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Grant Number DAMD17-96-1-6302

TITLE: Impulse Noise Exposures: Characterization and Effects on  
Fetal Sheep in Utero

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REPORT DATE: September 1997

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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19971008 057

# REPORT DOCUMENTATION PAGE

Form Approved

OMB No. 0704-0188

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1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 1997	3. REPORT TYPE AND DATES COVERED Annual (1 Sep 96 - 31 Aug 97)	
4. TITLE AND SUBTITLE Impulse Noise Exposures: Characterization and Effects on Fetal Sheep in Utero			5. FUNDING NUMBERS DAMD17-96-1-6302	
6. AUTHOR(S) Kenneth Gerhardt, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Florida Gainesville, Florida 32611			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) Progress has been made in addressing each of the three primary objectives: 1) characterization of the transmission of impulse noises into the uterus; 2) evaluation of the effects of impulse noise exposures on the hearing of the fetus <i>in utero</i> ; and 3) evaluation of the effects of impulse noise exposures on the behavioral state of the fetus. The shock tube produced impulses in air that averaged 169.7 dB peak sound pressure level (pSPL). In the uterus, the pSPL varied as a function of fetal head location. When the fetal head was against the abdominal wall, peak levels were within 2 dB of airborne levels and the morphology of the waveform resembled a Freidlander wave. When the fetal head was deep within the uterus, the duration of the impulse waveform increased and the peak amplitude decreased. In some instances the decrease in pSPL exceeded 10 dB. Data from six fetuses exposed at 117 days gestational age (dGA) and from six fetuses exposed at 127 dGA revealed slight post-exposure threshold elevations for low-frequency auditory brainstem response eliciting stimuli. Cochleae from these animals are currently being evaluated using scanning electron microscopy. Behavioral state (rapid eye-movement sleep and heart rate) data from eight fetuses taken before, during and after exposure to impulses are under evaluation.				
14. SUBJECT TERMS Defense Women's Health Research Program			15. NUMBER OF PAGES 24	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

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## INTRODUCTION

### Subject

This research addresses the effects of impulse noise exposures on the hearing and sleep state of fetuses. Fetal sheep are used as a model to evaluate the effects of exposures delivered to the flank of pregnant animals. Comparisons of evoked potentials, electrocortical activity and heart rate before and after exposure to 20 impulses with peak levels of 170 dB will be made to determine deleterious effects on the fetus. Scanning electron microscopy will be used to evaluate any histological changes within the cochleae.

### Purpose

Knowledge of the transmission of exogenous sounds into the uterus and the effects that these sounds have on fetal hearing is incomplete. Safe maternal exposure levels have yet to be determined. The purposes of this study are to measure the transmission of impulse noise into the uterine environment and to evaluate the effects of impulse noise exposure delivered to pregnant sheep on the hearing and sleep state of the fetus.

The following hypotheses will be addressed: 1) Low-frequency components of impulse noises will be transmitted into the uterus with little loss in energy, whereas the high-frequency components will be greatly attenuated; 2) Impulse noise exposures will produce elevations in fetal auditory evoked potential thresholds that are dependent upon the frequency content of the eliciting stimulus and the age at which the exposure occurred; 3) behavioral state will be temporarily disrupted by an exposure to impulse noises; and 4) histology of inner ear tissue will reveal significant hair cell damage in the middle and apical turn of the fetal cochlea and the damage will be greater when the exposure occurs earlier in gestation.

### Scope of Research

Impulse noise transmission to the uterine environment will be calculated by comparing noise levels near the maternal flank to noise levels in the uterus. It is known that externally-generated sound transmission to the fetal head is more efficient for low frequency, steady-state sounds than for higher frequency, steady-state sounds (Gerhardt, et al. 1990). Transmission of impulse noise to the fetal head has not been measured, although it would be expected to follow the same pattern.

Assessments of auditory evoked responses (auditory brainstem response [ABR] and amplitude modulation following response [AMFR]) and inner ear tissue (scanning electron microscopy) after impulse noise exposures will provide important information regarding the potential hazards of military noise exposure on fetal hearing. It is anticipated that animals exposed to noise at an earlier gestational age (117 days) will not demonstrate immediate post-exposure threshold shifts, but will show an arrest in the development of more sensitive evoked potential thresholds, particularly for low frequencies. It is expected that sensory cell loss will be apparent in the middle and apical region of the inner ear. Animals exposed to noise at a later gestational age (127 days) will show an immediate threshold shift, which will probably recover to near normal levels after the exposure. Histopathologic procedures are expected to show modest hair cell loss.

The central effects of exposure to impulse noises will be evaluated through assessment of fetal behavioral state. Fetal sleep state is an important event that relates directly to fetal brain growth and development. Behavioral state will be evaluated before, during and immediately after exposure to impulses. Potential deleterious effects on the central nervous system may be apparent from these assessments.

#### Background of Previous Research

The relation between exposures to intense sound and decreases in hearing acuity of adult male workers was first reported in the early 18th century. Since then, noise-induced hearing loss (NIHL) has been widely studied. The permanent and handicapping effects of intense noise on adult hearing have been well-documented. Recently, attention has shifted to the possibility of NIHL during fetal life (Gerhardt, 1990).

Significant numbers of American working women of childbearing age are noise exposed. The Committee on Hearing, Bioacoustics, and Biomechanics (CHABA, 1982), attempting to protect fetal hearing, suggested that pregnant women avoid noise exposures greater than 90 dB(A). Other investigators believed that these recommendations could needlessly exclude women from the work force (Niemtzow, 1993). There is a paucity of evidence to support either conviction. Two retrospective studies (Lalande et al., 1986; Daniel and Laciak, 1982) found increased risk of hearing loss in children with occupationally noise-exposed mothers. But these studies have been criticized for methodological shortcomings (Niemtzow, 1993, Henderson et al., 1993).

Findings from experimental animals have been paradoxical. Dunn et al. (1981) exposed pregnant sheep to intense steady-state noise for 4 hours a day, 5 days a week for several weeks. Thirty to forty days after the lambs were born, ABR thresholds were normal. The ABR, a far-field recording of a bioelectric response to sound from the auditory mechanism, is a common clinical and research hearing assessment tool. In contrast to the Dunn study, Griffiths et al. (1994) measured the ABR from fetal sheep *in utero* before and after a single 16-hour, broad-band noise exposure (100 Hertz [Hz] to 10 kHz) at 120 dB sound pressure level. The investigators found significant changes in ABR thresholds and latencies immediately following noise exposure, although these changes were temporary. In a related study (Pierson, 1993), the fetal sheep ABR was recorded over a 23-day period following a similar noise exposure delivered at 113 days gestation (gestation for sheep is 145 days). No immediate changes in ABR thresholds were found, but thresholds for the noise-exposed group were significantly higher than for an age-matched, nonexposed group after 2 weeks or more. Cook et al. (1981) demonstrated ABR Wave IV latency differences between guinea pigs exposed to textile noise during the last trimester of gestation and a non-exposed control group.

In most instances, postnatal noise exposures occur in air. Prenatally, externally generated sounds must pass from air to the fluid medium of the uterus to reach the fetus. The intrauterine sound environment is dominated by frequencies below 0.5 kHz (Gerhardt et al., 1990). Externally generated sound transmission to the fetal head is more efficient for low frequency, steady-state sounds than for higher frequency, steady-state sounds. Low frequencies penetrate the uterus with little reduction in sound pressure and higher frequencies are reduced by about 20 dB.

Transmission of impulse noise to the fetal head has not been measured, although it would be expected to follow the same pattern.

## **BODY OF THE ANNUAL REPORT**

### Methods, Assumptions and Procedures

During sterile surgery, the instrumentation for chronic recording of the evoked potentials was implanted in two groups of sheep, either at a gestational age of 117 days (N=6) or 127 days (N=11). The fetus was exteriorized and the fetal head prepared for evoked potential and behavioral state recordings. A hydrophone was sutured near the fetal head in some preparations. The purpose of the hydrophone was to record acoustic levels in the intrauterine environment during the impulse noise exposure.

