Fetal facial expression in response to intravaginal music emission

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Abstract
This study compared fetal response to musical stimuli applied intravaginally (intravaginal music [IVM]) with application via emitters placed on the mother’s abdomen (abdominal music [ABM]). Responses were quantified by recording facial movements identified on 3D/4D ultrasound. One hundred and six normal pregnancies between 14 and 39 weeks of gestation were randomized to 3D/4D ultrasound with: (a) ABM with standard headphones (flute monody at 98.6 dB); (b) IVM with a specially designed device emitting the same monody at 53.7 dB; or (c) intravaginal vibration (IVV; 125 Hz) at 68 dB with the same device. Facial movements were quantified at baseline, during stimulation, and for 5 minutes after stimulation was discontinued. In fetuses at a gestational age of >16 weeks, IVM-elicited mouthing (MT) and tongue expulsion (TE) in 86.7% and 46.6% of fetuses, respectively, with significant differences when compared with ABM and IVV (p = 0.002 and p = 0.004, respectively). There were no changes from baseline in ABM and IVV. TE occurred /C21 5 times in 5 minutes in 13.3% with IVM. IVM was related with higher occurrence of MT (odds ratio = 10.980; 95% confidence interval = 3.105–47.546) and TE (odds ratio = 10.943; 95% confidence interval = 2.568–77.037). The frequency of TE with IVM increased significantly with gestational age (p = 0.024). Fetuses at 16–39 weeks of gestation respond to intravaginally emitted music with repetitive MT and TE movements not observed with ABM or IVV. Our findings suggest that neural pathways participating in the auditory–motor system are developed as early as gestational week 16. These findings might contribute to diagnostic methods for prenatal hearing screening, and research into fetal neurological stimulation.

Keywords: Ultrasound, fetal movements, hearing, music, prenatal diagnosis

Introduction
The behavior of the fetus and its response to stimuli as a measure of its well-being and normal neural development is of great interest and has been the object of previous studies.¹² In this context, the advent of 3D/4D ultrasound has been a major breakthrough in the field: the fetus can be observed in real time, and very small-scale movements can be identified.³–⁷

Variations in fetal heart rate (FHR) or nonspecific movements on ultrasound have led to reports that the fetus can respond to sounds perceived through the amniotic fluid from at least weeks 19–20 of gestation.⁸,⁹ Nevertheless, hearing should be theoretically possible from week 16, when the auditory structures are formed.¹⁰,¹¹ We also know that as it matures, the fetus is increasingly able to discriminate frequencies between 100 and 3000 Hz, starting with the lowest frequencies.⁸,¹²,¹³ It is widely agreed that the fetus can hear external stimuli through the mother’s abdomen,¹⁴ but after crossing the maternal abdominal tissues and amniotic fluid, the quality and intensity of the stimulus have diminished substantially by the time it reaches the fetal ear.¹⁵,¹⁶ Highly intense external sounds (higher than 100 dB) are reduced to levels typical of human conversation (~40 dB) in the fetal ear, although this varies according to the frequency of the vibroacoustic stimuli used;⁸,¹⁷,¹⁸ frequencies higher than 500 Hz can be attenuated by up to 50 dB.¹⁷ It is also estimated that a maternal voice at 60 dB reaches the fetus at ~24 dB (distortions aside), which is equivalent to a quiet conversation. Furthermore, any external sound must be distinguished from the background noise of the uterus, which has been established in some studies as between 28 dB and 50 dB.¹⁹ Thus, acoustic stimulation methods that guarantee a level of sound reaching the fetus, with the least distortion possible, merit serious
consideration. Moreover, given its great potential, modern 3D/4D ultrasound may also be useful for identifying specific movements that might be more reliably associated with fetal response.7,20

The main aim of this study was to analyze fetal response to an acoustic stimulus emitted by a device which, due to its location and characteristics, might provide better sound intensity and quality. To that end, we used a device specifically designed to emit a melody or vibration from inside the mother’s vagina. This location is closest to the fetus, so there are fewer obstacles to attenuate the acoustic waves. The secondary objective was to identify quantifiable fetal movements that could be associated with the acoustic stimulus.

Materials and methods
Study design and patients
A single-center, prospective, stratified randomization study was conducted. Women ≥14 and <40 weeks pregnant attending the Gynecology and Obstetrics Department at the Institut Marqués (Barcelona, Spain) between May and August 2014 were invited to participate. For recruitment, consecutive patients meeting the inclusion criteria were informed in detail of the purpose and procedures of the study, the placement of abdominal and intravaginal music and vibration emitters, and safety issues, and they were assured that medical care would be of the same quality in case of refusal. The protocol was approved by Hospital Sanitas CIMA (Barcelona) Clinical Research Ethics Committee and all participants gave written informed consent.

