# Ovarian Steroid Hormones and Auditory Function

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# Statement of Originality

I, Deena Al-Mana, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

# Abstract

Considerable anecdotal evidence and information from previous studies suggest that auditory function may be influenced by hormones. This thesis reviews in detail the potential role of hormones in modulating the auditory system and in the development of pathological conditions in the auditory system with an emphasis on the effect of the ovarian hormones.

Ovarian steroids may influence auditory function directly through their receptors, which have been detected in the auditory system, or indirectly through their effects on the blood supply, the fluid electrolyte balance of the cochlea, and the neurotransmitters of the auditory system. Effects on other parts of the central nervous system connected to the auditory system may also be of importance.

The aim of the study was to investigate whether physiological alterations in ovarian hormones in women with normal hearing, during the natural ovarian cycle and assisted conception treatment were associated with changes in auditory function at the cochlear and brain stem level, and whether these variations were not seen in men over a similar period of time.

The auditory tests evaluated auditory function from the outer ear to the brainstem in both the afferent and efferent system. Hormone levels were assayed only in the female subjects at the same time as the auditory testing, four times during the ovarian cycle, or three times during the assisted conception treatment. Auditory tests were undertaken in the male subjects once a week for four consecutive weeks to correspond with the ovarian cycle measurements.

A number of changes in auditory function were observed during the ovarian cycle and assisted conception treatment, and gender differences were noted. The OAE results may suggest either excitation of the cochlea with higher levels of oestrogen, or suppression of the cochlea with higher level of progesterone. The longer ABR latency following ovarian stimulation and in the follicular phase of the ovarian cycle is consistent with the inhibitory effect of neurosteroids on ABR associated with higher levels of oestrogen. The variation in auditory function were not observed in men.

# Acknowledgements

# Publications and Presentation

## Publications

Al-Mana, D., Ceranic, B., Djahanbakhch, O., & Luxon, L. M. (2008). **Hormones and the auditory system: A review of physiology and pathophysiology**. Neuroscience, 153(4), 881-900.

Al-Mana, D., Ceranic, B., Djahanbakhch, O., & Luxon, L. M. (2010). **Alteration in auditory function during the ovarian cycle**. Hear Res, 268(1-2), 114-122.

(Copies in Appendix IV)

## Published abstract

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## Presentations

**"The effect of reproductive hormones on auditory system: Modulation of cochlear function during the hormone replacement therapy."**

- Oral presentation at 3rd Meeting of the British Society of Neuro-Otology, 22 September 2003. Charing Cross Hospital, London.

**"The Influence of Ovarian Hormones on Auditory Function"**

- Poster presentation at British Society of Audiology Short Papers Meeting on Experimental Studies of Hearing and Deafness, 16-17 September 2004. University College London, London.
- Poster and Oral presentation at Annual Research Forum 2004: 'Chronic Disease Research: addressing the challenges', 11 November 2004. Academic Centre, Newham University Hospital, London.

- Oral presentation at Neuro-Otology Scientific Meeting, 28 January 2005. The National Hospital for Neurology and Neurosurgery, London.
- Oral presentation at XVIII International Federation of Oto-Rhino-Laryngological Societies (IFOS) World Congress, 25-30 June 2005, Rome, Italy

**"Ovarian Steroids Influence the Gender Differences in Auditory Function"**

- Poster presentation at British Society of Audiology Short Papers Meeting on Experimental Studies of Hearing and Deafness, 14-15 September 2006. Cambridge University, Cambridge.
- Oral presentation at Annual Research Forum 2006: 'The Next Generation - Junior Researchers', 16 November 2006. Academic Centre, Newham University Hospital, London.
- Oral and poster presentation at Bart's International Conference in Reproductive Medicine, 14-16 May 2008. St. Bartholomew's Hospital, London.
- Oral presentation at IAPA 2008- XIV International Symposium in Audiological Medicine, 18-21 September 2008. Ferrara, Italy.