Two days after surgery the ewe was placed in a sound-treated booth and fetal evoked potential thresholds were assessed using tone bursts and clicks. Behavioral state and heart rate were assessed for at least one hour before exposure. After pre-exposure testing, ewes were exposed to 20 impulses produced by a shock tube. A second hydrophone connected to one channel of a spectrum analyzer and positioned close to the maternal flank recorded the pressure-time history generated by the shock tube. In addition, a simultaneous recording (channel 2) from the hydrophone *in utero* was obtained, thus yielding transmission characteristics for the impulse. The post-exposure evoked potentials were followed for 20 days in fetuses exposed at 117 days gestational age and for 10 days in the fetuses exposed at 127 days gestational age. At 137 days gestational age, the ewes and fetuses were sacrificed and cochleae removed and prepared for scanning electron microscopy.

Measurements of heart rate, electrocorticogram and electrooculography were simultaneously recorded on strip chart paper and FM magnetic tape. These measurements were obtained at least one hour before exposure to 20 impulses, during exposure and for at least one hour post exposure. The analog signals from the magnetic tape were subjected to spectral analyses in order to evaluate changes in behavioral state and heart rate responses produced by the stimulus.

We assumed that the impulses would be affected by transmission through maternal tissues and fluids, resulting in a reduction of peak sound pressure and a reduction in high-frequency spectral energy. We further postulated that fetal evoked potential thresholds would be elevated for low-frequency stimuli following exposure to impulses and scanning electron microscopy would reveal hair cell loss concentrated in the middle and apical turns. In addition, behavioral state cycling would be disrupted during and for a period of time immediately after the exposures.

### Results and Discussion

Shock Tube Construction. The shock tube that we are using for this project was obtained from the Army Research Laboratory at Aberdeen Proving Ground, MD. It was mounted into our sound-treated booth through a hole in the back of the room (Figure 1). The shock tube is 10 feet long with a 4 inch bore. An exponential horn as seen in Figure 2 was modeled after the horn used in the USAARL, Ft. Rucker, Alabama. The horn helps to match the impedance of the air at the end of the tube to that of the air in the sound-treated booth.

The inner walls of our 10'x 11' sound-treated booth have been covered with 4" sound absorbing material. We determined from tests performed in air with both a hydrophone and 1/8" microphone that 70 psi of nitrogen produces a peak sound pressure level (pSPL) of 170 dB at a distance of four feet from the horn. The waveform shown in Figure 3 was recorded with a hydrophone in the sound booth four feet from the horn and in-line with the flank of a pregnant ewe. The pSPL in this example is 169.6 dB and closely resembles a Freidlander wave. Approximately 30 ms after the over-pressure caused by the blast, two smaller peaks are consistently recorded. These paired positive peaks are reflections coming back down the shock tube and consequently cannot be eliminated. The pSPL of each peak is greater than 30 dB below the pSPL of the primary stimulus.

Evaluation of more than 120 impulses recorded both with and without the presence of a ewe averaged 169.7 dB pSPL with a standard deviation of less than 1 dB. The pSPL measured at other locations in the room varied by no more than 4 dB.

Impulse Transmission into the Uterus. Figure 4 includes a representative waveform of the impulse recorded with a hydrophone sutured near the pinna of the fetus *in utero*. What appears to be a negative peak in this waveform is actually positive. The second channel of the frequency analyzer inverts the waveform. Peak levels averaged 9.52 dB less when recorded *in utero* compared to levels recorded in air.

Waveforms, sampled for 100 ms, were processed through one-third octave-band filters from 100 to 10,000 Hz. The amplitude in each band was averaged across six animals. The averages were converted to decibels (re: 20  $\mu$ Pa) and plotted in Figure 5. The peak level for the impulse recorded in air occurred at 315 Hz compared to 160 Hz which was the peak level recorded from the uterus. Note that high-frequency sound pressure was attenuated by the tissues and fluids of the ewe, findings similar to those reported in pregnant sheep and humans from earlier studies (Gerhardt, et al. 1990; Peters, et al. 1993).

One complicating finding from these data is illustrated in Figure 6. The position of the hydrophone within the uterus appears to influence both pSPL as well as the spectral distribution of energies created by the impulse. This Figure includes two selected waveforms recorded from within the uterus. The upper panel in the Figure 6 is the waveform recorded with the hydrophone 2 inches from the surface of the flank. The fetal head is often in this position late in gestation. The peak levels were approximately 2 dB less than the peak levels recorded in air. With the hydrophone deep in the middle of the uterus, the morphology of the waveform changed as evidenced in the lower panel. Peak levels were 10 dB less than those recorded in air. We plan to investigate positional effects more thoroughly during the second year of this contract.

Evoked Potentials. Auditory brainstem response (ABR) and amplitude modulation following response (AMFR) recordings obtained from 12 fetal sheep are summarized. Of these animals, six were in the early blast exposure group and six were in the late blast exposure group. Figure 7 is a display of ABR waveforms obtained at a range of stimulus levels from a fetal sheep. ABR latency and thresholds and AMFR thresholds were measured prior to and following the blast exposures.

An example of AMFR results obtained from one of the fetal sheep is displayed in Figure 8. Determination of thresholds for the AMFR was based on the comparison of energy in the recordings at 50 Hz (the stimulus modulation frequency) to energy at other frequencies near 50



Hz. Threshold was defined as the lowest level at which energy at 50 Hz exceeded the average of that at four nearby frequencies (all below 100 Hz). Threshold is identified in this Figure with an arrow.

The mean ABR and AMFR thresholds across time for the late-exposed group of animals are shown in Figure 9. Elevations in the mean thresholds for the 1000 and 500 Hz stimuli are noted in the post-exposure measures. No similar elevations were noted for the higher frequency stimuli. Statistical analyses will be undertaken when measurements are completed on the full number of animals. In the early-exposed group of animals, average ABR and AMFR thresholds did not reveal any similar trend of post-exposure elevation of low-frequency thresholds. The average electrophysiologic thresholds for the early-exposed animals are displayed in Figure 10. ABR thresholds decrease over time, consistent with our previous description of developmental trends in fetal sheep.

Mean latency-intensity functions for wave IV of the click-evoked ABR across time are shown in Figure 11 for the late-exposed group of animals and Figure 12 for the early-exposed group. These functions show the well-known reduction in latency for higher level stimuli. In addition, developmentally appropriate trends appear as reduced latencies at the later measurement times. When data become available for the full number of animals in each group, statistical analyses will be undertaken to examine any exposure-related effects in the latency data, and whether or not any of these effects are level or age-dependent.

Histology. Cochleae from ten fetuses are in various stages of preparation for scanning electron microscopy. The first of these specimens was scanned and stored on disk in August, 1997. No cochleograms have been prepared at this time. The analysis of early- and late-noise exposed cochleae will be undertaken separately. At this time we have no information for early noise-exposed fetuses.

Behavioral State and Fetal Heart Rate. To date, we have collected behavioral state and heart rate data from eight fetuses. Measurements were obtained from the fetuses exposed to noise at 127 days gestational age. Behavioral state does not develop in the fetus until after about 120 days gestational age and therefore the fetuses exposed at 117 days gestational age were not evaluated. The strip charts and magnetic tapes have been sent to colleagues in Jena, Germany where analyses will be performed following protocols that have been developed for evaluation of spectral changes in electrocortical activity before, during and after exposure to vibroacoustic stimulation (Bauer et al., In Press).

### Recommendations Related to Statement of Work

Below is a summary of the tasks proposed in the Statement of Work:

#### Tasks Proposed:

#### Status:

Order ABR and AMFR recording equipment	Completed on schedule
Construct shock-tube	Completed on schedule
Test shock tube (air measurements)	Completed on schedule
Confirm shock tube signature in water	Completed on schedule
Collect data on ten animals (Study 1)	Completed on schedule
Submit annual Report	Completed on schedule
Collect data on 20 animals (Study 2)	Ahead of schedule
Evaluate histopathology	Ahead of schedule
Analysis data and submit reports to U.S. Army and the open-literature	April-September, 1998

Noise exposures have been completed on 12 animals and we are ahead of schedule with this portion of the project. Preliminary descriptive statistics have been generated for evoked potential data for all stimulus conditions. During the next year, the remainder of the animals will be tested and the statistical summaries up-dated. Histology is being assessed for the first few animals, and that work will continue throughout the fall. We are ahead of schedule with this portion of the project. Data collection and analysis will be more time-consuming than originally planned. We are forced to adjust to a tight schedule in order to gain access to the new scanning electron microscope in the University's Core Microscope Center, so this portion of the project will take more time than originally planned.

