

Evaluation of fetal exposure to external loud noise using a sheep model

Pierre GÉLAT, PhD, Anna L DAVID, MD, PhD, Seyyed Reza HAQHENAS, PhD,
Julian HENRIQUES, PhD, Aude THIBAUT de MAISIERES, MSc, Tony WHITE, MD,
PhD and Eric JAUNIAUX, MD, PhD, FRCOG

From the Department of Mechanical Engineering, University College London, London,
UK (Drs Gélát and Haqshenas); Department of Media, Communications and Cultural
Studies, Goldsmiths, University of London, London, UK (Pr Henriques); Sonic Womb
Productions Limited, London, UK (Mrs Thibaut de Maisieres); EGA Institute for
Women's Health, Faculty of Population Health Sciences, University College London,
London, UK (Prs Jauniaux and David), The Royal Veterinary College, London, UK (Mr
White)

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Corresponding author: Eric Jauniaux, MD, PhD, FRCOG e.jauniaux@ucl.ac.uk

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7 **Condensation:** Measurement of in utero acoustic attenuation in fetal sheep across the
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9 audio range (100Hz–20kHz, at 6Hz intervals) revealed significant transmission of
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11 external noise sources.
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16 **Short version of title:** Evaluation of fetal exposure to external sound sources
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21 **AJOG at a glance:**
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24 **A.** There are no prior studies providing fine frequency measurement data of in utero
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26 acoustic attenuation characteristics across the audio range, between 100 Hz and 20
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28 kHz.
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31 **B.** Measurement of sound attenuation in the uterus of pregnant ewes shows that, at
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33 specific frequencies and relative to a given microphone location, the external noise
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35 source is attenuated by as little as 3 dB through the abdominal wall and uterine cavity.
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38 **C.** Measurement of the attenuation of acoustic sources through the abdominal wall
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40 indicates that significant frequency content above 10 kHz is transmitted inside the
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42 uterus.
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45 **D.** The measured transfer functions were used to determine an impulse response function
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47 which was convolved with a music sample to provide a qualitative representation of
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49 the degree of in utero attenuation across the audio range.
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Abstract

Background: There is mounting evidence that hearing develops mainly in the third trimester of pregnancy 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 levels within the womb and the fetal ear. Non-auditory effects could result in mechanical damage to the fetus as a result of noise exposure. 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 protection from the sound levels and frequencies at which harm may be caused.

Objective: To measure the in utero acoustic transfer characteristics on a fetal sheep model in fine frequency steps between 100 Hz and 20 kHz, i.e. across most of the human audio range.

Study Design: We measured acoustic transfer characteristics in vivo in six time-mated singleton pregnant Romney ewes (gestational age 103–130 days, weight 54–74 kg). Under general anaesthesia 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 theatre, close to the head and to the body of each ewe. Initial experiments were carried out on three pregnant ewes three days after transport recovery, to inform the data acquisition protocol. This was followed by detailed data acquisition of three pregnant ewes under general anaesthesia, using external white noise signals. Voltage signals were acquired with two calibrated microphones, located near the head and the body of each ewe and with a calibrated hydrophone located in the amniotic fluid.

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4 Acoustic attenuation values were obtained from transfer functions estimated using
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7 Welch's averaged modified periodogram method on the deconvolved microphone and
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9 hydrophone signals.

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11 **Results:** Measurement of acoustic attenuation through the abdominal wall indicates that
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13 significant frequency content above 10 kHz is transmitted into the amniotic sac, and that
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15 some frequencies are attenuated by as little as 3 dB. The measured transfer functions
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17 were obtained throughout the human audio range in of 6 Hz steps.
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21 **Conclusion:** This study provides key information about in utero sound transmission of
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23 external noise sources, beyond physiological noise (cardiovascular, respiratory, and
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25 intestinal sounds). It demonstrates the requirement for fine frequency acoustic attenuation
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27 characteristics to be obtained to inform standards and clinical recommendations on
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29 exposure of pregnant women to noise. Such transfer functions may also inform the design
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31 of filters to produce an optimal acoustic setting for occupational noise exposure and for
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33 neonatal incubators.
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40 **Key Words:** in utero acoustics, convolution, fetal auditory exposure, fetal sheep
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Introduction

Human hearing develops mainly in the third trimester of pregnancy. Anatomical studies have shown that the cochlea and peripheral sensory-end auditory organs of the human fetus are fully formed by 24 weeks.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, confirming that sound processing occurs beyond the reflexive sub-cortical 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 weeks¹ whereas maternal voice recognition in utero appears between 33 and 34 weeks of gestation.³

Auditory change detection is crucial for the development of the auditory system and 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 dB⁶, decreasing to a nearly adult level of 13.5 dB by 40-42 weeks of gestation, indicating continuing postnatal maturation of acoustic neural pathways.

During pregnancy, the fetus is exposed both to internal sounds, such as the maternal heartbeat, voice and other body sounds, as well as external sounds that cross the different tissue layers of the maternal abdomen.⁷ Fetal exposure to artificial environment

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4 noise has changed dramatically in the second half of the 20th century, in particular in
5 urban areas. Sound is transmitted easily into the uterine environment.⁸ A recent
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7 population based cohort study has shown an association between occupational noise
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9 during pregnancy and hearing dysfunction in children.⁹ In fetal sheep, exposure to intense
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11 broadband noise altered the fetal auditory brain stem response and damaged cochlea hair
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13 cells.¹⁰ Noise levels over 100 dB, that are capable of damaging cochlea hair cells, have
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15 been recorded in adult intensive care units.¹¹ Excessive noise is implicated in intensive
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17 care psychosis, increased pain sensitivity and high blood pressure, poor recovery and
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19 higher risk of readmission. In 1997, the American Academy of Pediatrics Committee on
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21 Environmental Health expressed concern about the effect of high noise levels on the fetus
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23 and newborn and recommended further research.¹²
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31 A systematic review using the World Health Organization (WHO)
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33 environmental noise guidelines for the European region found associations between
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35 environmental noise, specifically aircraft and road traffic noise and adverse birth
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37 outcomes including preterm birth and low birth weight.¹³ The quality of the evidence was
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39 found to be low in particular in older studies due to limitations in the recording
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41 technology. One of the most prevalent types of noise exposure in working women is
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43 occupational noise. A recent Swedish nationwide cohort study has shown that full-time
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45 exposure to high levels of occupational noise during pregnancy is associated with slightly
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47 reduced fetal growth but not with premature birth.¹⁴ The effect of intermediate
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49 occupational noise exposure (75–85 dBA) showed a small, but statistically increased risk
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51 for all studied birth outcomes, strengthening the evidence that pregnant women should
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53 not be subject to a long-term exposure to levels >85 dBA of occupational noise during
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4 pregnancy. Other high sound levels for fetuses include music concerts, environmental
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6 noise and fetal stimulation devices. The latter are of particular concern, as there is a
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8 pervasive myth, supported by a myriad of websites that playing music to your unborn
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10 fetus will enhance its cognitive abilities throughout its life.
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14 Previous experiments in sheep and goats have indicated that intra-uterine noise is
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16 predominantly low-frequency¹⁵, whereas energy above 0.5 kHz is attenuated by 40 to 50
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18 dB.^{8,16,17} Sheep experiments have also indicated that sounds in the environment of a
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20 pregnant woman penetrate the tissues and fluids surrounding the fetal head and stimulate
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22 the inner ear through a bone conduction route.⁸ These data are limited by the quality of
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24 recording technology and access to computer models enabling the translation of animal
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26 data into human data. Amid somewhat inconsistent literature findings, it is critical to
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28 improve upon previous experimental designs to help quantify the potential for fetal
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30 physiological damage resulting from exposure to high levels of noise during pregnancy.
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32 The aim of our study was to use state-of-the-art data acquisition systems and calibrated
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34 instrumentation to measure the in utero acoustic transfer characteristics on pregnant ewes.
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43 **Materials and Methods**

