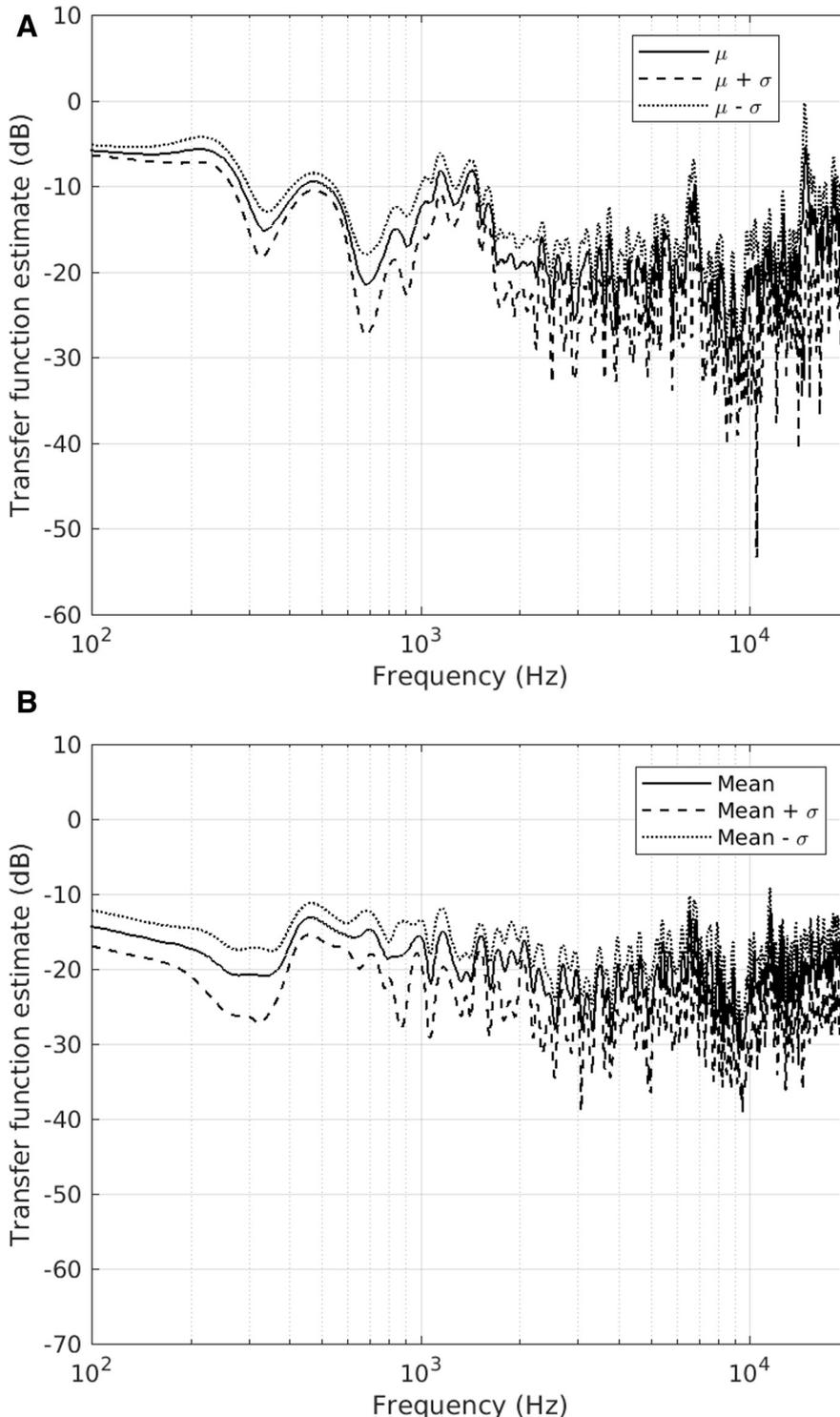
Recording traces showing **A,** Body microphone to amniotic sac hydrophone transfer characteristics for ewe 3 (main experiment). **B,** Head microphone to amniotic sac hydrophone transfer characteristics for ewe 3 (main experiment).

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sound is transmitted in utero and that attenuation is as little 3 dB at 11 kHz in one ewe.

These results are in accordance with those of Lecanuet et al,[23] who compared the characteristics of sound transmission between a similar sheep model and a nonbiological plain rubber sphere and support the hypothesis that internal resonances are responsible for the increase in in utero sound transfer characteristics at frequencies above 10 kHz.

Research implications

The possible fetal side effects associated with MRI include temperature increase because of radio frequency wave exposure, biological effects of a strong magnetic field, and acoustic noise from time-variant gradients.[24] A large controlled study including 1737 early pregnancies has shown that exposure to MRI without gadolinium contrast during the first trimester of pregnancy compared with nonexposure is not associated with an increased risk of congenital anomalies, neoplasm, or hearing loss from birth to age 4 years; however, the risk of vision loss was higher for exposure between 5 and 10 weeks of gestation.[25]

Prenatal exposure to MRI during the second or third trimester of pregnancy in a small cohort of 72 uncomplicated pregnancies is not associated with disturbances in functional outcomes or hearing impairment at preschool age.[26,27] These studies are retrospective and thus limited in the evaluation of actual noise fetal exposure.