### **Conclusions**

After the first year, we have succeeded in producing a repeatable impulse exposure of 170 dB pSPL recorded in air and have exposed a total of 12 animals each to 20 impulses. The levels recorded at the head of the fetus *in utero* averaged 160.19 dB pSPL. We have identified that the position of the fetal head within the uterus influences the pSPL and we plan to explore this during the second year of funding. Recordings of evoked potential and behavioral state plus heart rate activity have progressed ahead of schedule. Cochleae from 15 fetuses have been collected for scanning electron microscopy and 10 have been prepared for processing. One cochlea has been scanned but the cochleograms have not been developed from the images.

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## **Appendix**

Figures 1 through 12.

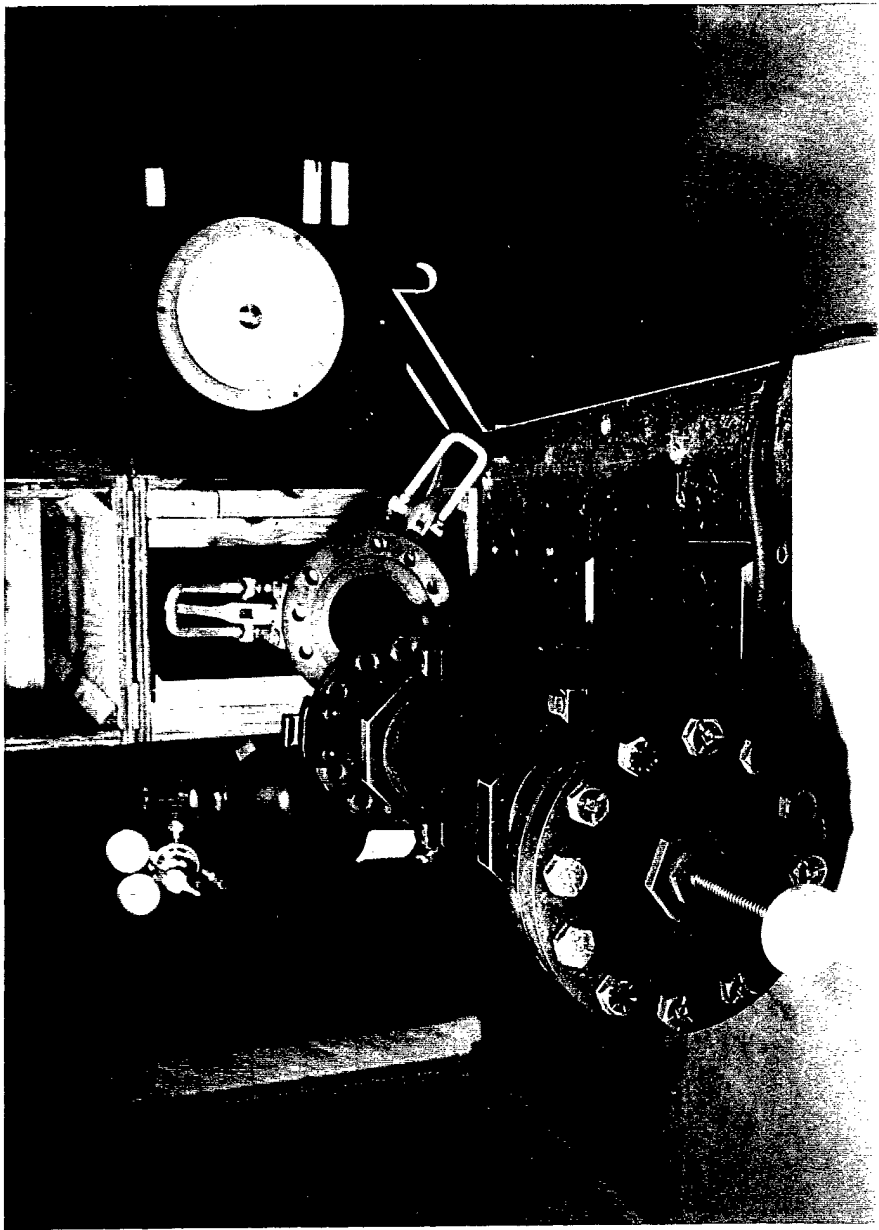


Figure 1. Photograph of the shock tube used to produce impulses of 170 dB peak sound pressure level (SPL).

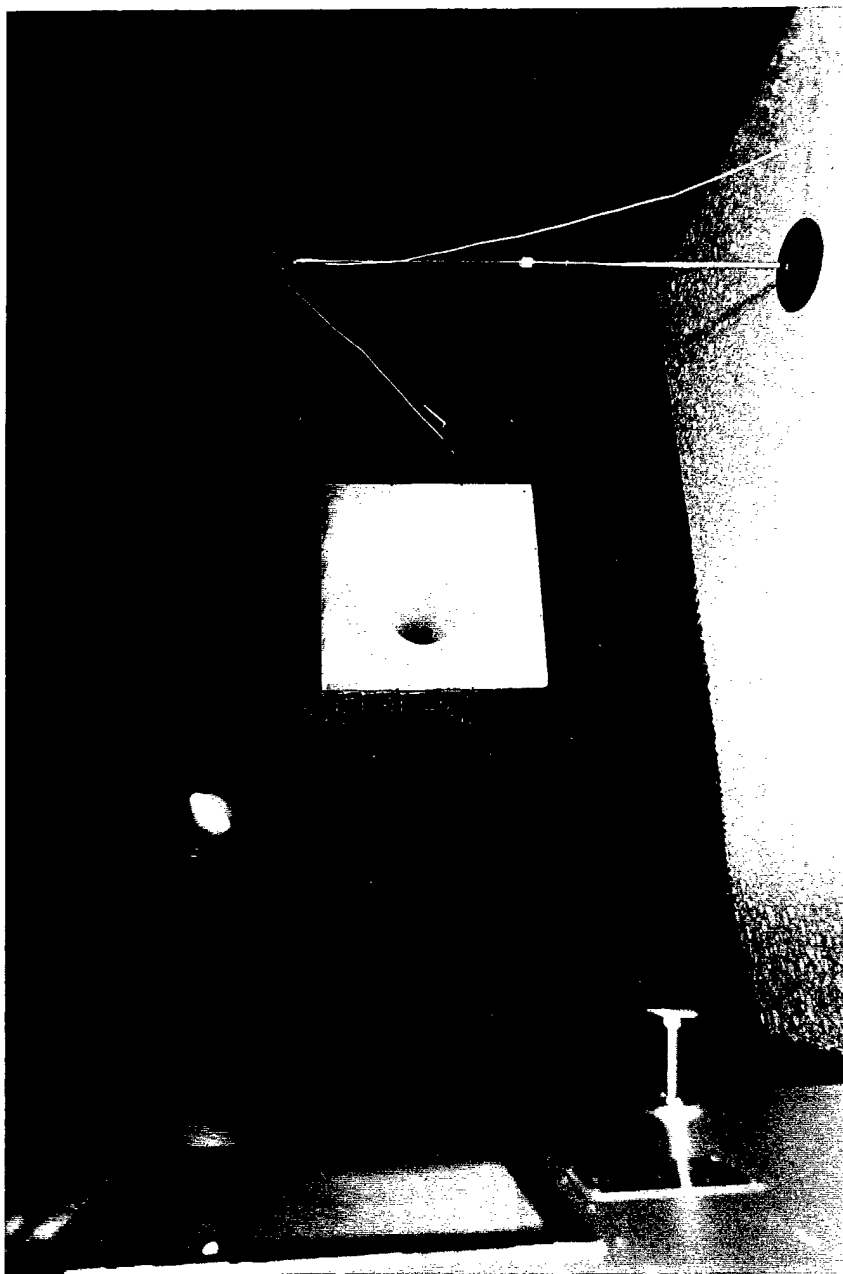


Figure 2. Photograph of the exponential horn fitted to the end of the shock tube. The horn has been mounted in a 10 x 11' sound-treated booth.