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# List of abbreviations

| | |
|---|---|
| AC | Auditory cortex |
| CN | Cochlear nucleus |
| CNS | Central nervous system |
| daPa | Deca Pascal |
| dB | Decibel |
| DPOAE | Distortion products otoacoustic emissions |
| ERα | Oestrogen receptor alpha |
| ERβ | Oestrogen receptor beta |
| FSH | Follicle stimulating hormone |
| GABA | γ-aminobutyrate |
| GnRH | Gonadotrophin releasing hormone |
| HL | Hearing level |
| kHz | Kilohertz |
| IC | Inferior colliculus |
| IHC | Inner hair cells |
| LH | Lutenizing hormone |
| LL | Lateral lemniscus |
| LOC | Lateral olivochoclear pathway |
| MGB | Medial geniculate body |
| MOC | Medial olivocochlear pathway |
| OAE | Otoacoustic emissions |
| OHC | Outer hair cells |
| PTA | Pure tone audiometry |
| SD | Standard deviation |
| SE | Standard error |
| SOAE | Spontaneous otoacoustic emissions |
| SOC | Superior olivary complex |
| SPL | Sound pressure level |
| TEOAE | Transient evoked otoacoustic emissions |

# Chapter 1 : General Introduction

The auditory system interacts with other system and structures in the central nervous system, which enables the auditory system to adjust to the acoustic environment. This is reflected in physiological modulation of the auditory system, as a part of the process of adaptation and survival, enabling interaction with other members of the species.

The aim of this research is to explore the possible effect of the endocrine system, particularly reproductive hormones, on the auditory system. Previous studies suggest that reproductive steroids, hormones that regulate the response to stress, fluid and electrolyte balance and circadian cycle are all relevant to auditory function. The recent advances in the fields of neuroendocrinology and neuropharmacology, together with the development of auditory assessment techniques have provided new insights into the contribution of hormones and neurotransmitters in modulating the auditory function and possible mechanisms of certain pathological conditions.

This chapter includes a review of two relevant areas:
- The functional anatomy of the auditory and endocrine systems.
- The assessment of auditory function.

The following chapters (Chapters 2 and 3) will review hormones and the basis for their physiological action on the auditory system, and the hormonal cycles that may influence the auditory function and possible effect of hormones in the development of auditory pathology.

## 1.1 Review of auditory system: structure and physiology

The auditory system consists of the external, middle and internal ears at the periphery and pathways from the eighth cranial nerve to the auditory cortex in the temporal lobe, with Heschel's gyrus considered to be the primary auditory cortex. Connecting the ear with the auditory cortex are two parallel ascending (afferent) and descending (efferent) pathways. The afferent pathway primarily facilitates signal transmission, while the efferent pathway modulates auditory information through a complex regulatory feedback mechanism. Hence, the auditory system has an ability to modify its activity in response to the acoustic stimuli.

### 1.1.1 The external and middle ears

Sound waves are funneled into the external ear canal by the pinna to reach the tympanic membrane (a conical shaped translucent membrane which separates the external ear from the middle ear as illustrated in Figure 1.1.1). The external ear enhances the resonant frequency of the tympanic membrane by 10-15 dB around the 3 kHz frequency and assists in sound localization through the funneling effect of the pinna and the head shadow effect.

The sound waves lead to the vibration of the tympanic membrane, which is transmitted to the inner ear through the three inter-articulated auditory ossicles, the malleus, incus and stapes, as well as through the air filled cavity of the middle ear (Figure1.1.1). The middle ear acts as a transformer facilitating sound transmission from a medium with a low impedance for sound waves (air) to one of a high impedance (the fluid filled cochlea), with as little loss of sound energy as possible. The impedance matching is largely due to the transfer of sound pressure from the larger tympanic membrane area, to the smaller oval window at the stapes footplate, with the ossicles exerting a leverage effect, which increases the pressure gain by 25-30 dB. The optimal transmission of sounds is around the frequency range of 1-2 kHz. The stiffness of the ossicular chain is controlled by two muscles, the tensor tympani attached to the malleus and the stapedius attached to the stapes.

In humans, the stapedius muscle contracts with acoustic stimulation, while the tensor tympani muscle contracts if a startle reflex is elicited and it has a much smaller effect on acoustic transmission than the stapedius muscle. The contraction increases the stiffness of the ossicular chain leading to a reduction in the middle ear transmission of up to 15 dB in the low-frequency range (below 1 kHz). The stapedial reflex arc is integrated in the lower brainstem and has an ipsilateral and contralateral pathway, with the efferent pathway in the facial nerve. On the other hand, the tensor tympani is innervated by a branch of the trigeminal nerve. Beside contraction to acoustic stimuli, these muscles also contract in response to other motor events such as vocalization and chewing. Thus these middle ear muscles may provide some protection to the auditory system from low frequency sounds and reduce distortion from sounds produced by an individual's own vocalization and mouth movement (reviewed by Yost, 2000).