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45 Two sets of experiments were carried out, each involving three time-mated pregnant
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47 Romney ewes carrying singleton fetuses (103–130 days of gestation, weight range 54–74
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49 kg) supplied by the Royal Veterinary College (Hertfordshire, UK). All procedures on
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51 animals were conducted in accordance with U.K. Home Office regulations and the
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53 Guidance for the Operation of Animals (Scientific Procedures) Act (1986). Ethics
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55 approval was provided by the animal studies committees of the Royal Veterinary College
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4 and the University College London, United Kingdom. General anaesthesia was induced
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7 with thiopental sodium 20 mg·kg⁻¹ intravenously (Thiovet; Novartis Animal Health UK
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9 Ltd, Hertfordshire, UK) and after intubation, animals were maintained with 2–2.5%
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11 isoflurane in oxygen (Isoflurane-Vet; Merial Animal Health Ltd, Essex, UK) as
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13 described.¹⁸ The gestational age was confirmed using ultrasound examination of fetal size
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15 according to standard measurements. The abdomen was sheared, cleaned with povidone
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17 iodine and draped. Laparotomy was performed using a midline incision below the
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19 umbilicus and the abdomen was opened in layers. In an area of myometrium away from
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21 placentomes, a 5 cm uterine incision was performed over the fetal head which was then
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23 exteriorized through the uterine incision.¹⁹ Babcock clamps were used to compress the
24
25 amniotic membrane against the uterine wall in order to minimize bleeding and reduce
26
27 amniotic fluid loss. A calibrated hydrophone was sutured to the occiput of the fetal head
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29 and cabling was secured to the posterior aspect of the fetal neck using 2.0 Prolene
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31 (Ethicon, Ohio, USA). This hydrophone features a flat frequency response (± 1 dB)
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33 between 100 Hz and 20 kHz. The uterine incision was closed in two layers as described.¹⁹
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41 The cabling from the hydrophone was then exteriorized onto the ewe's right flank and
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43 secured to the skin. Antibiotics for infection prophylaxis and analgesia were given to the
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45 ewe at the end of the procedure as described¹⁹. The abdomen was then closed in layers.
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4 killed with an overdose of Pentobarbitone (Thiovet; Novartis Animal Health UK Ltd,
5 Hertfordshire, UK). The preliminary experiments investigated whether the study carried
6 out by Gerhardt et al.⁸ could be replicated using modern hydrophones and digital
7 recording technology. The preliminary experiments (Appendix 1) were needed to
8 overcome the challenges of positioning the hydrophone inside the amniotic sac and were
9 of a more qualitative nature than the main experiments (Appendix 2). The experimental
10 setup is shown in Figure 1.
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21 The Waveform Audio File Format (WAV) files corresponding to the hydrophone
22 and microphone output voltage signals were imported into Matlab for signal processing.
23 The methodology used to estimate the attenuation resulting from the maternal tissues and
24 fluid is based on a transfer function estimate, whereby the frequency content of the
25 hydrophone signals is compared with that of the microphone signals and frequency
26 domain transfer characteristics derived. This data processing protocol is described in
27 Appendix 2.
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41 **Results**

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44 Figures 2 (a & b) show the microphone and hydrophone white noise excitation acoustic
45 pressure signals obtained on ewe 2 for the 6th repeat of the main experiments,
46 respectively. Calibration correction factors were applied. The signals were de-trended and
47 then filtered using a 5th order Chebyshev bandpass filter with upper and lower –3 dB cut-
48 off points at 100 Hz and 25 kHz, respectively.
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56 From the acoustic pressure data displayed in Figure 2a, the sound pressure level
57 (SPL) for each white noise burst was calculated using the RMS pressure value. On this
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4 particular repeat, a logarithmic average of the 20 SPL values was found to be 106 dB at
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6 the head microphone location and 107 dB at the body microphone location. The ambient
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8 SPL inside the operating theatre, calculated from a logarithmic average of four repeat
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10 measurements each of 98 s duration, with the loudspeaker switched off, was found to be
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12 72 dB at the head microphone location and 77 dB at the body microphone position. The
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14 background noise recorded by the amniotic sac hydrophone for corresponding
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16 measurements was found to be 96 dB. Since physiological noise (breathing, digestive
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18 noises, etc.) was reported by Gerhardt et al.⁸ to be of the order of 50 dB, it is likely that
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20 this noise is a result of RF interference. This was indeed confirmed by looking at the
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22 spectral content of the signal. A logarithmic average of the SPL during the white noise
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24 excitation intervals was obtained as 102 dB. Assuming that the RF noise is not coherent
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26 with the white noise excitation signal, the SPL inside the amniotic sac is therefore 101
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28 dB, i.e. only 5-6 dB lower than at the two operating theatre microphone locations.
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36 For each repeat measurement, 20 transfer functions estimates were obtained, i.e.
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38 one for each white noise burst (see Appendix 2). Figures 3(a & b) show the microphone
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40 to hydrophone transfer function estimates, as a function of frequency between 100 Hz
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42 and 20 kHz. The mean together with the mean μ plus or minus the standard deviation σ is
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44 plotted, based on the data set comprising 80 transfer function estimates from the four
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46 repeat measurements. The above procedure was repeated to derive the corresponding
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48 microphone and hydrophone output signals for ewe 3.
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53 From the data in Figure 4a, the logarithmic average of the SPL for the 20 white
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55 noise bursts was found to be 107 dB for the body microphone and 109 dB for the head
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57 microphone. The background SPL in absence of any loudspeaker excitation was found to
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4 be 72 dB at the body microphone locations and 77 dB at the head microphone location.
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6 These figures are comparable with those recorded with ewe 2 inside the operating theatre.
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8 The hydrophone inside the amniotic sac recorded a logarithmic average SPL of 103 dB
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10 over the 20 white noise bursts. The background noise recoded by the amniotic sac
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12 hydrophone for was found to be 98 dB. Hence the SPL recorded by the hydrophone is
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14 102 dB when removing contribution from the noise, assuming that the latter is not
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16 coherent with the loudspeaker signal. This indicates that the SPL inside the amniotic sac
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18 is therefore only 5–7 dB lower than at the two operating theatre microphone locations.
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20 Again, this result is comparable to that obtained for ewe 2. Following the same procedure
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22 as for ewe 2, the transfer function estimates are displayed in Figures 5a and 4b, for both
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24 microphone locations.
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33 **Comment**