Gestational age was confirmed by first trimester ultrasound. Twin or multiple pregnancies were excluded, as were patients with poor obstetric history, high-risk pregnancies (diabetes mellitus, high blood pressure, uterine malformations, threatened preterm labor, premature rupture of membranes), conditions that contraindicated remaining in supine decubitus for prolonged periods, repeated or active vaginal or urinary infections, and vaginismus or major vaginal malformations.

Procedure and equipment
For randomization purposes, participants were stratified into four groups: pregnancies ≥14 to ≤16, >16 to ≤24, >24 to ≤32 and >32 to ≤40 weeks of gestation. Fetuses aged ≤16 weeks were considered with no functional inner ear,10 and with hearing ability those >16 weeks. Each group was randomized to receive one of three types of stimuli (Figure 1): (a) a flute monody lasting 5 minutes with no repetitions, emitted through headphones placed on the mother’s abdomen, at a mean intensity of 98.6 dB (based on a loss of ∼30 dB,15,17 human voice range: 40-70 dB); (b) an intravaginal device emitting the same monody at 53.7 dB; or (c) the same intravaginal device emitting vibration only (125 Hz) at 68 dB. Both emitters were placed appropriately in all participants, regardless of the device finally used for the stimulus. Headphones were placed on the sides of the lower abdomen in all cases, and the intravaginal emitter was also always in the same position, so the ultrasound probe could be used freely to obtain good-quality images of the fetus head. The mother was isolated from the environment with relaxing music emitted through headphones.

The intravaginal device was a patented design prototype (PCT/ES2014/070227) with certified sound calibration provided by MusicInBaby S.L. (Barcelona, Spain). The device consists of an insulating capsule of a size and shape suitable for intravaginal use, containing emitters connected by a cable to audio equipment and a control system. The abdominal music was emitted using headphones MDR-XD150 (frequency response 0.12-22kHz; Sony, Japan).

Fetal movements were observed by 2D/3D/4D transabdominal ultrasound (GE Voluson E6; GE Healthcare; Little Chalfont, United Kingdom). Each 15-minute ultrasound session was subdivided into three 5-minute periods. Once all the devices had been placed, with the patient in supine decubitus, the following data were recorded: (1) fetal activity (FA) at baseline, without any stimuli; (2) its activity during stimulation with one of the three modalities, and (3) its activity in silence after discontinuing stimulation.
Ultrasound analysis and variables recorded

Routine assessments, including FHR during the three stages of the session, were carried out at each ultrasound; middle cerebral artery pulsatility index (MCA-PI) was also measured in fetuses >20 weeks. All ultrasounds were recorded, and two investigators, blind to the fetal age, and type and presence or absence of acoustic stimuli in the video sections, provided independently analyzed fetal movements. FA was observed and the occurrence of facial movements was counted in each recording. FA was defined as fetal rotation and column flexion–extension movements, or limb movements in front of the area under study (cerebral pole/faces of the fetus). Facial movements quantified were mouthing (MT: mouth opening or tongue movements inside the oral cavity) and tongue expulsion (TE: protrusion of the tongue over the lower lip).

Statistical procedures

Sample size was calculated using the infinite population formula, with unknown proportion (p=q=50%), 95% confidence level, and 5% standard error. The final sample size needed was 96 patients.

Group analysis was performed by gestational age and type of stimulation. Data are expressed as mean and standard deviation for the continuous variables (FHR and MCA-PI), and as frequencies for the dichotomous and discrete variables (FA, MT, and TE). The pattern of movements was compared between fetuses aged > or ≤16 weeks using comparison tests for paired data (Wilcoxon test for two level variables and Friedman’s test for TE). Fetal movements were analyzed using the χ² test. Multiple linear or logistic regression models were adjusted according to the type of variable, using the type of stimulus, fetal sex, and previous parity as explanatory variables. Finally, the relationship of the variables with gestational age stratified into the four age groups was analyzed by analysis of variance, the Student’s t test, or Kruskal–Wallis test according to the levels of each variable. All analyses were performed with the package R version 14.0.