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**Figure 1.1.1:** The basic anatomy of the ear (adapted from Vitrualmedicalcentre.com, 2008)

(Retrieved Febuary 2010, from http://www.virtualcancercentre.com/uploads/VMC/TreatmentImages/2191_ear_anatomy _450.jpg)

### 1.1.2   The internal ear

The internal ear contains the organs of balance (the cristae of the semicircular canals and otolith organs of the vestibule) and hearing (the cochlea). The cochlea is a coiled tube like structure of two and a half turns composed of a bony and membranous labyrinth, that spiral around a central axis known as the modiolus. The interior of the bony labyrinth is partitioned by the Reissner's membrane and basilar membrane into three fluid filled spaces; the scala vestibuli, tympani and media. The scala vestibuli and scala tympani contain perilymph and communicate with each other at the apex of the cochlea and with the subarachnoid space of the posterior cranial fossa via the Sylvian aqueduct. The scala media is continuous with the vestibular membranous labyrinth and contains endolymph. There is no communication between the spaces filled with perilymph and those filled with endolymph (Figure 1.1.2).
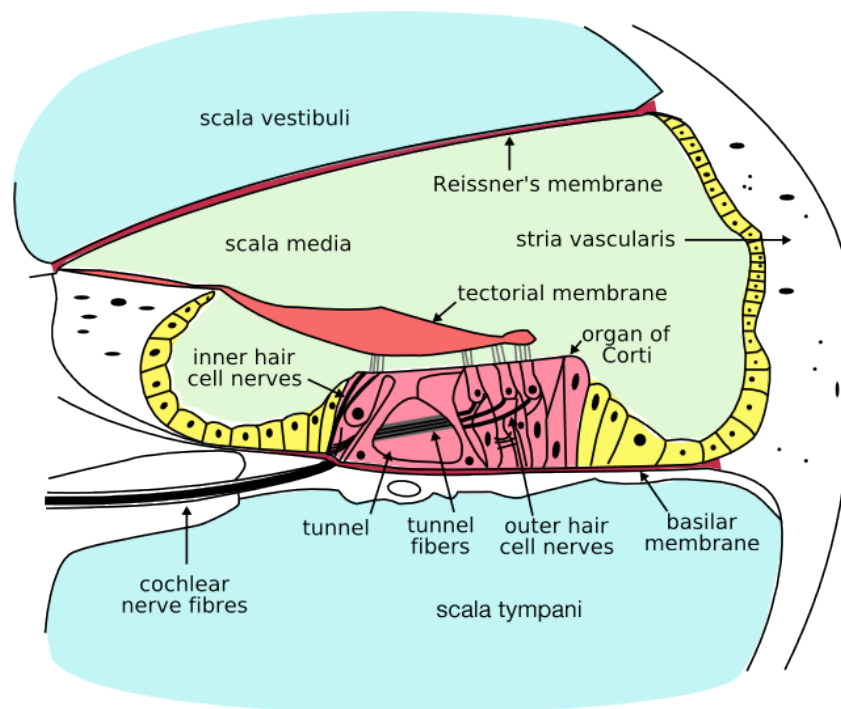


**Figure 1.1.2**: Cross section of the cochlea showing the fluid filled chambers and the organ of Corti (From Wikimedia Commons, 2004).