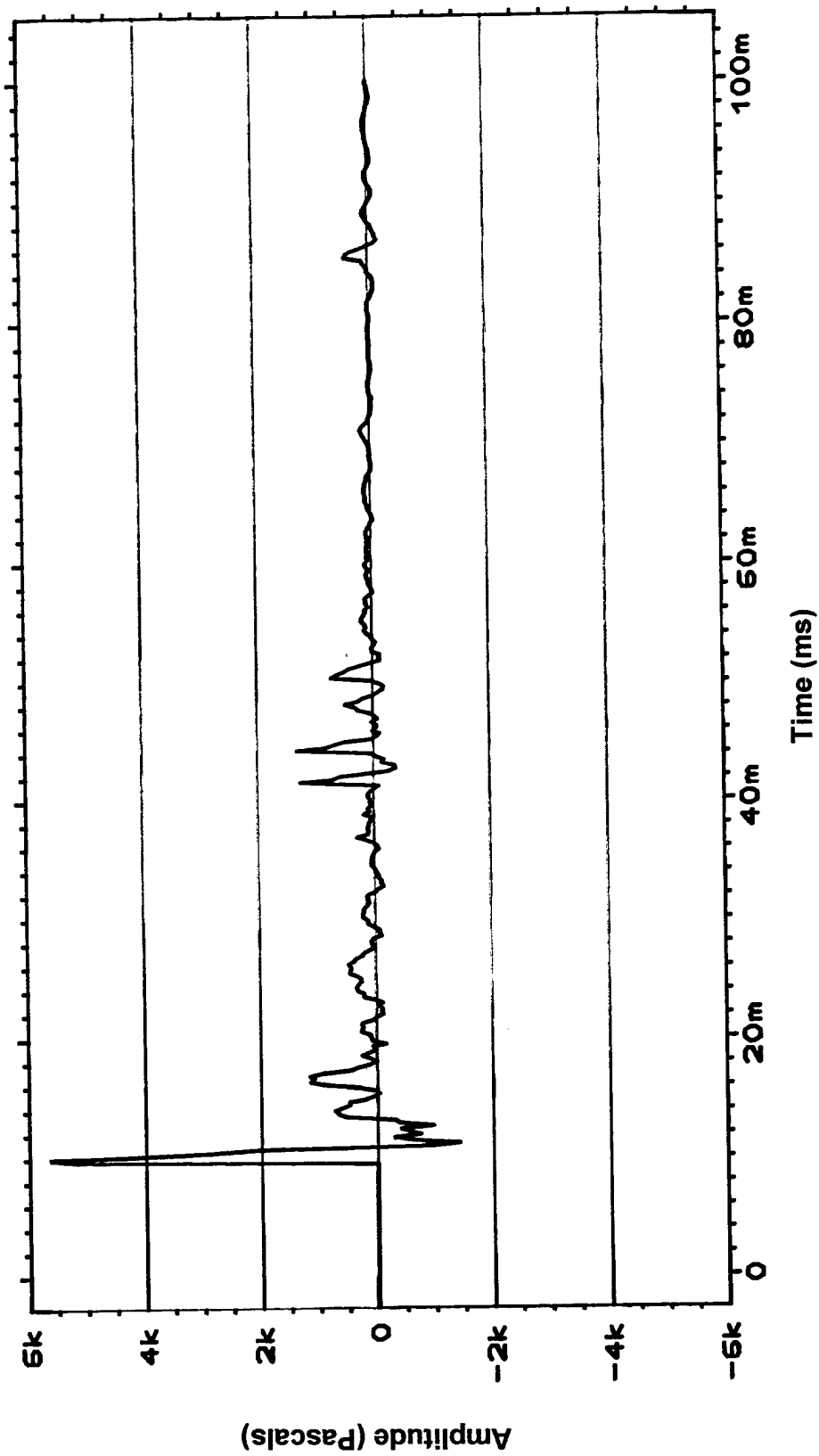


Figure 3. Waveform of the impulse from the shock tube recorded with a hydrophone positioned in the middle of the sound-treated booth. The peak SPL is 169.1 dB. Duration of the window is 100 ms. Positive deflection in this figure records positive pressure.

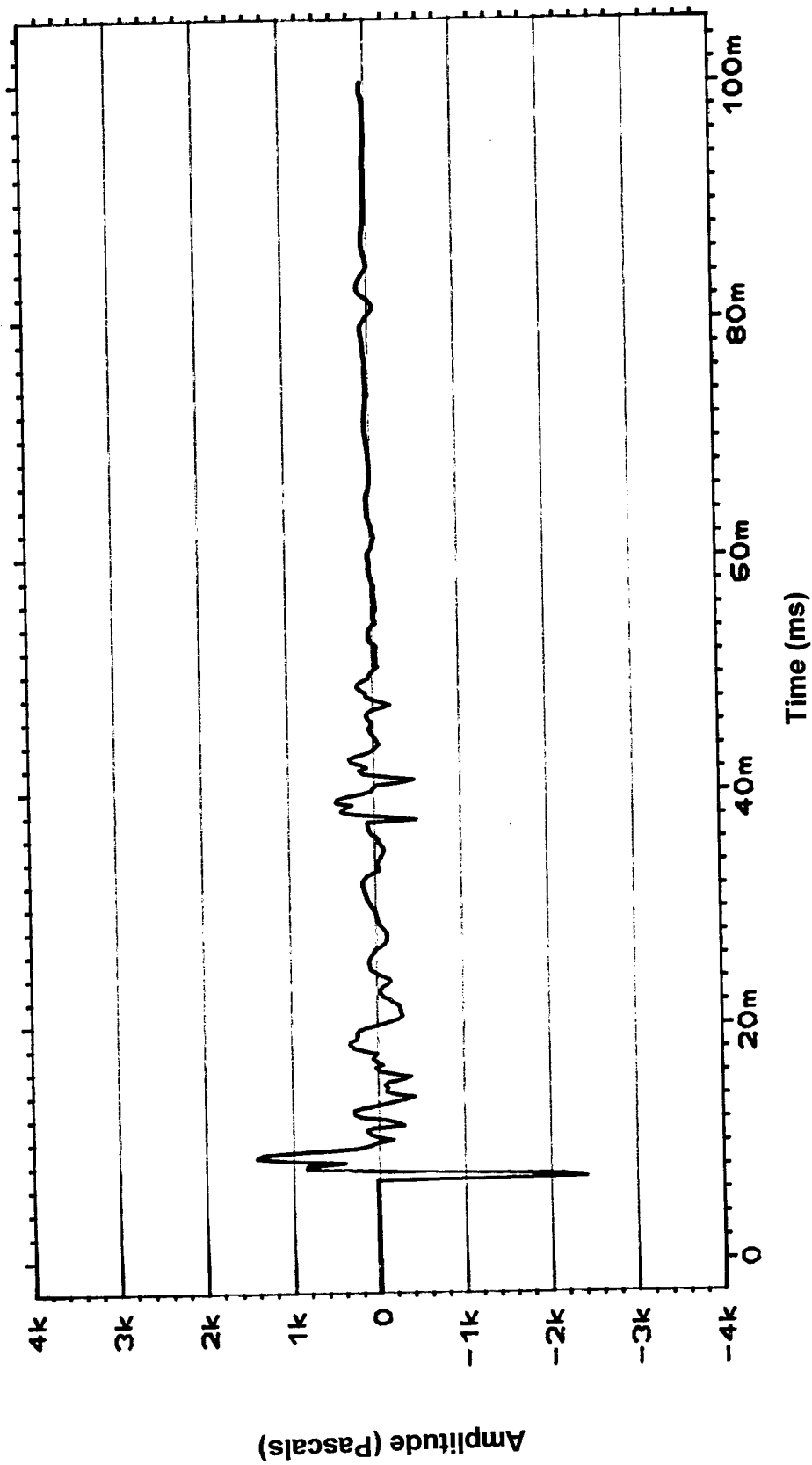


Figure 4. Waveform of the impulse recorded with a hydrophone positioned in the uterus of a pregnant sheep standing in the middle of the sound-treated booth. The peak SPL is 161 dB. Negative deflection in this figure records positive pressure.