Results

Twenty-four fetuses at ≤16 weeks gestation and 82 fetuses >16 weeks gestation (mean age 24.5±7.5 weeks) were included, and distributed randomly to exposure to intravaginal music (IVM, n=38), intravaginal vibration (IVV, n=34), or abdominal music (ABM, n=34). Mean age of the participants was 34.3±4.5 years; 80 were nulliparous and 26 had had previous pregnancies. All pregnancies were normal, as per the inclusion criteria. In the overall sample, there were no significant differences in any baseline ultrasound variables (FHR, MCA-PI, fetal movements) with respect to fetal sex or parity of the mothers, and all obstetric parameters were within reference ranges. In the baseline ultrasound, all fetuses in all stimulus groups had a similar level of activity, with a low frequency of spontaneous facial movements (>65% of fetuses with no MT movements). There were no differences in baseline between the gestational age groups over or under 16 weeks.

Fetuses <16 weeks did not show any significant variation during stimulation in FA or MT in any of the modalities, and none of them showed TE. Fetuses aged >16 weeks in the three stimulus groups had a similar baseline status, but a significant increase in FA, MT, and TE was found in the IVM group only (Video 1-available with the online version of this article at: http://ult.sagepub.com). In this group, MT movements were observed in 86.7% (n=26/30) of fetuses, and TE in 46.6% (n=14/30). Both these increases were significant compared with the other two groups (Figure 2). In fetuses aged >16 weeks, only those in the IVM group made five or more TE movements in the 5 minutes of exposure to the stimulus (13.3%; n=4/30; Figure 2(c)). The IVV and ABM groups showed similar frequencies for FA and MT movements that were significantly lower than in the IVM group compared to the other two groups (p=0.008; Table 1).

In the regression models constructed with the data taken during the stimulation, neither previous parity nor sex was found to be related with the study variables. However, IVM was related with greater FA (odds ratio [OR] = 4.662; 95% confidence interval [CI] = 1.334–18.708), and a higher occurrence of MT (OR = 10.980; 95% CI = 3.105–47.546) and TE (OR = 10.943; 95% CI = 2.568–77.037).

Finally, variables were compared by weeks of gestation in four ranges (Figure 3). Fetal age was related with baseline FHR and MCA-PI (p=0.002 and p=0.001, respectively), but no differences were observed in baseline MT or TE according to gestational age. During stimulation, a relationship was found between age and FA (p=0.0002), and between age and the number of fetuses with TE (p=0.002); a higher percentage of fetuses aged between 24 and 39 weeks protruded their tongue (up to 38.5% of fetuses between 32 and 39 weeks).

Discussion

This prospective, randomized study used ultrasound to examine fetal response to intravaginally emitted music and identified movements that could be significantly related to acoustic stimulation. A previous pilot study had been initially conducted that suggested oral movements as possible candidate variables. A musical stimulus was chosen, consisting of a flute monody, which exposed the fetus to fundamental frequencies and harmonics within the Western tonal system; this was compared with the noise generated by a vibration with a principal frequency component at 125 Hz. Our results show that the musical stimulus was significantly associated with a fetal response in the form of fetal mouth and tongue movements (Video 1-available with the online version of this article at: http://ult.sagepub.com) that was not observed during stimulation with vibration at a frequency considered to be
within the fetus’ preferential auditory range, according to the literature.\textsuperscript{8,15,17} The extensive TE observed with IVM did not occur in the 5-minute baseline recording. TE of any magnitude is very rare among spontaneous movements observed with 3D/4D ultrasound to date,\textsuperscript{4,21–23} especially in fetuses under 25 weeks.\textsuperscript{4,22,24} We may note that previous studies have reported a frequency of 0–1 TE in 15-minute recordings in fetuses < 20 weeks\textsuperscript{22} that increases slightly in fetuses of 20–25 weeks, median 1 (range 0–2)\textsuperscript{22} or 0 (1–2)\textsuperscript{24} depending on reports. Sato et al.\textsuperscript{24} reported that 3/23 (13\%) fetuses aged 20–24 weeks performed TE movements in 15 minutes, while Kanenishi et al.\textsuperscript{4} observed this movement in 7/23 (30.4\%) fetuses aged 25–27 weeks (median 1.5 [0–5]), and TE was observed in 6/10 fetuses aged 28–34 weeks in a related study (median 1.5 [0–5]).\textsuperscript{23} In this regard, we detected 7.4\% of 16- to 23-week-old fetuses performing at least one TE during the 5 minutes of baseline recording, increased to 13.8\% in those aged 24–31 weeks.