34 *Main findings*

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39 Acoustic transfer characteristics measured on two pregnant Romney ewes between 100
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41 Hz and 20 kHz at 6 Hz intervals, reveal that significant transmission of external noise
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43 sources occurs across much of the audio range. In one ewe, a hydrophone inserted inside
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45 the amniotic sac recorded a 4-dB attenuation between 200 Hz and 400 Hz, with respect to
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47 a microphone inside the operating theatre, placed near the body of the ewe. A common
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49 increase in the transfer characteristics above 8 kHz was observed in both ewes.
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53 *Strengths and limitations*

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56 A key strength of this study is that the excitation protocol used, involving a broadband
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58 loudspeaker to reproduce segments of white noise, enables the transfer functions from
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4 microphone locations inside the operating theatre to a hydrophone location inside the
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6 amniotic fluid, to be evaluated with fine frequency resolution throughout most of the
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8 human audio range. Furthermore, the use of the H_1 estimator (see Appendix 2) helps
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10 remove contamination from uncorrelated output noise, helping to overcome the poor
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12 signal-to-noise ratio of the hydrophone signals. A key factor not previously studied in
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14 the ovine experiments is the quality of the transmission of the maternal voice to the
15
16 fetus and the interaction between internal and external sounds and their cumulative
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18 effects. That there is significant transmission is supported by the growing evidence that
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20 the third trimester fetus responds to the maternal voice³, and is suggested by the fact
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22 that neonates respond to native language immediately post-birth.²⁰
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28 Differences in attenuation response trends are reported, depending on the animal,
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30 which are likely to be due to both variations in anatomy and in acoustic tissue properties.
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32 It is possible that such behaviour results from internal acoustic modes which are specific
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34 to the animal. We found that the microphone location may have an impact on the overall
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36 transfer function estimate (Figures 3 and 5). This is likely to be due to the complex
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38 acoustic environment inside the operating theatre. Reflections on surfaces within the
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40 room, together with acoustic resonances will impact on the transfer function estimate.
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42 Ideally, such experiments would be carried out in an anechoic room but such an
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44 undertaking would be technically very challenging. Depending on the position of the
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46 microphone inside the room, and with respect to the animal, the acoustic transfer
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48 characteristics between the loudspeaker and the microphone vary. The existence of anti-
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50 resonances in the transfer functions may in some cases be due to room modes which may
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52 be picked up more prominently at one microphone positions rather than another. For
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4 example, it is likely that the trough at around 750 Hz in Figure 3b results from a room
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6 resonance that is more prominent at the location of the head microphone than that of the
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8 body microphone.
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10 11 *Comparison with existing literature*

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13 Like previous authors, we have used the sheep model because the similarities between the
14
15 sheep and human fetus in size and function make it the most widely used animal for
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17 studying fetal physiology and give clinical relevance to these experiments. Previous in
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19 vivo experiments in the near-term ewe have indicated that of the external airborne
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21 sounds, only the low frequency (below 0.3 kHz) components reach the womb interior,
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23 and that frequencies above 0.5 kHz are attenuated by 40 to 50 dB.^{7,8} Overall, previous
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25 studies show that low-frequency sound energy easily penetrates to the fetal head, with
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27 less than 5 dB attenuation for frequencies below 0.5 kHz, whereas higher frequencies are
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29 attenuated by up to 20 to 30 dB. The sound energy in amniotic fluid stimulates fetal
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31 hearing through a bone conduction route rather than through the external and middle ear
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33 systems²¹. Intrauterine sound recording in humans during labour after membrane rupture
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35 showed that low-frequency sounds (0.125 kHz) generated outside the mother were
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37 enhanced by an average of 3.7 dB.²² There is a gradual increase in attenuation for
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39 increasing frequencies, with a maximum attenuation of 10.0 dB at 4.0 kHz but the
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41 interpretation of these data is difficult in the absence of amniotic fluid around the fetus.
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50 The study described in this paper confirms that, at specific frequencies, the
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52 abdominal wall, together with overlying tissue and the amniotic fluid provide as little as
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54 2–3 dB acoustic attenuation of external airborne sound inside the womb environment at
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56 frequencies below 1 kHz, confirming the results of previous studies.^{7,8} Above 1 kHz, the
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4 attenuation increases to 20–40 dB, depending on the animal and position of the external
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6 microphone. Above 10 kHz, the improved measurement protocol used in this study
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8 reveals that significant external sound is transmitted into the womb and that attenuation is
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10 as little 3 dB at 11 kHz in one ewe. These results support the study carried by Lecanuet et
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12 al.²³ and may corroborate the theory that internal resonances are responsible for the
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14 increase in in utero sound transfer characteristics at frequencies above 10 kHz.
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19 Exposure to MRI during the first trimester of pregnancy compared with non-
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21 exposure was not associated with increased risk of harm to the fetus or in early
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23 childhood²⁴. Prenatal exposure to 1.5-T MRI during the second or third trimester of
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25 pregnancy in a cohort of healthy fetuses is not associated with disturbances in functional
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27 outcomes or hearing impairment at preschool age.²⁵ These are epidemiological
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29 (retrospective) studies and thus limited in the evaluation of actual noise exposure.
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34 Premature newborns are exposed to a non-physiological environment to keep the
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36 baby alive without medical support until its lungs and digestive tubes have fully
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38 developed. There is evidence that improving the quality of the environment of the
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40 premature baby in a neonatal intensive care unit (NICU) from birth can positively
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42 influence cognitive and social development later in children. A recent review²⁶ has shown
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44 that all recordings of maternal voice at sound levels are above current recommendations
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46 and that few of the findings on the positive effects of these recordings reached statistical
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48 significance.
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52 ***Conclusions and implications***