Music devices designed for fetal acoustical stimulation have become increasingly popular over the last decade. Based on the assumption that playing music to the unborn fetus will enhance its cognitive abilities throughout its life.

Jahn et al[28] have recently evaluated the sound characteristics of 3 different music devices and have shown that sound pressure levels are hardly detectable under attenuated conditions. Their measurements were obtained in open air,

using a pork uterus of 5 mm thickness covered by abdominal porcine tissue, which is very different from the in utero experimental conditions of the present study. We agree with Jahn et al[28] that there is no evidence that. isolated auditory stimulation using loudspeakers with poor sound characteristics, may support the development complex multimodal maturation of the fetal sensory system.

However, our data, obtained in a physiological uterine environment, indicate that some frequencies are attenuated by as little as 3 dB by the maternal abdominal and uterine wall. In addition, unlike the acoustic noise exposure associated with a regular 20—60 min MRI examination, music devices for fetal sound simulation are often used for several hours per day therefore exposing the fetus to nonphysiological sound. These findings highlight the need for further research into the potential negative impact of these devices, particularly when used internally.

Music devices have also been used in neonatal intensive care units with evidence that their use has positive short- and long-term impact on preterm infant cognitive and emotional development.[28–31] However, a recent review[25] has shown that all recordings of maternal voice at sound levels are above neonatal intensive care units acceptable levels of 45dB, recommended by the American Academy of Pediatrics, and that few of the findings on the positive effects of these recordings reached statistical significance.

The integration of the in vivo data of the present study into a computerised model together with available anatomical human data should enable us to develop a sound filter representative of the in utero physiological environment, which may then be integrated in a prototype womb-like incubator.

Conclusions
The results of the present study highlight the importance of acoustic studies during pregnancy and support the epidemiological evidence that pregnant women should not be exposed long term

to high-level occupational noise. New sources of nonphysiological external loud noise of fetal exposure during pregnancy include those produced by medical equipment such as MRI and by musical devices designed for fetal acoustical stimulation. The latter are often used daily for many hours by parents-to-be without any evidence of benefit for the fetal development but with potential harm associated with chronic exposure to high-level noise. ∎

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Appendix 1. Glossary of acoustic terminology.

Acoustic pressure is the difference between the pressure caused by a sound wave and the ambient pressure of the media the sound wave is passing through. It is usually measured in Pascal (Pa).

Audio Range the human ear can respond to minute sound pressure variations in the air if they are in the frequency range of roughly 20 Hz to 20 kHz. In air, this corresponds to a wavelength of 17 m to 0.017 mm.

Bandpass filter is a device that passes frequencies within a certain range and rejects (attenuates) frequencies outside that range.

Frequency is the number of occurrences of a repeating event per unit of time. It is usually measured in Hertz (Hz).

Hydrophone is a microphone designed to be used underwater for recording or listening to underwater sound. Most hydrophones are based on a piezoelectric transducer that generates an electric potential when subjected to a pressure change, such as a sound wave.

Resonance in acoustic, mechanical and electrical is a phenomenon that occurs when the frequency at which a pressure, force or voltage is periodically applied, is equal or nearly equal to one of the natural frequencies of the system on which it acts. This causes the system to oscillate with larger amplitude than when the force is applied at other frequencies.

Root mean square of a time-varying quantity is obtained by squaring the amplitude at each instant, obtaining the average of the squared values over the interval of interest, and then taking the square root of this average.

Sound pressure level is 20 times the decimal logarithm of the ratio of a given sound pressure to a reference sound pressure in decibels is 20 times the logarithm to the base ten of the ratio. The reference sound pressure is 20 μPa for airborne sound. It is usually expressed in decibels (dB). 40 dB corresponds to the sound pressure level in a library whereas typical sound pressure levels at a rock concert are around 110 dB. Sound pressures are understood to be expressed as root mean square (RMS) values.

Transfer characteristic describes how acoustic signals are modified by any structure or medium they encounter. This is generally expressed as 20 times the logarithmic ratio of the acoustic pressure at the receiver location and at the source location.

White noise is a type of noise whose power spectral density is essentially independent of frequency. It is commonly used as an excitation signal to measure a transfer characteristic as a function of frequency, since it can be thought of as containing "every frequency".

Windowing is a technique used to reduce spectral leakage by multiplying the time domain waveform by a window function. The process of windowing reduces the amplitudes of discontinuities at the edges of a waveform, thereby reducing the spectral leakage.

Appendix 2. Preliminary experiments in pregnant ewes to establish proof-of-concept.

