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ORIGINAL ARTICLE

Distortion Product Otoacoustic Emissions in Infant, Pregnant and Non-pregnant Adult Rabbits: Comparison for Different Stimulus Levels

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Objectives: In the present study, we investigated Distortion Product Otoacoustic Emissions in pregnant (Group 1); non-pregnat adult female rabbits (Group 2) and infant rabbits (Group 3). We assessed Distortion Product Otoacoustic Emission amplitudes in both stimulus levels of F2/F1=1.22 and 1.14; and analyzed the amplitude differences in different groups.

Methods: Thirty-six New Zealand White rabbits were included into the study. They were divided into three groups. Group 1 consisted of 9 each 13-month-old, adult, pregnant female rabbits. Group 2 consisted of 9 each 13-month-old, adult, non-pregnant female rabbits. Group 3 consisted of 18 each one-month-old, infant rabbits (Nine of them, male; and nine of them, female). In all groups, cochlear functions were assessed by Distortion Product Otoacoustic Emissions at 1.0-8.0 kHz. Stimulus parameters were used as F2/F1=1.22 in the first recording; and 1.14, in the second recording for each of the ears.

Results: In all groups (1 to 3), Distortion Product Otoacoustic Emission amplitudes were found as higher with F2/F1:1.22 measurements than F2/F1:1.14 measurements. In F2/F1:1.22; and F2/F1:1.14 measurements seperately; at each Distortion Product Otoacoustic Emission frequencies (1.0-8.0 kHz), the difference between Distortion Product Otoacoustic Emission amplitudes of Group1-3 were analyzed by "Kruskal Wallis Variance Analysis": The statistically significant difference were present at frequencies of 1.5-2.0 kHz and 8.0 kHz for F2/F1:1.22 measurements; and 1.0-2.0 kHz and 4.0-8.0 kHz for F2/F1:1.14 measurements. In F2/F1:1.22 measurements, at 1.5 kHz, the mean value of Group 1 (Pregnant rabbits) was significantly higher than that of Group 3 (Infant rabbits). In F2/F1:1.14 measurements, at 1.0, 4.0 and 8.0 khz, the mean values of Group 1 (Pregnant rabbits) was significantly higher than those of Group 3 (Infant rabbits); and at 1.0, 2.0 and 4.0 kHz, the mean values of Group 2 (Non-pregnant rabbits) were significantly higher than those of Group 3 (Infant rabbits)

Conclusion: Our study demonstrated that, in pregnant rabbits, higher corticosteroid levels may cause higher DPOAE amplitudes than infant rabbits by F2/F1:1.14 measurements. In all rabbits and especially in infant rabbits, Distortion Product Otoacoustic Emissions could be taken by F2/F1:1.22 measurements with higher amplitudes. The importance of our study is, when Distortion Product Otoacoustic Emission measurement is planned, measurements should be done using F2/F1: 1.22 to get healthy and accurate results in experimental studies. In measurements made by F2/F1: 1.14, amplitudes can be observed as lower than F2/F1: 1.22 measurements. This decline is evident especially in infant rabbit groups. Water containing medium in the middle ear of infant rabbits may cause the reduce in Distortion Product Otoacoustic Emission amplitudes than adult rabbits at both F2/F1:1.22 and 1.14 measurements.

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Introduction

Distortion product otoacoustic emissions (DPOAEs) provide relatively easily accessible external information about internal properties of the inner ear. A primary result is that the detection of normal DPOAE components is a strong indicator of normal functioning of the cochlear mechanics. The details of amplitude and phase behavior of the DPOAEs, and the development over time (DPOAE delay) are not fully understood and subject of some debate[1].

Detailed characteristics of the phase of the DPOAE have been described by Knight and Kemp[2-4], who noted that the phase gradient against frequency, obtained using fixed frequency ratio sweeps, is

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consistent with a combination of two different DPOAE emission components. They refer to these components as place- and wave-fixed, based on the assumed site of generation.

Distortion generated at the F2 place also propagates in the forward direction to the distortion product (DP) place, where it may be reflected. Zweig and Shera[5] have proposed a series of reflecting or scattering sites existing along the basilar membrane and a mechanism of coherent reflection involving the sharply tuned basilar membrane excitation pattern. As a stimulus is swept in frequency and its excitation pattern moves along the basilar membrane, the stimulus phase at the reflection site will change, thus changing the otoacoustic emission (OAE) phase and creating a steep gradient. Because these two components are generated by different processes, at different sites along the basilar membrane, it is of interest to understand whether they each change as a result of an interaction between outer hairy cells (OHC) and electromagnetic Field (EMF)[5,6].