# Spectra of Impulse Recorded in Air and Uterus

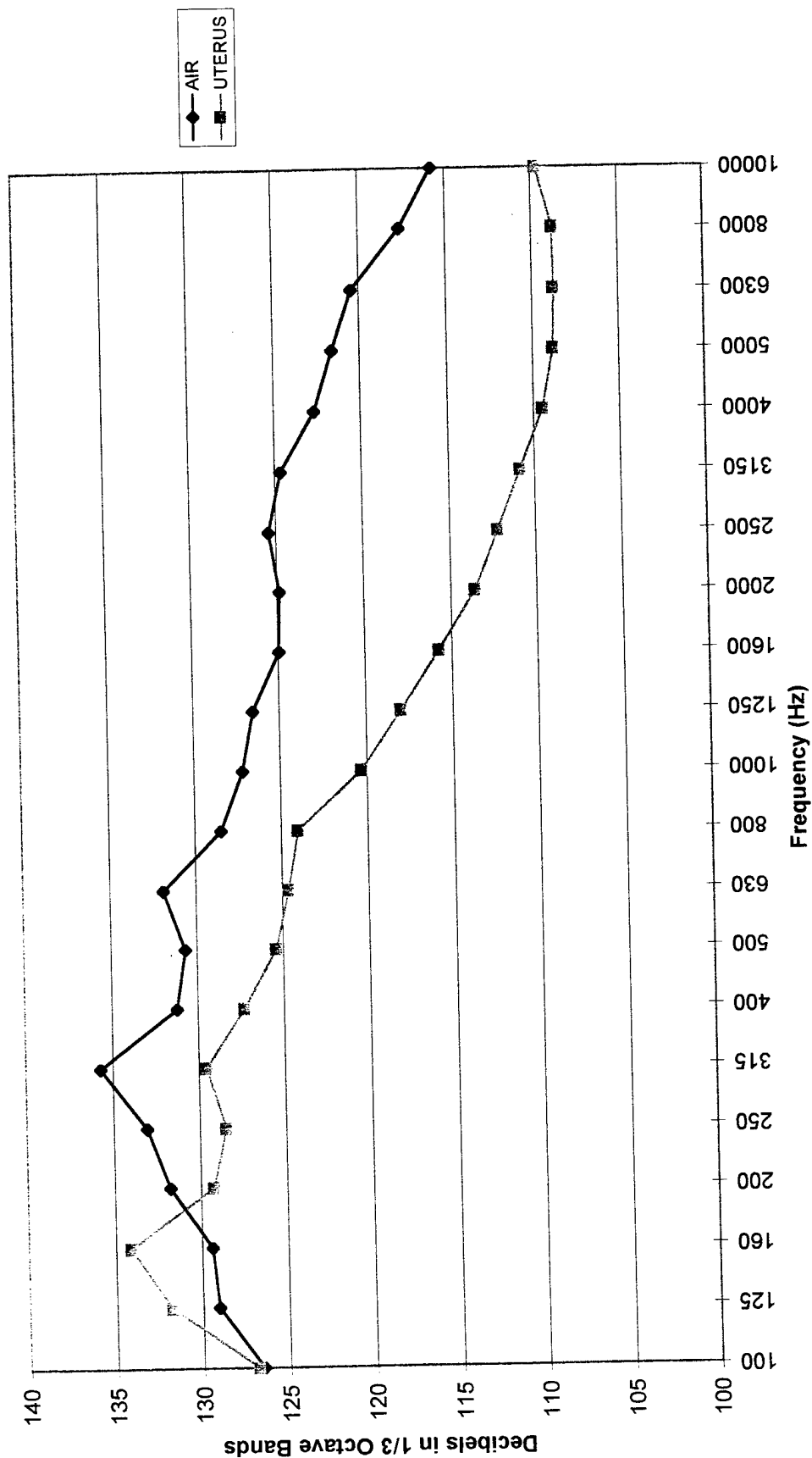


Figure 5. Spectra of impulses recorded in air and in the uterus of pregnant sheep. Each data point represents the average of 20 impulses from each of six ewes.

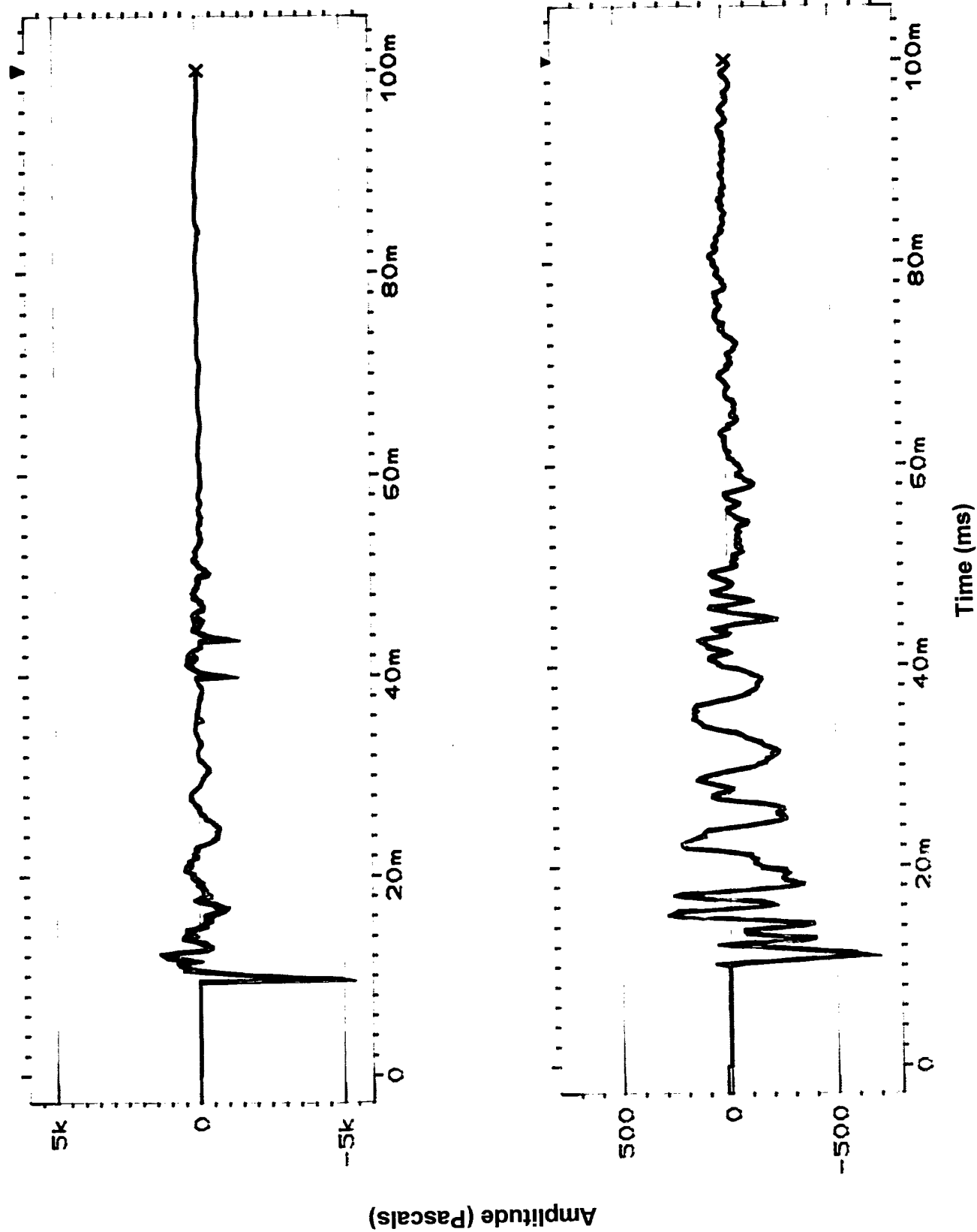


Figure 6. Two impulses recorded with a hydrophone positioned 2" inside the abdomen of a ewe (top panel) and at midline of the abdominal cavity (lower panel). Negative deflection in this figure records positive pressure.