(Retrieved December 2008, from http://upload.wikimedia.org/wikipedia/commons/0/0c/Cochlea-crosssection.png)

### 1.1.2.1 The cochlear fluids

The perilymph composition is similar to other extracellular fluids. The origin of perilymph is still unclear, but it seems that the fluid in the scala vestibuli originates from plasma, while the perilymph in the scala tympani comes from both the cerebrospinal fluid and plasma (Sterkers, et al., 1988). The endolymph, on the other hand, is a unique extracellular fluid with a composition similar to that of intracellular fluid with a high potassium (K+) and low sodium (Na+) concentration (Slepecky, 1996). It is widely accepted that the endolymph is formed by the stria vascularis (Figure 1.1.2), which is a multilayer highly vascular epithelial tissue. The stria vascularis contains a high concentration of Na+, K+-ATPase, adenyl cyclase and carbonic anhydrase enzymes, which are associated with ion pumping and fluid transport into the endolymph, as well as high levels of oxidative enzymes needed for glucose metabolism that provides the fuel for the active transport mechanism (Sterkers, et al., 1988; Ciuman, 2009). This latter mechanism is needed to maintain the positive electrical potential of +80 mV, known as the endolymphatic potential. The main role of the cochlear fluids is to transmit the mechanical acoustic stimuli to the organ of Corti, as well as participating in the transduction mechanism through ionic exchange with the cochlear hair cells (Salt, 2001).

### 1.1.2.2 The organ of Corti

The organ of Corti is the sensory organ of hearing and contains two types of sensory cells, the inner and outer hair cells as well as supporting epithelial cells and neural elements. It is located on the basilar membrane within the scala media (Figure. 1.1.2).

The processes of the hair cells, stereocilia, are bathed in endolymph. The stereocilia are formed of packed actin filaments and linked together by fine extracellular filaments, some of which are known at "tip links", which play an important role in the mechanical transduction system of the hair cells (Pickles, et al., 1984). There are tight junctions present between the apical parts of the hair cells and the adjacent supporting cells which prevents endolymph reaching the base of the cells. However, the basilar membrane is permeable to perilymph that

bathes the base of the cells. The inner hair cells (IHC) transform the acoustical information to electrical impulses that are conveyed to the type I auditory afferent fibers. The outer hair cells (OHC) on the other hand, are characterized by the presence of an actin-myosin complex in their cytoskeleton, which makes the cells contractile. The cell structure of the OHC, as well as their greater efferent innervations (see section 1.1.2.3), suggest they act as a modulator and amplifier capable of fine-tuning the receptive function of the cochlea (Santos-Sacchi, 2001).

The sound induced vibration of the stapes footplate in the oval window leads to a passive dynamic displacement of the membranous cochlea producing a travelling wave that results in the basilar and Reissner's membranes swinging from side to side. This mechanical vibration of the basilar membrane is translated by the organ of Corti into neural responses as a consequence of bending of the stereocilia of the hair cells. The deflection of the stereocilia leads to stretching of the tip links and thus activate the mechanotranducer channels of the hair cells membranes (Pickles et al, 1984). However, the cochlea is not only a passive mechanical signal analyser, but it also plays an active role in processing sound which is brought about by the contractile action of the OHC. The OHC are capable of fast and slow contractions. The fast contractions (Brownell, et al., 1985) are phase locked to the stimulating sound and help in enhancing the vibration of the basilar membrane and thus amplify sound by about 40 dB near threshold. On the other hand, the slow tonic contractions of the OHC (Zenner, 1986) alters the stiffness of the basilar membrane and, thus, reduces the movement of the basilar membrane, as a consequence of the action of the efferent system (see section 1.1.4).

### 1.1.2.3 *Cochlear innervation and blood supply*

The sensory cells of the organ of Corti have both afferent and efferent innervation. The afferent fibres are dendrites from cell bodies of the afferent auditory nerve located in the spiral ganglion within the modiolus and are of two types. Type I fibres are thicker and myelinated and form 90-95% of the afferent fibres. The remaining 5-10% of fibres are thinner and unmyelinated and are known as type II afferents. Type I pathway is the main sensory pathway that transfers the acoustic information to higher centers, while little is known about type II function (Brown,

2001). The efferent fibres on the other hand arise from the superior olivary complex, which gives rise to two pathways; the lateral and medial olivocochlear pathways. These efferent fibers provide feedback from higher auditory structures to either enhance or inhibit cochlear function (for more details see section 1.1.4).