The general assumption with a wave-fixed mechanism is that the emission is generated by distortion at a site that is an integral part of and moves smoothly with the stimulus travelling wave envelope in the cochlea as stimulus frequency is swept[7]. For the 2F1–F2 DP, the wave-fixed component is considered to be generated close to the F2 place on the basilar membrane and reaches the ear canal via a travelling wave propagating in the reverse direction along the basilar membrane. The phase at any point moving with the travelling wave envelope changes little; therefore, any OAE contribution from that point would have a shallow phase gradient using fixed frequency ratio sweeps.

DP-grams were recorded over a restricted frequency range and using frequency-scaled stimuli. Frequency sweeps were performed with the primary-frequency ratio F2/F1 and the F1 frequency step held constant. Specifically, the following stimulus parameters were used: L1/L2 of 60/50, F2/F1 ratio of 1.22, 1.15 or 1.05, and F1 frequency ranges of 1216–2432, 1280–2496, 1408–2624 Hz, respectively, with sweeps having steps of 32 Hertz (Hz)[6].

In the present study, we investigated DPOAEs in pregnant (Group 1); non-pregnat adult female rabbits (Group 2) and infant rabbits (Group 3). Stimulus parameters were used as F2/F1 was 1.22 in the first recording; and 1.14 was in the second recording for each of the ears. We assessed DPOAE amplitudes in both stimulus levels of 1.22 and 1.14 and analyzed the amplitude differences in different groups.

Materials and Methods

The study was assessed in Gazi University Faculty of Medicine. Adaptation and care of the animals were taken by Experimental Animal Breeding and Experimental Studies Center of Gazi University. During both adaptation and experiment periods, the animals were treated in compliance with the principles of the Declaration of Helsinki[8].

Animal Subjects

Animals in the study were consisted of three groups:

1. **Group 1 :** Nine each 13-month-old, adult, pregnant, New Zealand White female rabbits
2. **Group 2 :** Nine each 13-month-old, adult, non-pregnant, New Zealand White female rabbits
3. **Group 3 :** Eighteen each one-month old New Zealand infant rabbits (Nine of them, male; and nine of them, female)

These 36 rabbits were used in the present study. Rabbits were obtained from Laboratory Animals Breeding and Experimental Researches Center of Gazi University. The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All the animal procedures were performed in accordance with the approved protocol.

Rabbits were housed under the same conditions in temperature and humidity controlled room (20±1°C, 50 ± 10% relative humidity) and 14-16 h light/dark cycle conditions. Except during exposure periods, tap water and standard pelletized food are provided ad libitum.

Experimental Design

DPOAE Recordings

Prior to the experimental DPOAE measurements, ear examination of the infant and adult rabbits was managed by otoscope and any foreign body, if present, was removed from the external auditory canal by curette. The animals having external auditory canal or eardrum pathologies that could prevent noise transmission were excluded from the study. Included animals were anesthetized both during examinations and experiments via intramuscular injection of 40 mg/kg ketamine hydrochloride [(Ketalar, Parke-Davis, United States of America (USA)] and 5 mg/kg

xylazine hydrochloride (Rompun, Bayer, Germany). Eye-blink reflexes and respiratory rhythms were followed during the experiments and deep anesthesia was achieved by repeated doses. Earlier studies investigated the effects of certain types of anesthesia (acetylpromazine-ketamine, xylazine-ketamine, and sodium pentobarbital), and it was shown that these agents did not differentially alter the DPOAE onset levels[9]. It could be said that in the present study, the anesthetic agent ketamine hydrochloride did not significantly affect the OAE amplitudes.

The recordings were performed in an isolated quiet environment and the female rabbits were followed to be totally sedated and motionless condition with regular spontaneous breathing, in order to minimize the noise contamination originating from the environment or the muscular activity of the animals. The plastic tubing adapters that presented the optimum fit to the external auditory canal were attached to the emission probe and a closed cavity was formed by placement of the probe into the external auditory canal.