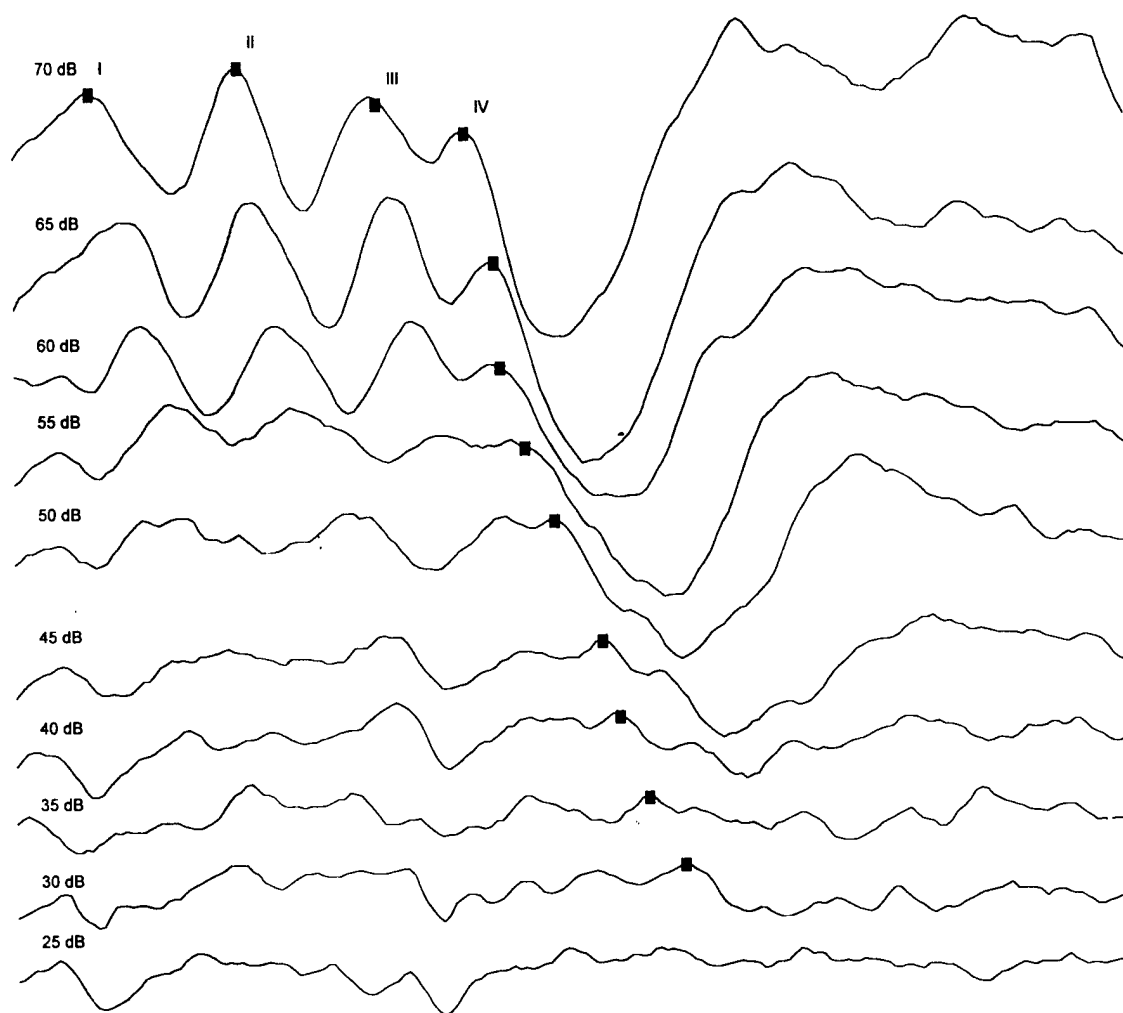


Figure 7. A series of auditory brainstem responses recorded with different stimulus levels from the same fetus. Waves I-IV are indicated along the top waveform and wave IV is marked along the subsequent waveforms. Threshold in this figure is 30 dB.

# AMFR Intensity Series

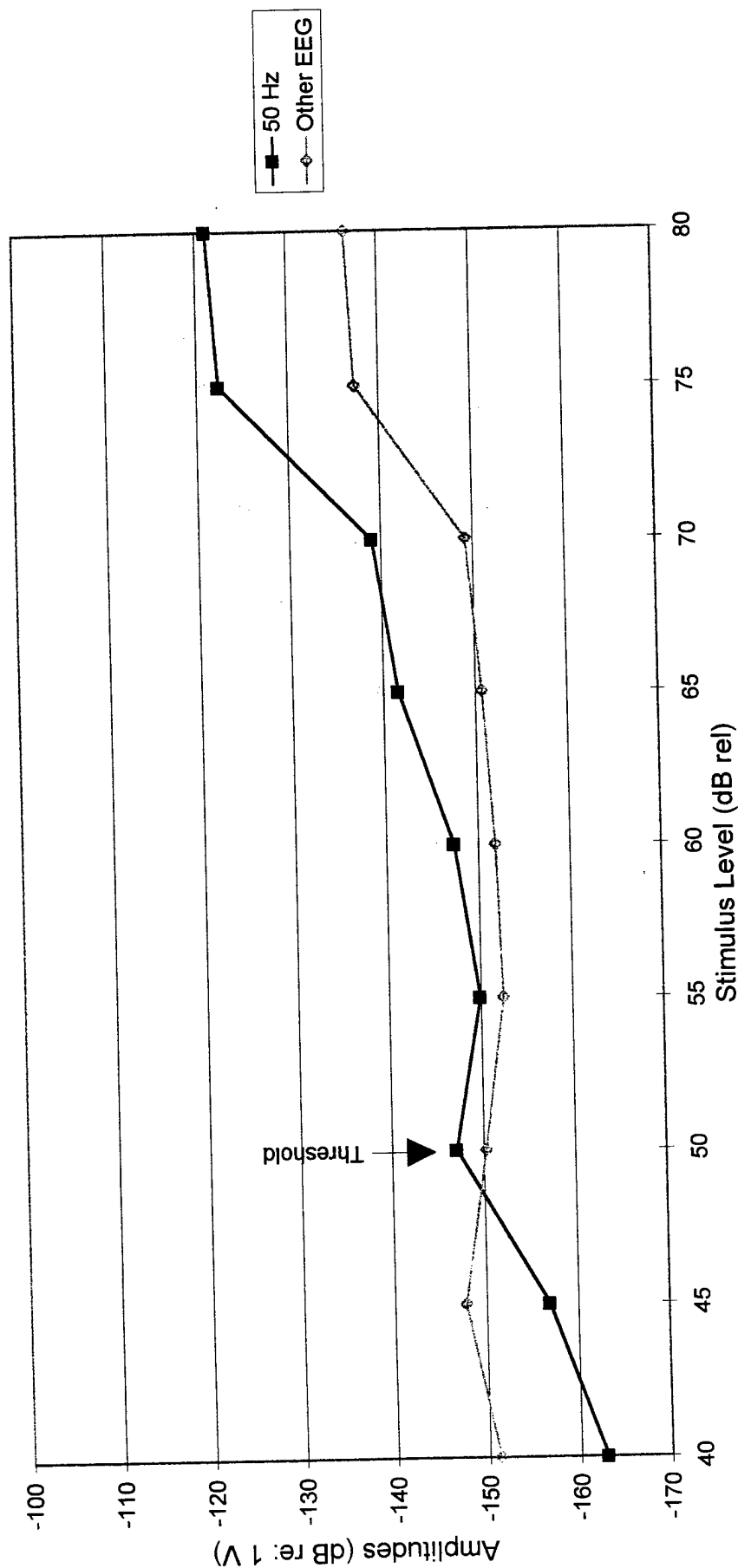
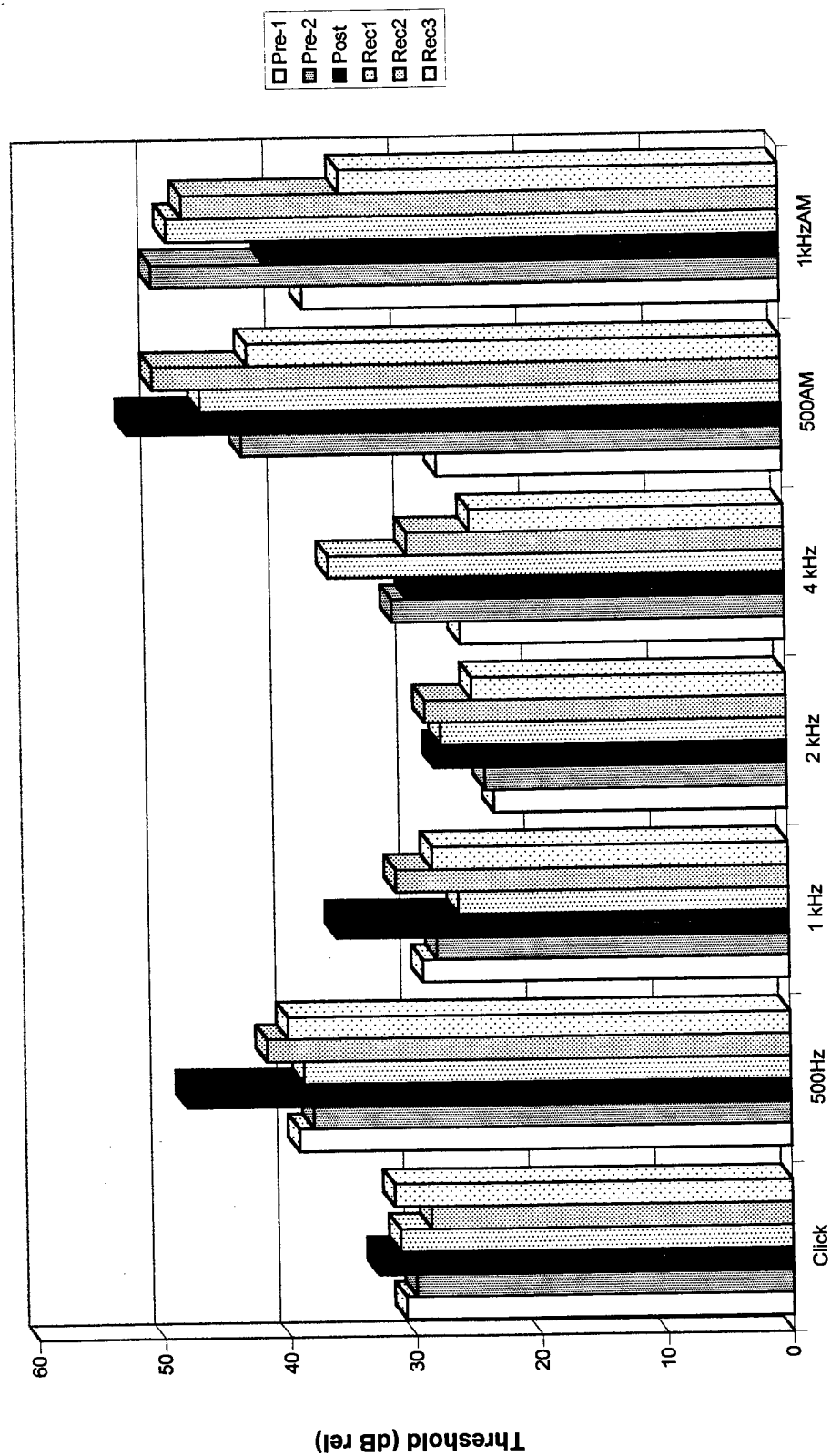