### *Inner hair cell innervation:*

Each IHC has synapses with 20-30 type I afferent fibres which innervates one IHC (Liberman, et al., 1990), and the likely neurotransmitter is glutamate (Table 1.1-A). The efferent innervation of the IHC is from the lateral olivocochlear pathway (LOC), which mainly arises from the ipsilateral superior olivary complex. The efferent fibres synapse with the Type I afferent fibers at the base of the IHC as demonstrated in Figure 1.1.3. The LOC fibers contain several neurotransmitters (see Table 1.1-B) that have both inhibitory and excitatory action on the IHC and Type I afferent fibres.
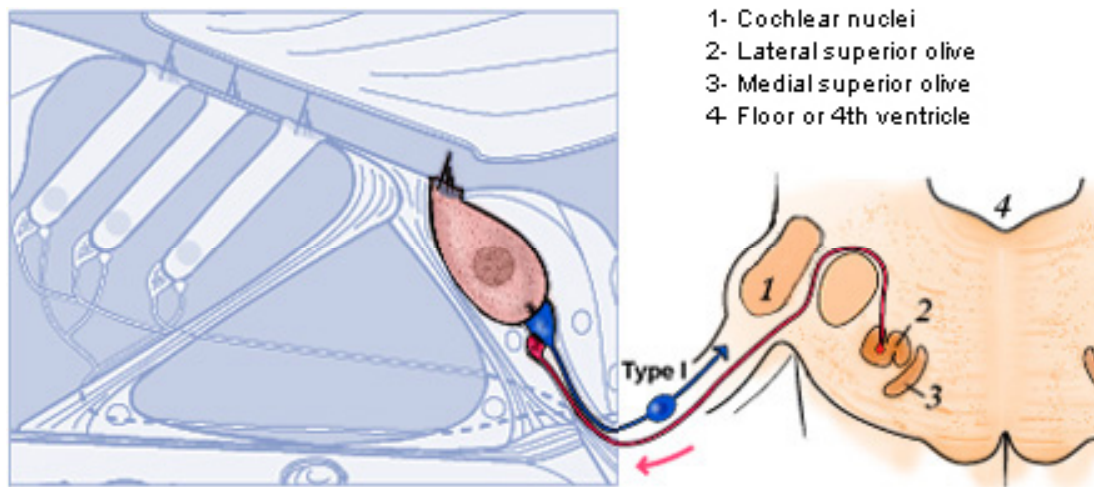


**Figure 1.1.3:** Diagram of the afferent (blue) and efferent (red) innervations of the IHC. There is some contribution to the efferent fibres from the contralateral lateral superior olive (from a drawing by Blatrix, 2007a, permission to reproduce granted kindly by R.Pujol).

### *Outer hair cell innervation:*

Each type II afferent fibre innervates several OHC as displayed in Figure 1.1.4, and their synapses are small and little is known about their function. However, the synapses of the efferent fibers with OHC are large and vesiculated. The efferent fibres that innervate the OHC are from the medial olivocochlear pathway (MOC) that arise mainly from the contralateral superior olivary complex with a small

contribution from the ipsilateral superior olivary complex (Figure 1.1.4). The main neurotransmitter of the MOC fibers is acetylcholine with γ-aminobutyrate (GABA), which is present mainly in the apical region of the cochlea (Le Prell, et al., 2001).
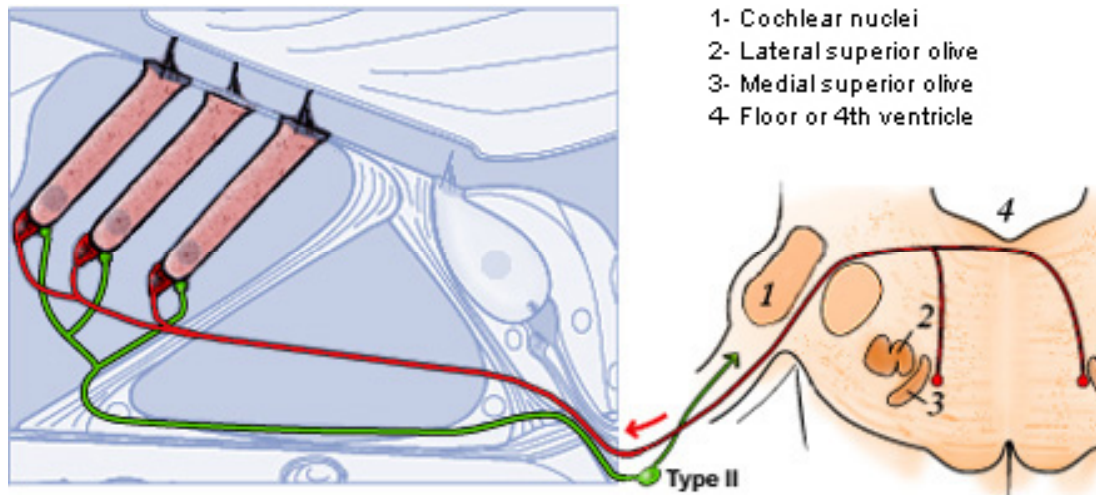