![Figure 2](image)

**Figure 2** Percentage of fetuses ≥ 16 weeks that are active (FA) and make mouthing (MT) and tongue expulsion (TE) movements, according to type of acoustic stimulus. In the group with intravaginal music, there was more FA during the stimulation than in the other two groups (a). Stimulation (stimulus ON) with vaginal music elicited MT (b) and TE (c) in significantly more fetuses than in the other groups. This effect remained in the 5 minutes after the stimulus was discontinued (stimulus OFF). TE was identified between 1 and 4 times in some fetuses in all groups, but ≥ 5 TEs during stimulation were seen only in fetuses in the vaginal music group (13.3\%, white box in c). IV: intravaginal; AB: abdominal.

|                      | Baseline US | Stimulus ON | Stimulus OFF | p Value (difference baseline–ON) | p Value (difference baseline–OFF) |
|----------------------|-------------|-------------|--------------|----------------------------------|----------------------------------|
| **FHR (M ± SD)**     |             |             |              |                                  |                                  |
| IV music             | 143.7 ± 8.6 | 150.3 ± 12.2| 148.2 ± 8.6  | 0.003                            | 0.008                            |
| IV vibration         | 150.3 ± 12.2| 146.7 ± 8.7 | 145.6 ± 9.4  |                                  |                                  |
| AB music             | 148.2 ± 8.6 | 147.9 ± 8.9 | 143.2 ± 7.5  |                                  |                                  |
| P value              | 0.04        | 0.206       | 0.105        |                                  |                                  |
| **MCA-PI (M ± SD)**  |             |             |              |                                  |                                  |
| IV music             | 1.98 ± 0.32 | 1.77 ± 0.25 | 1.74 ± 0.26  | 0.204                            | 0.061                            |
| IV vibration         | 1.95 ± 0.34 | 1.85 ± 0.33 | 1.99 ± 0.44  |                                  |                                  |
| AB music             | 1.87 ± 0.55 | 1.82 ± 0.45 | 1.79 ± 0.44  |                                  |                                  |
| P value              | 0.636       | 0.724       | 0.188        |                                  |                                  |

Note: IV: intravaginal; AB: abdominal.

Comparisons by analysis of variance (ANOVA). Individual data for the difference between each stimulation period were calculated and compared by type of device by ANOVA.
However, our recording of spontaneous movements was much shorter, intended to serve as a control to observations made during vibroacoustic stimulation, and previously observed percentages of occurrence might have been achieved with longer baseline explorations. In view of those reports, such a high number of TE in such a short period during stimulation in our study (>4 TE in 5 minutes with IVM in 14.6%, i.e., 4/55 fetuses aged 24–39 weeks) is therefore remarkable. This high frequency of TE did not appear at baseline in any case, and disappeared or diminished when stimulation ended, thus appearing to indicate its association with music. It is also notable that MT movements, especially TE, were uncommon among fetuses exposed to ABM in the conditions described. Although IVM, unlike the vibratory stimulus, was significantly associated with TE, fetuses with ABM showed no such response with the same melody. This suggests that the intensity of the ABM may have been too low for the fetal ear, or that the distortion with this route is too high to elicit a response. We used standard headphones in our study, and the loss of distortion with this route is too high to elicit a response. We attempted to ensure that IVM and ABM were as similar as possible, while avoiding harm to the fetus at all times.

IVM and ABM may have been too low for the fetal ear, or that the ABM intensity described in the literature (30–50 dB) was taken into account when designing the methodology, in an attempt to ensure that IVM and ABM were as similar as possible, while avoiding harm to the fetus at all times. FHR and MCA-PI indicate that the fetus was not startled by any of the stimuli, although the IVM caused a slight, but not significant increase in FHR. As regards potential losses in sound intensity described in the literature, the ABM delivered in this study was more intense between 0.4 and 3 kHz; it has been reported that frequencies > 0.5 kHz suffer losses of up to 50 dB before they reach the fetus. 17 However, the IVM was emitted at an intensity of only ~30 dB between 0.1 and 2 kHz, and at human conversation levels between 2.5 and 4 kHz, which apparently did not prevent the fetus from responding to it.

Our results also suggest that the fetus can hear musical stimuli from an early age. However, studies based on trans-abdominal stimulation have determined that a younger fetus would perceive frequencies between 0.25 and 0.5 kHz, while responses to frequencies over 1000 Hz have been described only in fetuses older than 30 weeks. 8 In this respect, it should be remembered that the intravaginal vibroacoustic stimulus at 125 Hz did not elicit any response. It may be that a noise at such low frequency is masked by surrounding sounds, which have been reported to be between 200 and 500 Hz with an intensity of 65 dB at times. 25,26 Moreover, these sounds may not elicit a reaction in fetuses that have become habituated to them. In any case, determining a distinction between a sustained frequency and a sequence of tones, as in the case of a melody, is an area to be explored in future studies.