Figure 8. Amplitude Modulation Following Response (AMFR) recorded from one fetus. To determine the threshold of the AMFR (marked with arrow), the spectral energy at 50 Hz is compared to the average spectral energy in that frequency region.

## Electrophysiologic Thresholds - Late Group



### Stimulus

Figure 9. Evoked potential thresholds to various stimuli from fetal sheep exposed to impulses at 127 days gestational age. Measurements were obtained before (Pre-1 and2), immediately after (Post), and every 2 days following (Rec1-3) the exposure.

# Electrophysiologic Thresholds - Early Group

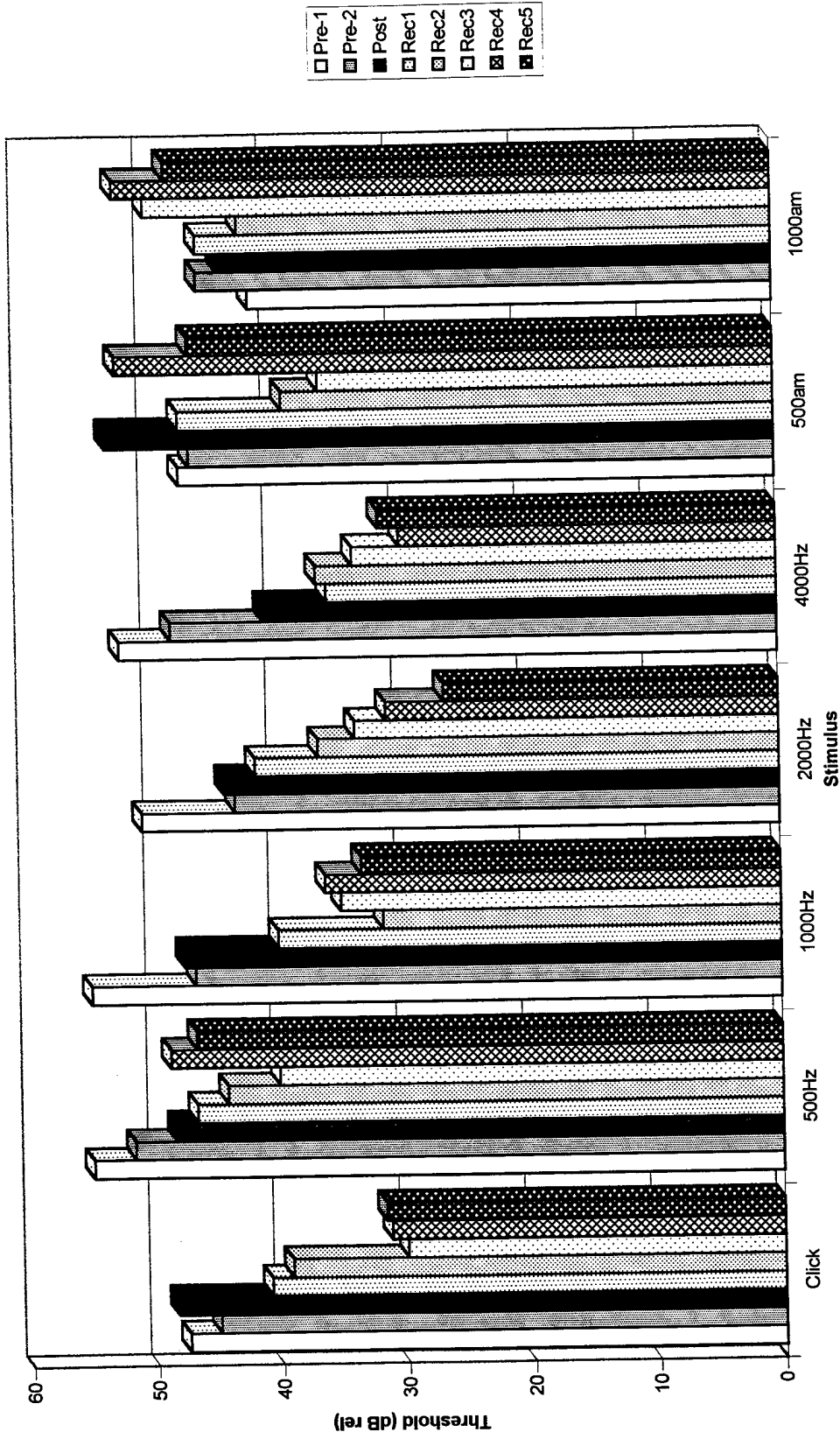


Figure 10. Evoked potential thresholds to various stimuli from fetal sheep exposed to impulses at 117 days gestational age. Measurements were obtained before (Pre-1 and 2), immediately after (Post) and every 2 days following (Rec1-5) the exposure.