In DPOAE recordings, two F2/F1 values were used:

1. DPOAE is generated in 72 ears of 36 rabbits by ILO 288 USB II (Otodynamics Ltd Clinical OAE System, England) cochlear emission analyzer. The acoustic stimulus consisted of two simultaneous continuous pure tones at different frequencies; F1 and F2 (F2/F1: 1.22). The general trend indicates that (for the 2F1–F2 DPOAE) there tends to be a predominance of the wave-fixed component if F2/F1 is equal to 1.22[6]. Intensities are L1 [dB sound pressure level (SPL)] and L2 (dB SPL)[10]. DP-grams were recorded over a restricted frequency range and using frequency-scaled stimuli. Frequency sweeps were performed with the primary-frequency ratio F2/F1 and the F1 frequency step held constant[6]. Specifically, the following stimulus parameters were usually used: L1/L2 of 65 dB SPL/55 dB SPL, F2/F1 ratio of 1.22[10]. F1 frequency ranges of 1216–2432, with sweeps having steps of 32Hz[6].

In our study, with L1=65 dB SPL and L2=55 dB SPL, the responses received were not sufficient. It is known that the variations in the external physical dimensions, such as the length and width of the ear canal (a wider ear canal in rabbits than in humans) and the differences in the depth of probe insertion, lead to complicating problems throughout the otoacoustic measurement; thus, they should be corrected (11-13). In view of this fact, the intensity of the stimuli was increased to L1=80 dB SPL and L2=70 dB SPL.

In the literature, it was reported that, for all experimental conditions, the frequency ratio was set to f2/f1 1.22[14].

2. The second DPOAE recordings were performed with the acoustic stimulus consisted of two simultaneous continuous pure tones at different frequencies; F1 and F2 (F2/F1: 1.14).

DPOAE recordings and assessments were performed at the laboratories of Physiology Discipline of Gazi University Medical School.

Method

In three groups (Group 1, 2 and 3), after infant and adult rabbits were kept in plexiglas cage; the rabbits were anesthetized, otoscopic examinations were performed; and DPOAE measurements were assessed both ears (Right and left) of each rabbits. F2/F1 was 1.22 in the first recording; and 1.14 was in the second recording for each of the ears.

Study were planned and continued in accordance with Gazi University Faculty of Medicine Local Ethics Committee.

Statistical Analysis: Statistical packet for SPSS (Version 9.0) was used for statistical evaluation.

In each of the groups (Group 1; Group 2; and Group 3), at each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of F2/F1:1.22 and 1.14 recordings were analyzed by Mann Whithey U Test.

In F2/F1:1.22; and F2/F1:1.14 measurements seperately; at each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of Group1-3 were analyzed by "Kruskal Wallis Variance Analysis". p value < 0.05 was considered as statistically significant.

When statistically significant result was present, "Wilcoxon Matched-Pairs Signed-Ranks Test" with Bonferroni correction was used to detect the time of value which had caused difference. p value < 0.0175 was considered as statistically significant.

Results

Table 1 and Figure 1-3 demonstrates DPOAE amplitudes of all groups (Group 1-3) which were recorded with F2/F1:1.22; and F2/F1:1.14 at 1.0 to 8.0 kHz . In each of the groups (Group 1; Group 2; and Group 3), at each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of F2/F1:1.22 and 1.14 recordings were analyzed by Mann Whithey U Test (Table 1):