At present, our data appear to suggest two interpretations: that intravaginal application, with fewer obstacles, could be more effective in transmitting music to the fetus,
and that the fetus might perceive these higher frequencies at an earlier age than reported to date. In this regard, we observed a response to IVM in fetuses of all ages from as early as week 16. Older fetuses responded more, as expected from the literature, since development, initially simple circuits are formed that grow in complexity and definition during pregnancy and the first few months of life. Cochlea and middle ear are already formed at week 15, although they are traditionally considered to become functional at week 20. However, such an early response registered in our study suggests the involvement of anatomical elements and neural substrates formed at an earlier stage. Thus, our results suggest possibly earlier functioning, although the intensity or quality of the perception remains unknown.

With respect to neural substrates, various studies in the literature suggest that these MT and TE movements, apparently induced by a musical stimulus, could be related to preparation for vocalization. Similarly to our findings, music has been observed to elicit TE in 4-week-old babies, which could be related to this fetal behavior. Certain spontaneous movements similar to the generation of vocals, including TE, have also been observed from week 18. These movements would require the participation of primitive structures, such as those found in the brainstem (Figure 4), which contains nuclei and pathways involved in phonation and oral movements that might establish indirect synaptic connections with the auditory nuclei, already identifiable in the eighth week. Similarly, neurofilaments associated with auditory structures are observed as early as week 16. Tracer studies in primates have located centers in the reticular formation that generate vocalization patterns, with connections to the cranial motor nuclei (trigeminal, facial, ambiguous, and hypoglossal) and to the premotor centers involved in these activities. These reticular centers also receive connections from the midbrain periaqueductal gray (PAG), which could function as a subcortical center responsible for the integration between hearing and phonation, as it is associated with the motor systems of the lingual, laryngeal, and pharyngeal musculature, and with speech processing in humans (Figure 4).

In this respect, there is evidence that the specific region of the PAG that produces vocalization in primates, when stimulated receives direct connections from the inferior colliculus, an auditory relay center, and from the superior colliculus; this has been related in the human brain with the perception of dissonance and musical memory. Future studies could shed more light on the significance of our findings and, most particularly, on the nerve pathways that could be involved from so early in fetal development. The possible participation of the PAG would have major implications, since it has been proposed as a node in the social behavior network, which is of great evolutionary importance in the evaluation of external stimuli and adaptive behavior.

This study has the limitation that it is a single center study and therefore sample size is small, although it was sufficient for the analyses performed. Postpartum follow-up of the neonates for correlation of the results with neurodevelopmental variables was not planned. Finally, working in the clinical setting presents methodological limitations that prevent characterization of the quantity and quality of the sound or noise that reaches the fetus, and exploration of the reasons for the events observed. These results indicate that intravaginal acoustic stimuli cause the fetus to make or increase the frequency of an uncommon movement, TE, as early as week 16 of pregnancy. In line with studies that suggest that music might have beneficial effects on the fetus, such stimulation could be used as a method for fostering fetal well-being, with the guarantee that the fetus hears it. It could also be used to evoke arousal responses of the fetus, and stimulate movements to facilitate and shorten obstetric ultrasound examinations. Moreover, use of IVM at home to produce fetal movements in gestations < 24 weeks (when the fetus is not yet viable even if urgent intervention were required) might contribute to the mother’s peace of mind and reduce health care costs associated with the recommended monitoring in this group. Some authors have advocated...
the usefulness of 4D ultrasound to evaluate fetal behavior, in order to increase knowledge regarding the central nervous system development, and also determine functional characteristics to predict possible developmental problems.\cite{4,5} Previously designed methods are based on spontaneous behavior only,\cite{5} and our findings might contribute to further research in methods to assess fetal neurodevelopment. From a clinical point of view, it would be interesting to conduct further studies to explore this approach as a possible diagnostic method for prenatal hearing screening, in addition to its possible contribution to the performance of tests of fetal well-being. Its potential to contribute to the field of research in fetal neurodevelopmental stimulation also merits attention.

DEclarations

Competing interests: Dr. López-Teijón participated in the design of the prototype for the intravaginal device, the patent for which belongs to MusicInBaby S. L. There are no other relationships with industry or other entities that could lead to conflicts of interest.

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Guarantor: M Lopez-Teijón

Contributorship: MLT and AGF conceived and designed the study, obtained the data, performed the analysis with support of a biostatistics service (Medical Statistics Consulting S.L., Valencia), and interpreted the data. APG performed bibliographical research and contributed significantly to data interpretation. All authors participated in the revision of manuscript drafts and approved the final version.

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