# ABR WAVE IV LATENCIES - Late Group

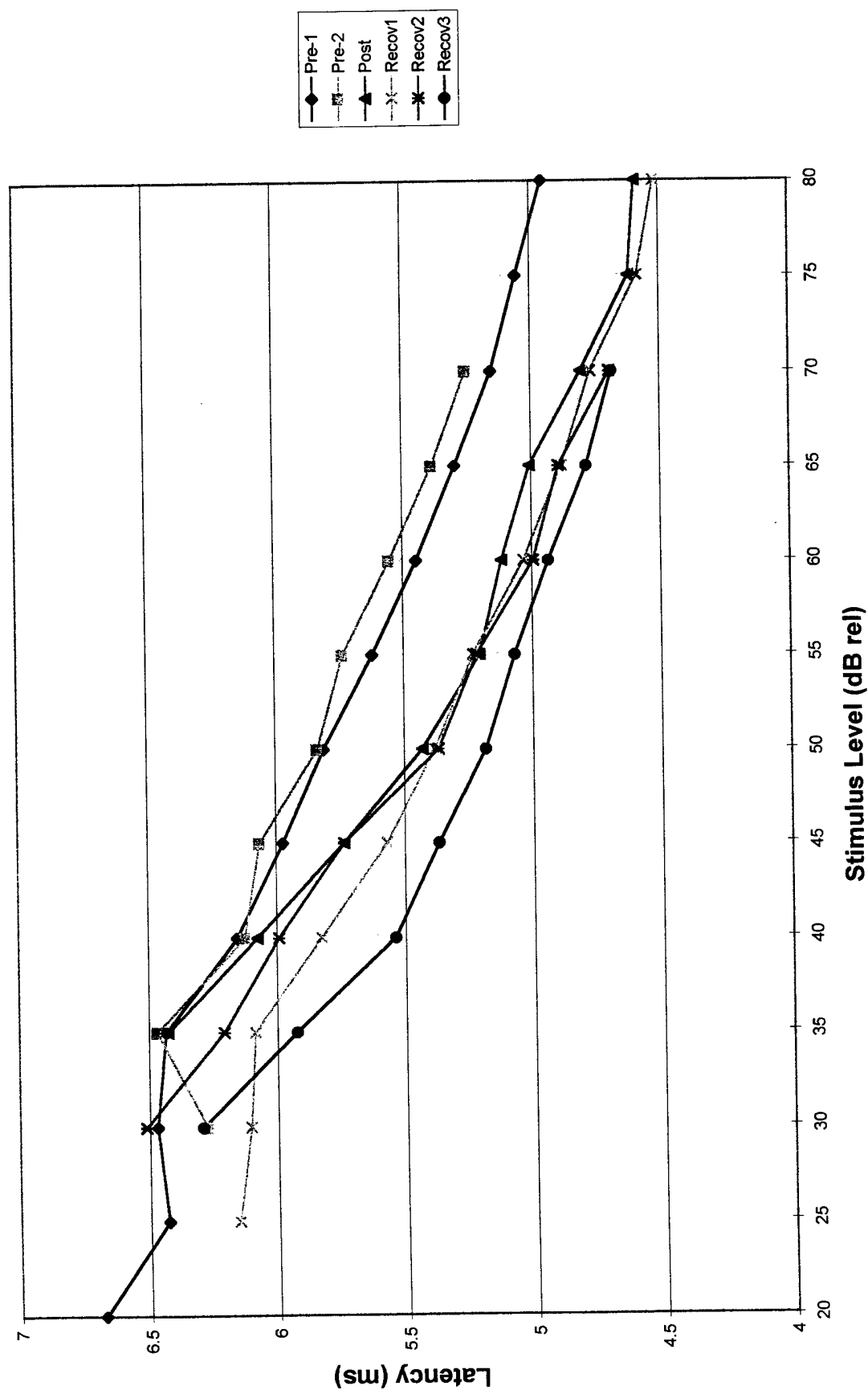


Figure 11. Click-evoked ABR latency-intensity functions from fetuses before (Pre-1 and 2), immediately after (Post) impulse exposure at 127 days gestational age, and every two days thereafter (recov1 through 3).

# ABR WAVE IV LATENCIES - Early Group

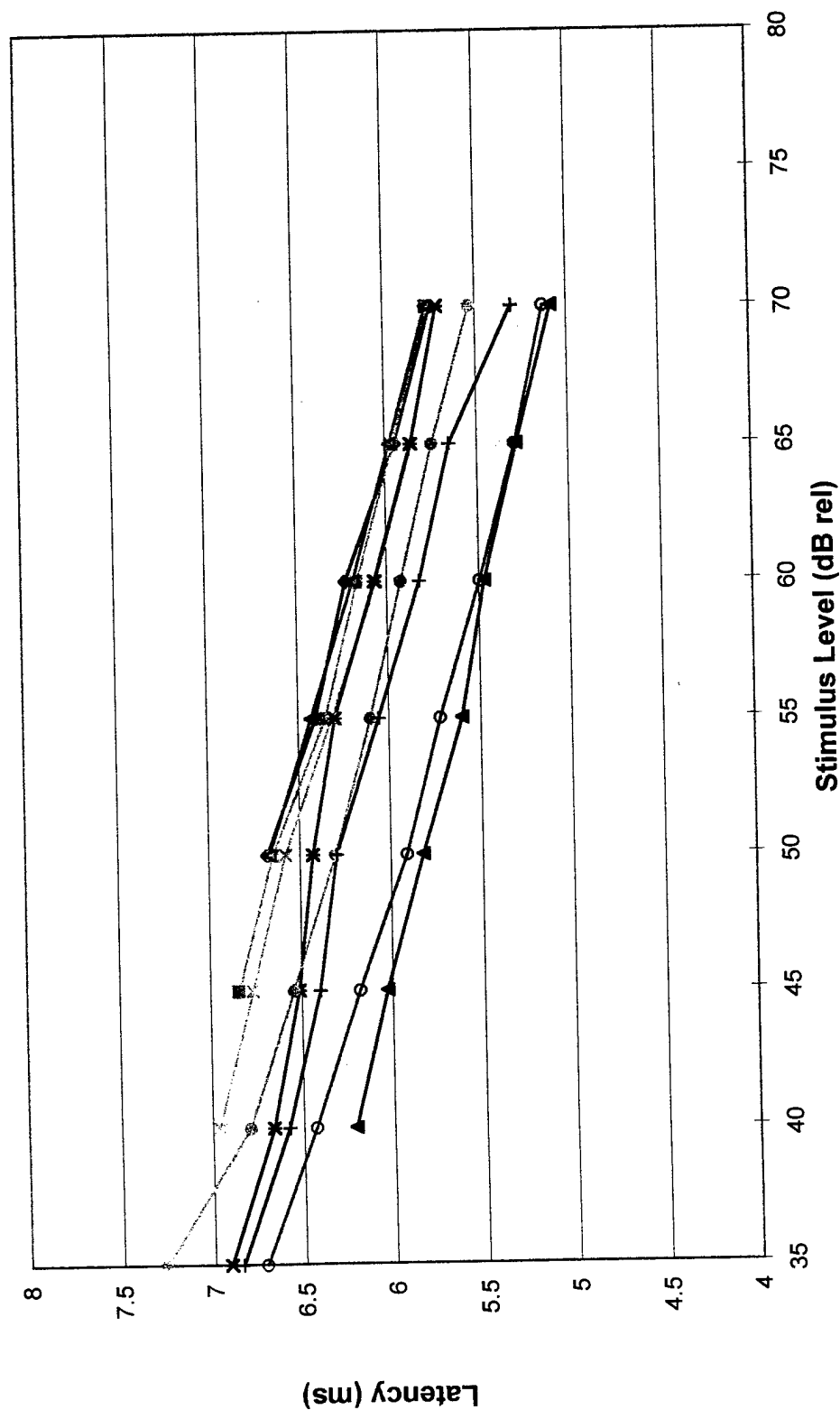


Figure 12. Click-evoked ABR latency-intensity functions from fetuses before (pre-1 and -2), immediately after (post) impulse exposure at 117 days gestational age, and every two days thereafter (recovery 1 through 6).