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54 Our findings support the epidemiological evidence that pregnant women should not be
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56 long-term exposed to high level occupational noise¹⁴ and highlight the importance of
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4 providing the premature neonate in NICU with a physiological environment as close as
5 possible to the environment to which it would have been exposed had the pregnancy
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7 continued to term.
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11 Improving the environment for preterm born babies has been highlighted in a top 15
12 priorities for preterm birth research by a recent Priority Setting Partnership with patients
13 and the public lead by the James Lind Alliance.²⁷ The integration of these in vivo data
14 into a computerised model together with available anatomical human data will enable us
15 to develop a sound filter representative of the in utero physiological environment, which
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17 may then be integrated in a prototype womb-like incubator.
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Figure legends

Figure 1. Schematic of 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 two microphones and the one hydrophone in the amniotic cavity and data transferred via cables to the computer.

Figure 2. (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.

Figure 3. (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).

Figure 4. (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.

Figure 5. (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).

Figure 1
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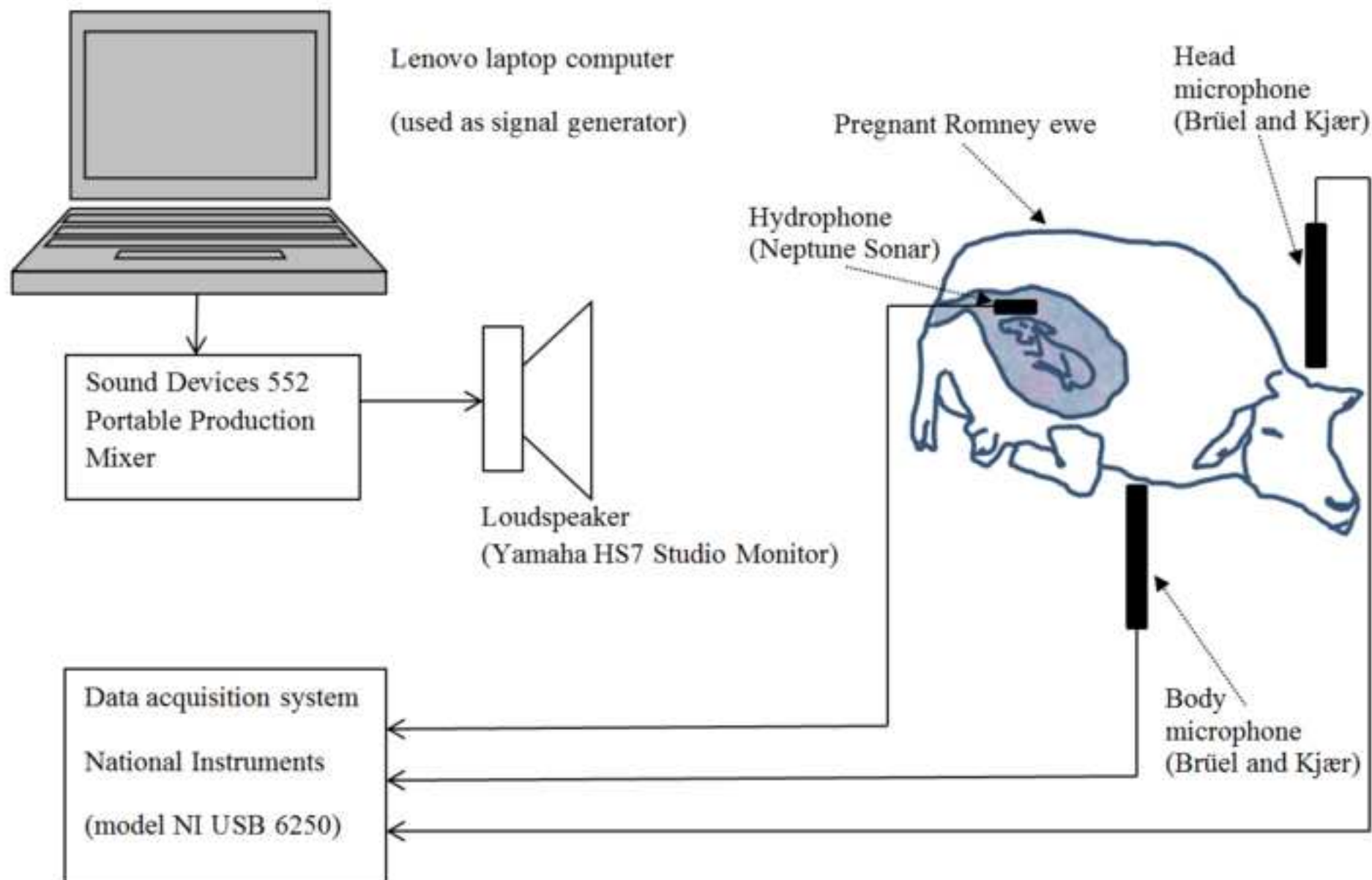