Table 1. DPOAE Amplitudes of Groups at 1.0-8.0 kHz

Groups	F2/F1	DPOAE Amplitudes						
		1.0 kHz	**1.5 kHz**	**2.0 kHz**	**3.0 kHz**	**4.0 kHz**	**6.0 kHz**	**8.0 kHz**
Group 1	**1.22**	5.06±7.71	18.71±13.59	22.80±21.75	19.57±22.59	32.21±23.86	26.14±24.99	17.78±25.09
(Pregnant	**1.14**	10.24±8.17	13.96±15.83	16.63±20.43	15.66±17.40	43.41±26.07	9.93±20.75	-0.983±18.26
Rabbits)	**p***	0.111	0.323	0.051	0.214	0.143	0.002	0.005
Group 2	**1.22**	8.67±9.29	17.67±19.39	26.10±19.08	18.10±19.06	35.13±22.03	26.07±24.27	18.52±29.63
(Non-pregnant	**1.14**	12.07±9.25	15.91±16.34	18.99±20.11	14.08±15.92	48.92±23.26	8.17±22.49	0.65±20.71
Adult Female	**p***	0.355	0.308	0.020	0.279	0.008	0.001	0.010
Rabbits)								
Group 3	**1.22**	4.18±5.85	9.28±7.02	17.72±8.62	26.75±11.99	31.90±11.58	30.55±13.25	30.85±17.80
(Infant rabbits)	**1.14**	0.66±5.17	2. 63±6.31	5.92±8.79	14.03±9.43	20.03±12.44	-0.54±9.85	-13.61±12.20
	p*	0.002	0.000	0.000	0.000	0.000	0.000	0.000
Kruskal-Wallis Variance Analysis								
	$p^{1.22}$	0.281	0.008	0.022	0.220	0.244	0.201	0.041
	$p^{1.14}$	0.000	0.006	0.005	0.415	0.000	0.008	0.002

p shows the results of Mann Whitney U test*

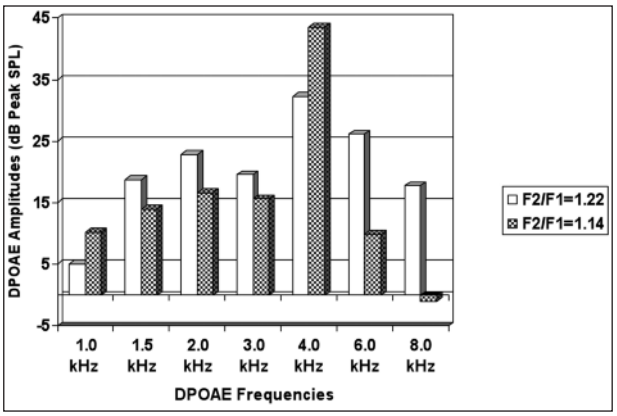

Figure 1. DPOAE amplitudes of Group 1 (Pregnant Rabbits) at 1.0-8.0 kHz** At 6.0 (p=0.002) and 8.0 kHz (p=0.005), the difference was significant by Mann Whitney U Test

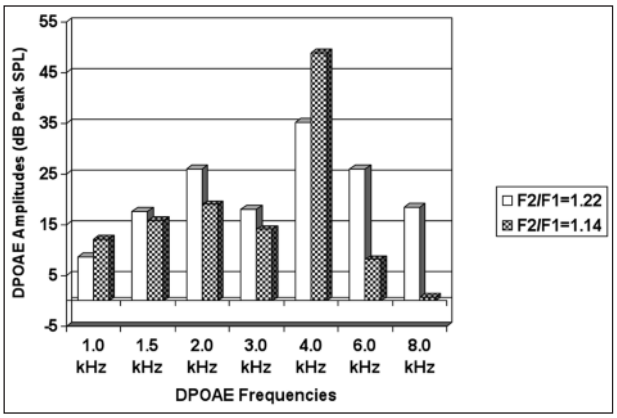

Figure 2. DPOAE amplitudes of Group 2 (Non-pregnantAdult Female Rabbits) at 1.0-8.0 kHz* * At 2.0 kHz (p=0.020), 4.0 kHz (p=0.008), 6.0 (p=0.001) and 8.0 kHz (p=0.010), the difference was significant by Mann Whitney U Test.

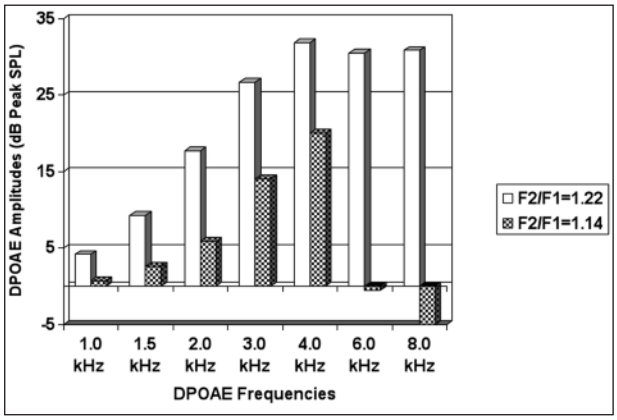

Figure 3. DPOAE amplitudes of Group 3 (Infant Rabbits) at 1.0-8.0 kHz**At 1.0 kHz (p=0.002), 1.5 kHz (p=0.000), 2.0 kHz (p=0.000), 3.0 kHz (p=0.000), 4.0 kHz (p=0.000), 6.0 (p=0.000) and 8.0 kHz (p=0.000), the difference was significant by Mann Whitney U Test.