A hydrophone (50 mm length and 9.5 mm diameter) was attached to a 3 m shielded Teflon cable. The hydrophone and amplifier were calibrated with a piston phone (B&K model 4223). Calibration signals of the hydrophone and microphone were recorded on tape to assure accuracy of sound pressure during playback. Acoustic signals were recorded with B&K a miniature hydrophone (model 8103), amplified (B&K model 2635 amplifier) and recorded (B&K FM tape recorder model 7005). The outputs from the sound level meter, located 50 mm directly above the back of each of the three Romney ewes and from the implanted hydrophone, were continuously recorded on two channels of the recorder. A separate channel was available for comments by the experimenter. Recordings were typically made for up to four hours. A place for tethering the ewe was chosen in a low-reflection acoustic environment in an open stable area. The output and direction of two matched speakers located 2 m distant were carefully adjusted to produce a uniform sound pressure level (+3 dB) in the space occupied by the ewe.

A range of audio material was used to excite the loudspeakers with. This included:

- Comparisons of room ambient noise − frequency responses to low level sound stimulus before the test recordings are played.
- Comparisons of frequency responses to white noise.
- Comparisons of frequency responses to the bleating of the ewe. This was chosen to simulate how the "mother's voice" is present inside and outside the womb.
- Analysis of moments when the internal sounds of the ewe (heartbeat etc.) are present and audible.

The analysis focused on one second bursts of sound during each of the events outline above. It should be noted that the hydrophone data which was acquired was not absolute, in that calibration factor of the hydrophone was not taken into account. These experiments were carried out with a primary aim of ensuring that signal inside the womb of each ewe could be acquired as a result of the loudspeaker excitation. Thus far, nearly all the literature suggests that external sounds are attenuated incrementally from around 200 Hz to an approximate 40 dB attenuation at 10 kHz. One study suggests that above around 12 kHz some frequencies are increased again.[23] The results from these preliminary experiments support previous evidence of a general attenuation from around 500 Hz to 1 kHz up to 10 kHz and beyond. In this study, attenuation ranges from 0−10 dB at around 1 kHz to up to 30 dB at 15 kHz. Some of the frequency responses charts also display an increase in frequency at around 15 kHz.[23]

Appendix 3. Measurement set-up and protocol used in main experiments on three pregnant ewes.

The sound source consisted of a Yamaha HS7 studio monitor, which was positioned on a stand in the operating theatre approximately 2 m from the ewes. This source is a two-way bi-amp powered studio monitor and was chosen for its flat frequency response between 43 Hz and 20 kHz (-3 dB). Additionally, the loudspeaker is able to generate sound pressure levels in excess of 100 dB re 2×10^{-5} Pa 1 m from the source, thus helping to overcome potential low in utero signal-to-noise ratios and to help overcome any physiological noise which may contaminate the transfer function measurements. Two calibrated Brüel and Kjær microphones were positioned inside the operating theatre: close to the head (condenser 0.5 inch cartridge type 4133) and near the body (free-field 0.5 inch type 4190) of each ewe. Both microphones feature a flat frequency response between 20 Hz and 20 kHz (-1 dB). The loudspeaker was connected to a Lenovo laptop computer via a Sound Devices 552 Portable Production Mixer. For ewes 1, 2 and 3, the excitation protocol consisted of 26 pure tone signals of frequency 39 Hz to 12 kHz, in third octave bands. For ewes 2 and 3, an additional excitation protocol was used, which featured 20 bursts of white noise. On ewe 2, each burst was of 5 s duration and interspaced by 3 s of silence. On ewe 3, due to time constraints, the duration of the white noise burst was reduced to 2 s, with 2 s of silence in between bursts. Four repeats of the pure tone excitation protocol were carried out on ewes 1 and 2 and two repeats on ewe 3. Four repeats of the white noise excitation protocol were carried out on ewes 2 and 3, thus a total of 80 white noise bursts. The inconsistency of the experimental protocol was essentially the result of time constraints associated with the availability of the operating theatre and associated staff. All time series waveforms were generated using Matlab and exported as WAV files, with a 96 kHz sampling frequency and a 24 dB dynamic range. The WAV files were subsequently imported into the Audacity digital audio workstation and MIDI sequencer software for playback.

All microphones and the Neptune Sonar D/140 spherical transducer hydrophone were connected to a National Instruments (model NI USB 6250) data acquisition system. All hydrophone and microphone signals were acquired using a sampling frequency of 100 kHz and with a dynamic range of 24 dB and were subsequently exported as WAV files.

In order to gain a further understanding of how effectively various sounds are transmitted into the abdomen, it is of interest to estimate the transfer characteristics as a function of frequency. Whilst the contamination of the output signal by uncorrelated noise may appear problematic in this context, there exist established signal processing tools which can help overcome this. If the output noise is uncorrelated with the input signal, a transfer function estimate can be obtained based on calculating the quotient of the cross power spectral density of the signals and the power spectral density of the input signal. These quantities may be calculated using Welch's averaged, modified periodogram method. In the presence of output noise uncorrelated with the input signal, the H_1 estimator option may be used within the Matlab *tfestimator* function. The relevant segments of the signals were selected by correlating the hydrophone and microphone voltage waveforms. A 1024 point Hanning window was used, with no overlap between the adjoining segments. 2^{14} sampling points were used to compute the discrete Fourier transforms.