Figure 2a
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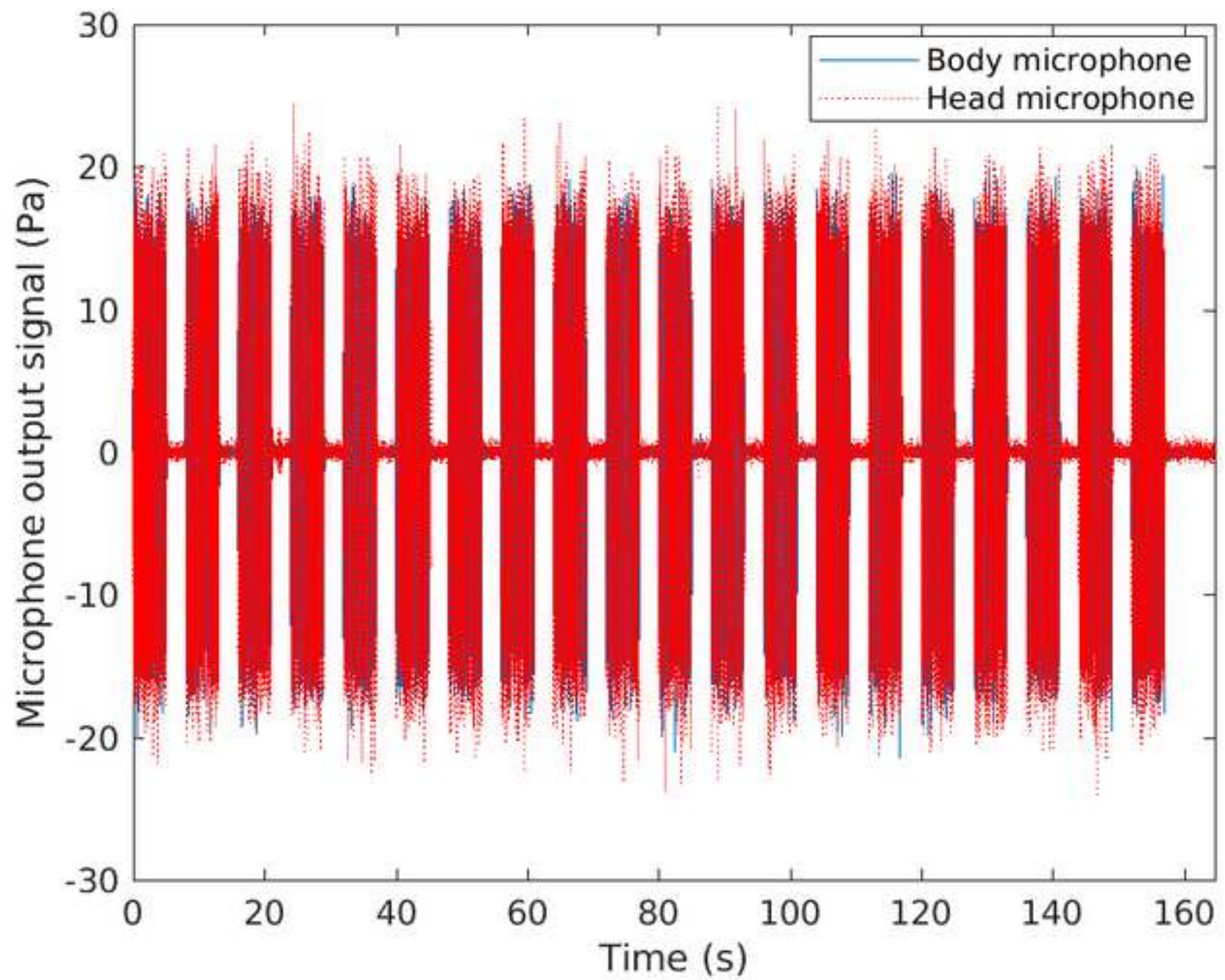


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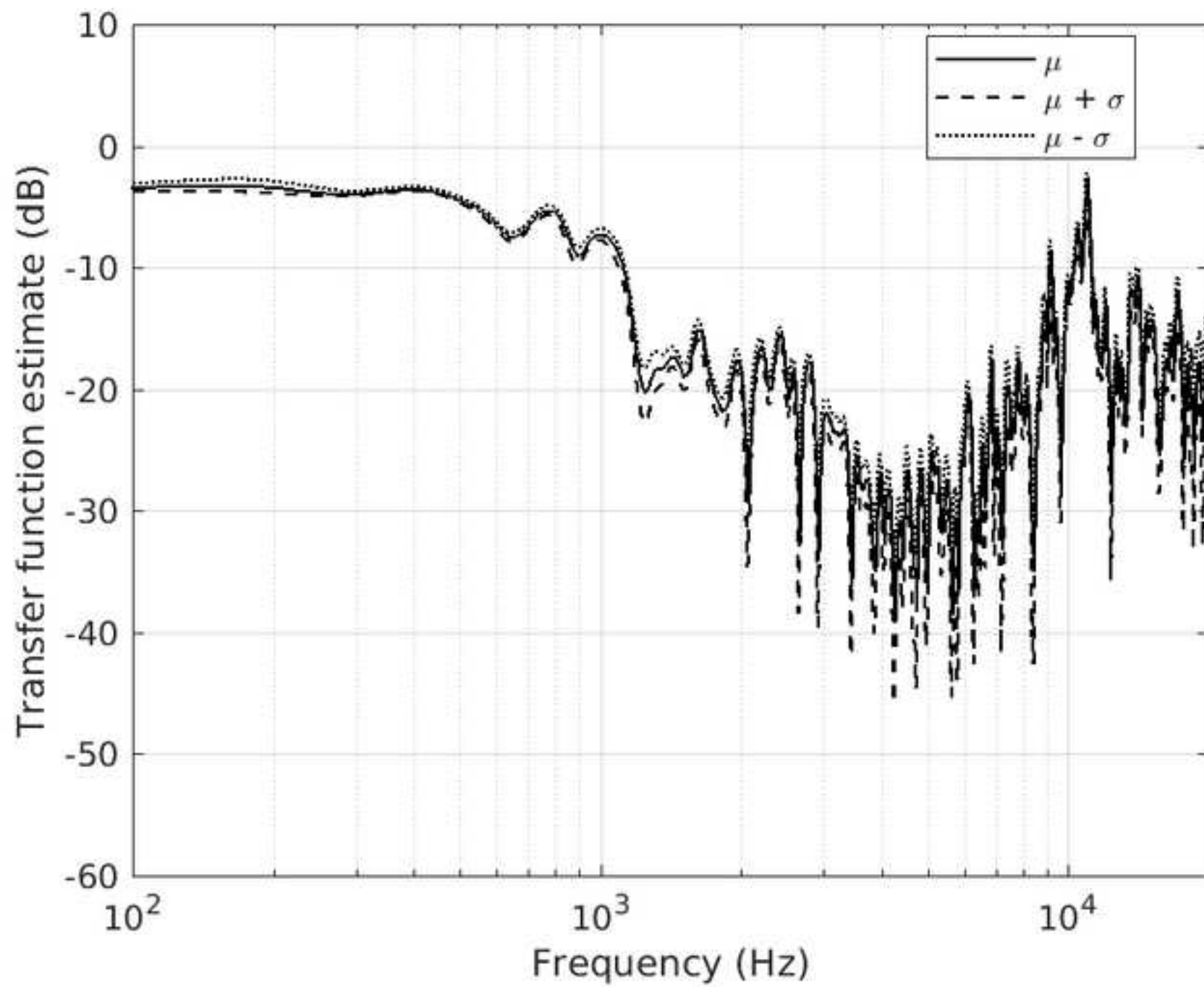