- In pregnant rabbits (Group 1), at frequencies of 6.0 (p=0.002); and 8.0 kHz (p=0.005), DPOAE amplitudes were significantly higher; and at 1.5-3.0 kHz, non-significantly higher with F2/F1:1.22 measurements than F2/F1:1.14 measurements (See on Table 1).

- In non-pregnant adult rabbits (Group 2), at frequencies of 2.0 kHz (p=0.020); and 4.0 kHz (p=0.008), 6.0 kHz (p=0.001) and 8.0 kHz (p=0.010), DPOAE amplitudes were significantly higher; and at 1.0 and 3.0 kHz, non-significantly higher with F2/F1:1.22 measurements than F2/F1:1.14 measurements (p<0.05) (See on Table 1).

-In Infant rabbits (Group 3), at all frequencies (1.0-8.0 kHz), DPOAE amplitudes were significantly higher with F2/F1:1.22 measurements than F2/F1:1.14 measurements (p<0.05) (See on Table 1).

In F2/F1:1.22; and F2/F1:1.14 measurements separately; at each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of Group1-3 were analyzed by "Kruskal Wallis Variance Analysis":

-For F2/F1:1.22 measurement, the statistically significant difference were present at frequencies, namely 1.5 kHz (p=0.008); 2.0 kHz (p=0.022); and 8.0 kHz (p=0.041) (See on Table 1). The confidence interval was 99 %.

-For F2/F1:1.14 measurement, the statistically significant difference were present at frequencies, namely 1.0 kHz (p=0.000); 1.5 kHz (p=0.006); 2.0 kHz (p=0.005); 4.0 kHz (p=0.000); 6.0 kHz (p=0.008); and 8.0 kHz (p=0.002) (See on Table 1). The confidence interval was 99 %.

When statistically significant result was present, "Wilcoxon Matched-Pairs Signed-Ranks Test " with Bonferroni correction was used to detect the time of value which had caused difference (See on Table 2) The confidence interval was 99 % :

In F2/F1:1.22 measurement:

At 1.5 kHz, the mean DPOAE value of Group 1 (Pregnant rabbits) (5.06 dB Peak SPL) was significantly higher than that of Group 3 (Infant rabbits) (4.18 dB Peak SPL) (p=0.015) (Table 2).

In F2/F1:1.14 measurement:

-At 1.0 kHz, the mean DPOAE values of Group 1 (Pregnant rabbits) (10.24 dB Peak SPL) (p=0.002); and Group 2 (Non-pregnant rabbits) (12.07 dB Peak SPL) (p=0.001) were significantly higher than that of Group 3 (Infant rabbits) (0.66 dB Peak SPL) (Table 2).

-At 1.5 kHz, the mean DPOAE value of Group 2 (Non-pregnant rabbits) (15.91 dB Peak SPL) was significantly higher than that of Group 3 (Infant rabbits) (2.63 dB Peak SPL) (p=0.012) (Table 2).

-At 2.0 kHz, the mean DPOAE value of Group 2 (Non-pregnant rabbits) (18.99 dB Peak SPL) was significantly higher than that of Group 3 (Infant rabbits) (5.92 dB Peak SPL) (p=0.011) (Table 2).

-At 4.0 kHz, the mean DPOAE values of Group 1 (Pregnant rabbits) (43.41 dB Peak SPL) (p=0.001); and Group 2 (Non-pregnant rabbits) (48.92 dB Peak SPL) (p=0.004) were significantly higher than that of Group 3 (Infant rabbits) (20.03 dB Peak SPL) (Table 2).

-At 8.0 kHz, the mean DPOAE value of Group 1 (Pregnant rabbits) (-0.983 dB Peak SPL) was significantly higher than that of Group 3 (Infant rabbits) (-13.61 dB Peak SPL) (p=0.006) (Table 2).