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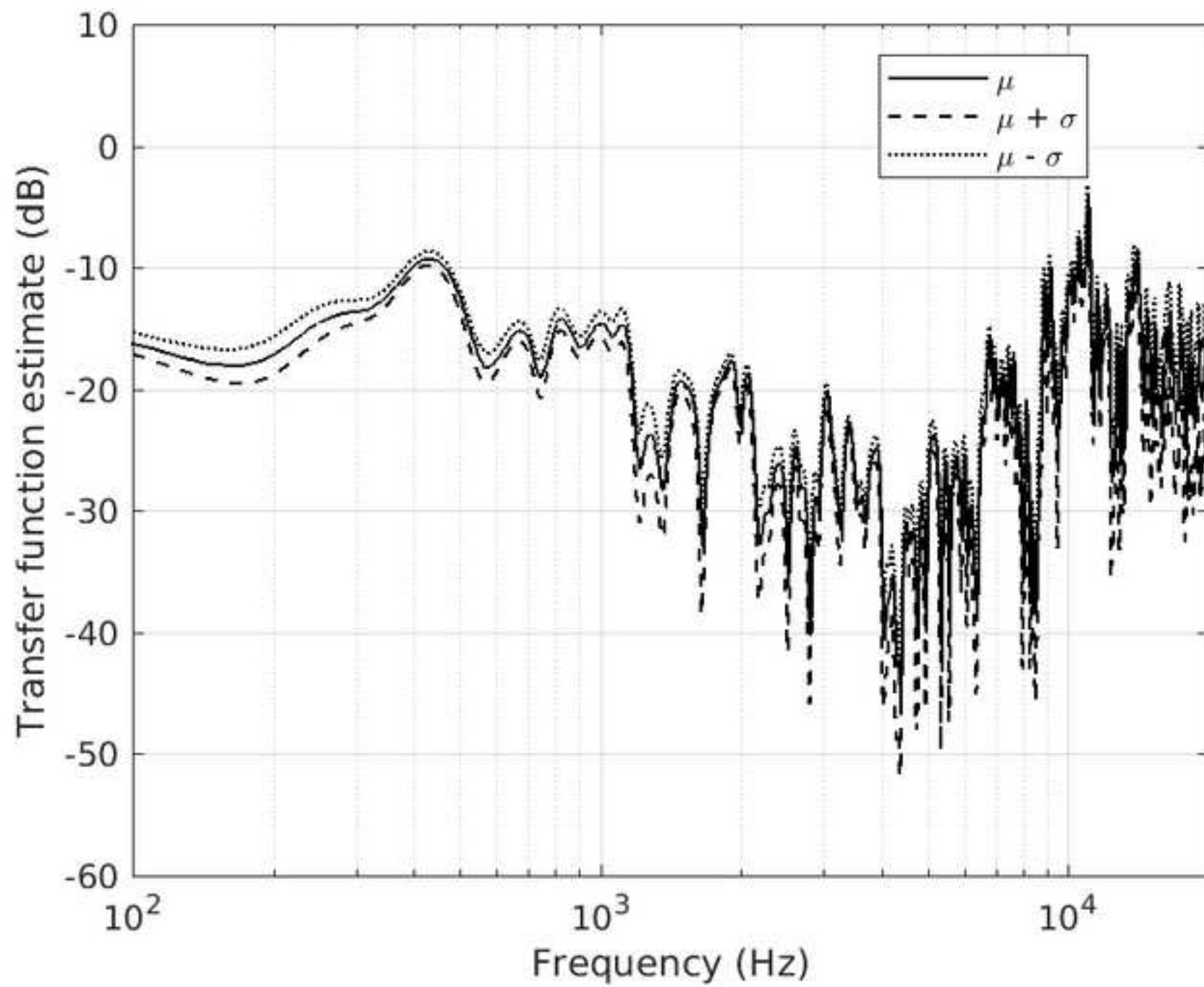


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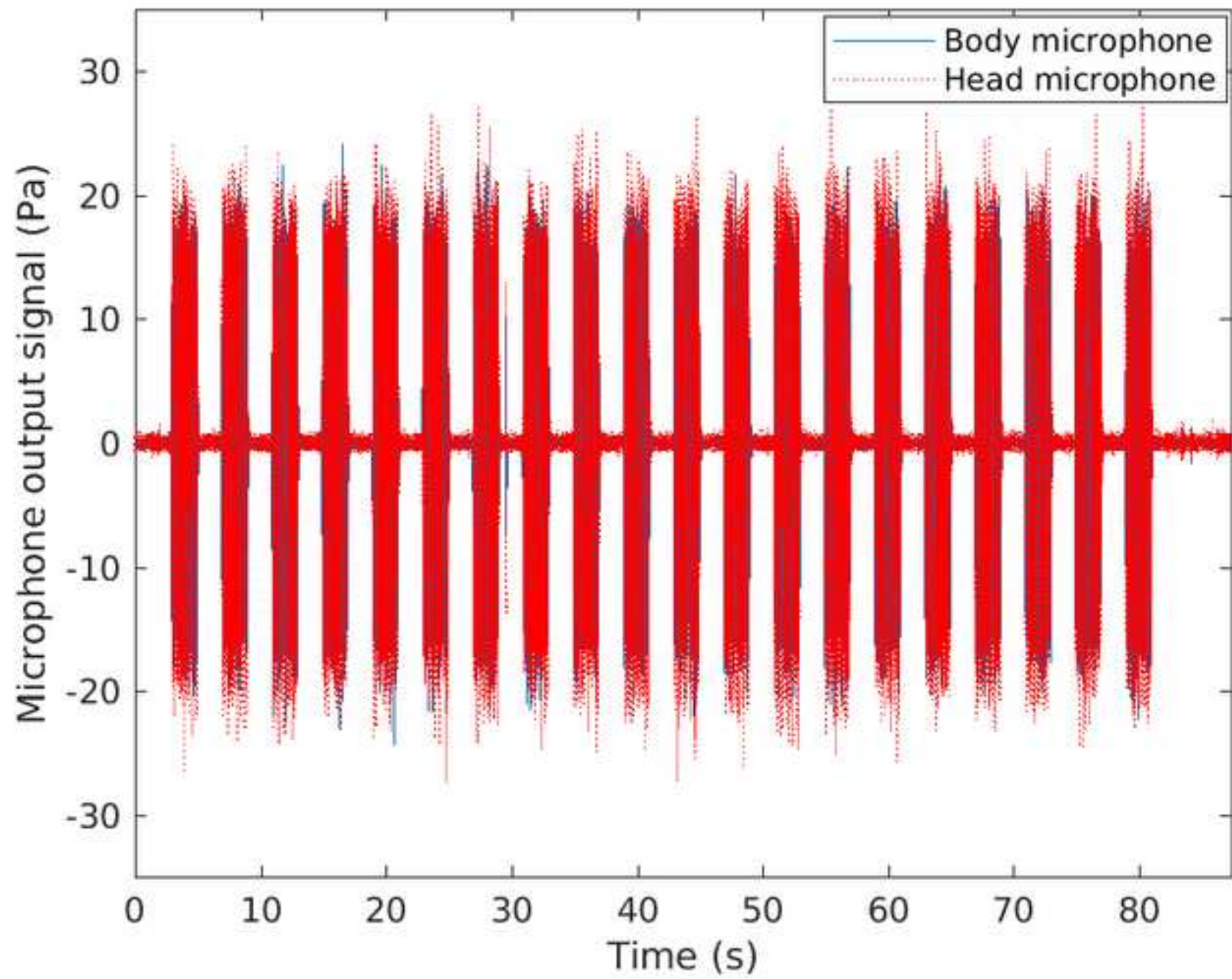