Table 2. The results of Wilcoxon Signed Ranks Test with Bonferroni Correction

Frequencies of DPOAE Recordings							
F2/F1=1.22		**1.5 kHz**	**2.0 kHz**				**8.0 kHz**
Group1-Group2		0.948	0.586				0.744
Group1-Group3		0.015	0.372				0.093
Group2-Group3		0.074	0.094				0.758
Frequencies of DPOAE Recordings							
F2/F1=1.14	**1.0 kHz**	**1.5 kHz**	**2.0 kHz**		**4.0 kHz**	**6.0 kHz**	**8.0 kHz**
Group1-Group2	0.513	0.085	0.679		0.616	0.913	0.811
Group1-Group3	0.002	0.170	0.018		0.001	0.071	0.006
Group2-Group3	0.001	0.012	0.011		0.004	0.306	0.022

Discussion

Otoacoustic emissions (OAEs) have become a commonly used clinical tool for assessing cochlear health status, in particular, the integrity of the cochlear amplifier or motor component of cochlear function. Predicting hearing thresholds from OAEs, however, remains a research challenge. Models and experimental data suggest that there are two mechanisms involved in the generation of OAEs. For distortion product, transient, and high-level stimulus frequency emissions, the interaction of multiple sources of emissions in the cochlea leads to amplitude variation in the composite ear canal signal. Multiple sources of emissions complicate simple correlations between audiometric test frequencies and otoacoustic emission frequencies[15].

Steep and shallow phase gradients have been observed in the 2F1–F2 DP (4) depending on whether a large or small frequency ratio is used. In particular, for a small frequency ratio, the phase gradient is steep, consistent with a predominantly place-fixed emission mechanism, while with a larger frequency ratio, the phase gradient becomes shallow and is more consistent with a wave-fixed mechanism.

The distortion products have been obtained using two stimuli tones with a ratio between the frequencies f2/fl = 1.22 and amplitudes A,=A, from 70 dB SPL down to the threshold, as suggested in[16]. The Distortion products at the frequency 2f1-f2 were therefore examined. In order to analyze the effects, for each experiment were carried out the DP-gram (distortion product gram) and the DP growth functions. For each DP gram the stimuli tones were varied across a wide range of frequencies, maintaining a constant f2/f1 ratio, and the 2f1-f2 distortion products were plotted as a function of the f2 frequency stimulus. For the DP growth functions the intensity of the two stimuli tones is decreased and the amplitude of 2f,-f2 distortion products was plotted versus the f2 stimuli intensity[17].

Stimulus conditions for the DPOAE sweeps may be summarized as[6]: (1) When F1=1216–2432Hz and F1 Frequency step = 32 Hz, F2/F1 = 1.22. (2) When F1 = 1280–2496 Hz and F1 Frequency step = 32 Hz, F2/F1 = 1.15. (3) When F1 = 1408–2624 Hz and F1 Frequency step=32Hz, F2/F1=1.05.

In the present study, we investigated DPOAEs in pregnant (Group 1); non-pregnant adult female rabbits (Group 2) and infant rabbits (Group 3). Stimulus parameters were used as F2/F1 was 1.22 in the first recording; and 1.14 was in the second recording for each of the ears. We assessed DPOAE amplitudes in both stimulus levels of 1.22 and 1.14 and analyzed the amplitude differences in different groups. In all groups (1 to 3), DPOAE amplitudes were found as higher with F2/F1:1.22 measurements than F2/F1:1.14 measurements.

In F2/F1:1.22; and F2/F1:1.14 measurements seperately; at each DPOAE frequencies (1.0-8.0 kHz), the difference between DPOAE amplitudes of Group1-3 were analyzed by "Kruskal Wallis Variance Analysis": The statistically significant difference were present at frequencies of 1.5-2.0 kHz and 8.0 kHz for F2/F1:1.22 measurements; and 1.0-2.0 kHz and 4.0-8.0 kHz for F2/F1:1.14 measurements.

In F2/F1:1.22 measurements, at 1.5 kHz, the mean value of Group 1 (Pregnant rabbits) was significantly higher than that of Group 3 (Infant rabbits). In F2/F1:1.14 measurements, at 1.0, 4.0 and 8.0 khz, the mean values of Group 1 (Pregnant rabbits) was significantly higher than those of Group 3 (Infant rabbits); and at 1.0, 2.0 and 4.0 kHz, the mean values of Group 2 (Non-pregnant rabbits) were significantly higher than those of Group 3 (Infant rabbits). Water containing medium in the middle ear of infant rabbits may cause the reduce in DPOAE amplitudes than adult rabbits at both F2/F1:1.22 and 1.14 measurements.