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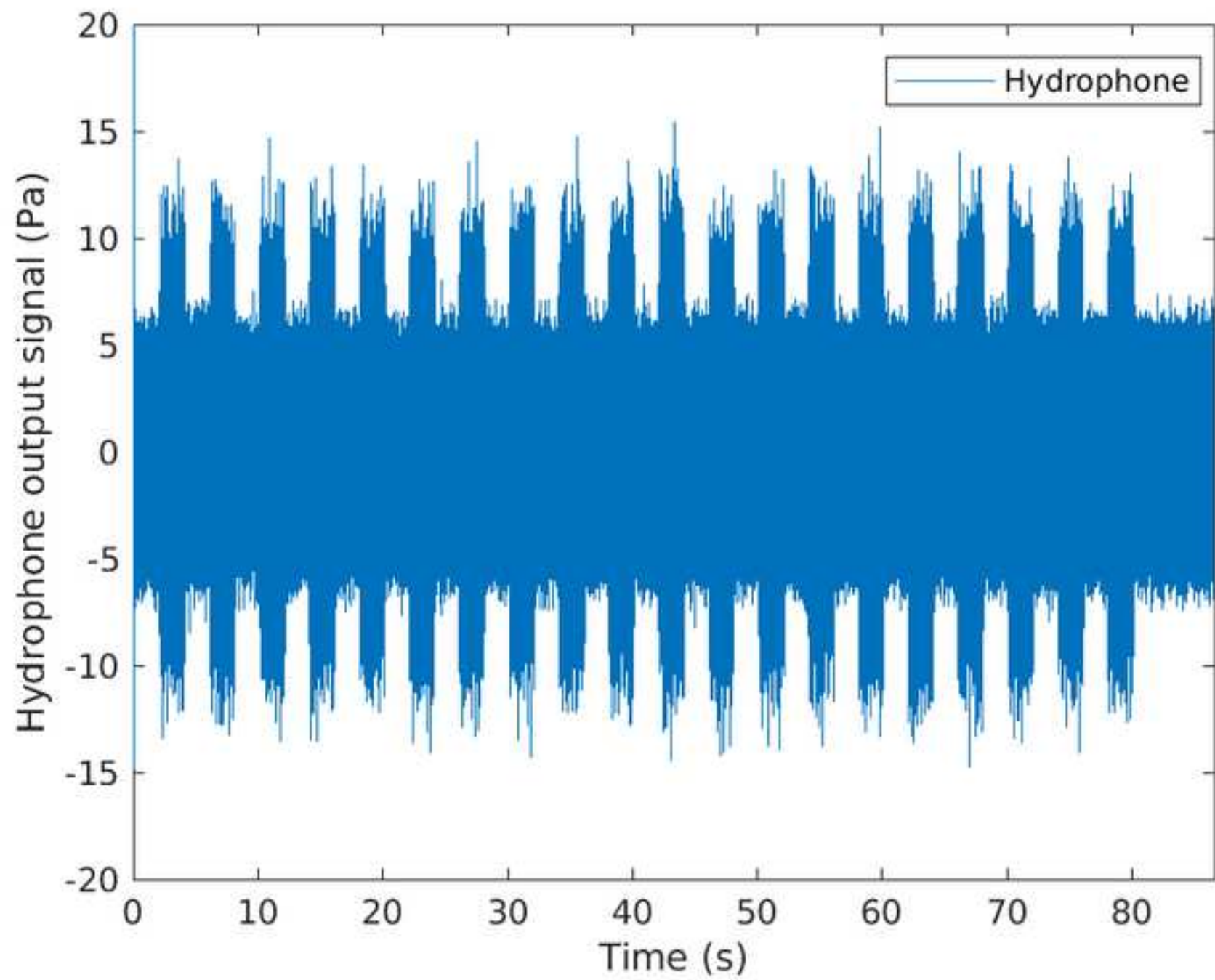


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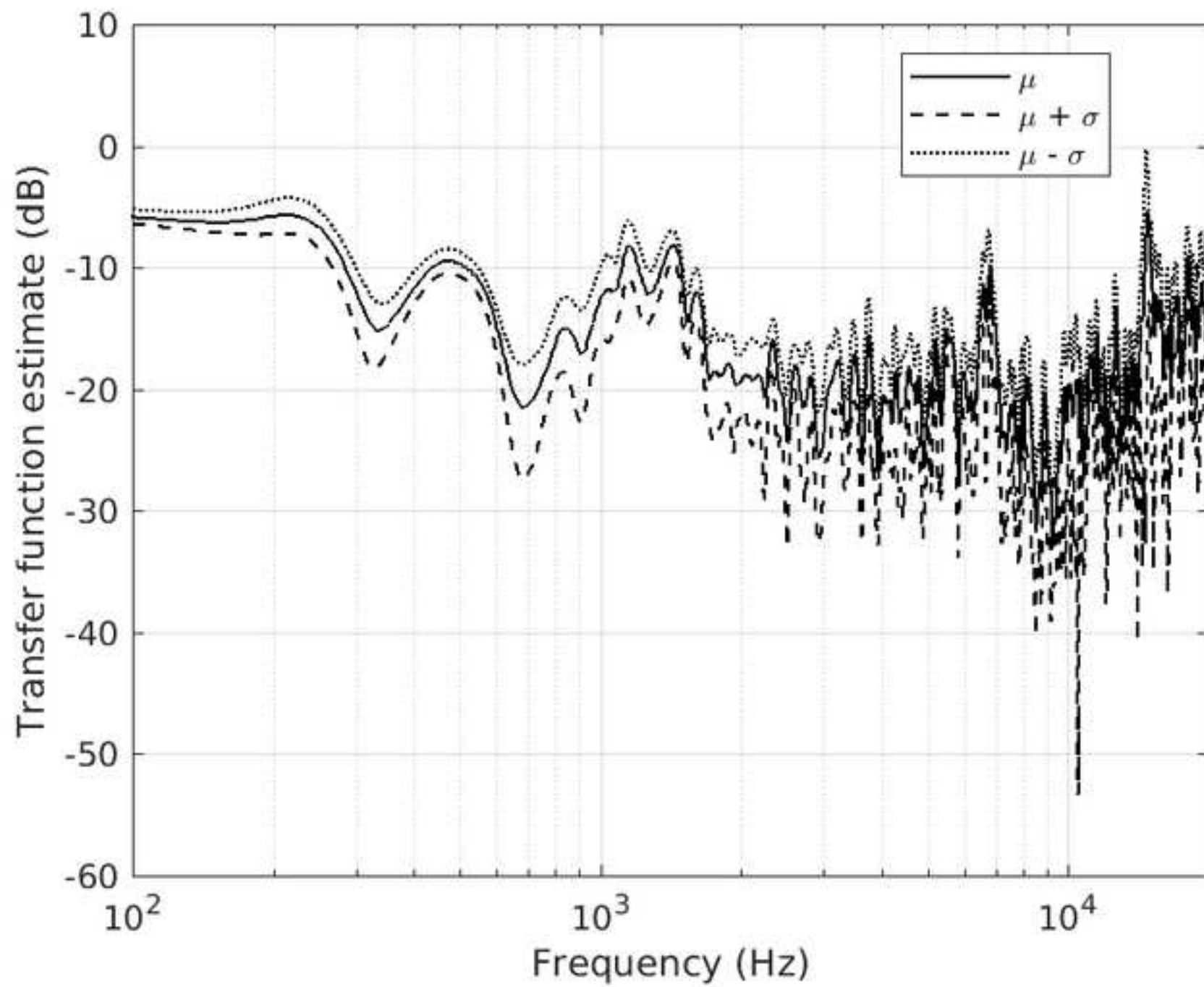
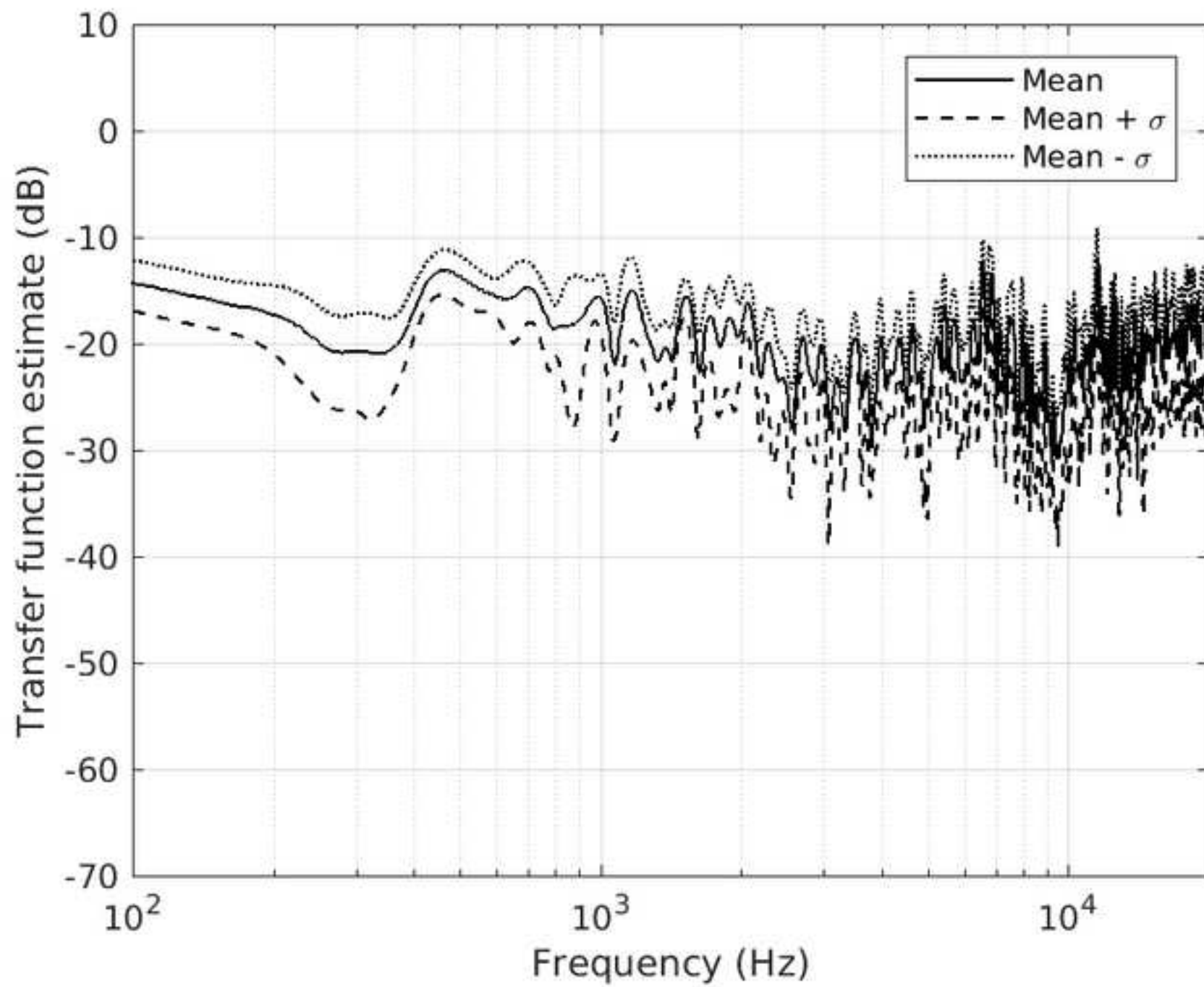


Figure 5b
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Appendix 1. *Preliminary experiments in pregnant ewes to establish proof-of-concept.*

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 considered. 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 2. *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. 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. 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 method²⁸. 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.