The possible explanations for higher DPOAE amplitudes in pregnant rabbits than infant rabbits by F2/F1:1.22; and F2/F1:1.14 measurements may be summarized as:

In pregnant rabbits, volume increase in the inner ear and total body are seen. Chen and Nathans showed that estrogen-related receptor beta (ERR-beta; NR3B2), an orphan nuclear receptor, is specifically expressed in and controls the development of the endolymph-producing cells of the inner ear: the strial marginal cells in the cochlea and the vestibular dark cells in the ampulla and utricle[18]. As estrogen increased during the pregnancy, endolymph production by stria vascularis also increase. In pregnancy, specifically estriol (E3) and progesterone hormones increase[19]. Since estrogen receptors are present in the inner ear; increased estriol levels and estriol-binded estrogen receptors may affect DPOAE levels. Meltser,

et al.[20] examined the role of estrogen receptors (ERs) in response to auditory trauma. They found a ligand-dependent protective role for ERbeta in the auditory system by investigating mice deficient in ERalpha, ERbeta, and aromatase. Their data indicated that ERbeta-mediated neuroprotection involving brain-derived neurotrophic factor (BDNF) in the auditory system of females.

Plasma CRH increases progressively during the second and third trimester, peaking at delivery. The placenta is the likely source[21]. ACTH concentrations also increase during pregnancy and may be of placental origin[22]; the diurnal variation of blood cortisol and ACTH, although blunted, is maintained. Corticosteroid-binding globulin concentrations increase by three times during pregnancy, resulting in an increase in the total plasma cortisol and a fall in its metabolic clearance. The unbound fraction also increases, however, and this is reflected by a rise in urinary free cortiso[19].

The exact mechanism in which steroids may improve hearing is unknown. Dexamethasone (9-fluro-11b,17,21-trihydroxy-16a-methylpregna-1,4-diene-3,20-dione), a corticosteroid, has antiinflammatory effects. The effects of steroids are mediated through receptors found within the cytoplasm[23]. Both glucocorticoid and mineralocorticoid receptors are found in the inner ear.[24] Steroids play a significant role in modulating cochlear function. Steroids decrease inflammation from labyrinthitis,[25] improve cochlear blood flow,[26] protect against cochlear ischemia,[27] protect against noise-induced HL,[28] and regulate inner ear de novo protein synthesis.[29] Stria vascularis, maintains Na+/K+ secretion, is necessary for maintenance of the endocochlear potential[30]; and steroids also improve stria vascularis function and morphology.[31] Na,K-ATPase, which is widely distributed in the cochlea, is activated by the steroids, leading to an immediate restoration of the disturbed cellular osmolarity, electrochemical gradients, and neuronal conduction[28].

In Parazzini, et al.'s study (6), the two 2F1–F2 components have been separated from each other using a time-domain windowing of the inverse fast Fourier transform (IFFT) of the DP-gram, following the method proposed by Kalluri and Shera (32). They showedg that there is a substantial variation among subjects concerning which is the predominant DP emission component, in relation to the frequency ratio used to evoke it. The general trend for F2/F1=1.22 is a predominance of the wave-fixed component, while for the F2/F1=1.05 there tends to be a predominance of the place-fixed component. However, there are exceptions to this rule. It follows that when F2/F1 = 1.15 there is greater variation: this frequency ratio appears to be a region of transition at which both emissions commonly predominate. The relative amplitudes of the two components, therefore, seem to be determined by stimulus conditions and also by subject-related factors. However, whichever component is stronger for any F2/F1, it appears that both components are repeatable across time within individual ear .

Our study demonstrated that, in pregnant rabbits, Higher corticosteroid levels may cause higher DPOAE amplitudes than infant rabbits by F2/F1:1.14 measurements. In all rabbits and especially in infant rabbits, DPOAEs could be taken by F2/F1:1.22 measurements with higher amplitudes. The importance of our study is, when DPOAE measurement is planned, measurements should be done using F2/F1: 1.22 to get healthy and accurate results in experimental studies. In measurements made by F2/F1: 1.14, amplitudes can be observed as lower than F2/F1: 1.22 measurements. This decline is evident especially in infant rabbit groups.

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