**Figure 1.1.4**: Diagram of the afferent (green) and efferent (red) innervations of the OHC (from a drawing by Blatrix, 2007b, permission to reproduce granted kindly by R.Pujol).

The cochlea also receives sympathetic, adrenergic innervation (Vicente-Torres & Gil-Loyzaga, 2002) that originates from both the superior cervical ganglion and the stellate ganglion (reviewed by Eybalin, 1993). The sympathetic fibres end on the blood vessels in the spiral lamina, some terminate near afferent fibres of the cochlear nerve (Brechtelsbauer, et al., 1990), and form part of perivascular fibres in the stria vascularis (Liu, et al., 1996). The presence of adrenergic innervation in the cochlea suggests its role in controlling vasomotor tone and influencing cochlear haemodynamics.

The main blood supply of the cochlea is from the spiral modiolar artery, which is a branch of  the cochlear artery, and the main drainage is from the spiral modiolar vein (Axelsson, 1988). The control of the cochlear blood flow is a combination of both local and systemic mechanisms including vasoactive hormones (Miller & Dengerink, 1988).

### 1.1.3  Afferent auditory pathway

The auditory signal from the organ of Corti travels along the auditory nerve to the ipsilateral cochlear nucleus and from there the majority of the afferent auditory fibers project to the contralateral superior olivary complex, the lateral lemniscus, inferior colliculus, medial geniculate body to the auditory cortex. The rest of the fibers from the cochlear nucleus project either directly towards the contralateral lateral lemniscus and inferior colliculus bypassing the superior olivary complex or project to the ipsilateral superior olivary complex, lateral lemniscus or inferior colliculus (Chermak & Musiek, 1997). The contralateral pathways from the cochlear nucleus carry the greater number of fibres with auditory information, as demonstrated graphically in Figure 1.1.5.

Image removed for copyright reasons

**Figure 1.1.5:** Diagram of the afferent auditory pathway showing the principle and secondary afferent auditory pathways (from Noback & Demarest, 1981).

The auditory signal is not transmitted to the auditory cortex passively, but processed at the different auditory nuclei. The cochlear nucleus enhances the contrast of the auditory stimuli (i.e. sharpens the auditory stimulus) through suppressing noise by lateral inhibition, while the superior olivary complex aids in sound localization in space as a result of binaural inputs. The inferior colliculus is the major integrator of the auditory information before relaying to the auditory cortex via the medial geniculate body. The sensory information is conveyed to the different auditory nuclei by neurotransmitters that are either excitatory or inhibitory and thus modulate the transfer and processing of the acoustical signal from one centre to another.

Table 1.1-A summarises the neurotransmitters that have been identified in the afferent auditory system and their possible actions. The main excitatory neurotransmitter of the afferent auditory system is glutamate, while GABA is the main inhibitory neurotransmitter.

Auditory information is not processed in isolation from other sensory stimuli, but is integrated with other sensory modalities such as vision and touch and may influence the processing of other stimuli (Shimojo & Shams, 2001; Foxe, 2009). Integration occurs in the cortex (Beauchamp, 2005) and subcortical areas such as the superior colliculus (Meredith & Stein, 1986; Kayser & Logothetis, 2007). This mutual interaction between the different sensory modalities may influence the way the individual responds to the environment.

The auditory information is also modulated by the efferent auditory pathway that arises from the auditory cortex and descends into the brainstem to reach the cochlea (Suga, et al., 2000) seen graphically in Figure 1.1.6.

**Table 1.1-A:** Neurotransmitters of the afferent system.

| Level | Afferent Auditory System | |
| --- | --- | --- |
| | **Neurotransmitter** | **Possible Function** |
| **Inner hair cells** | Glutamate | Excitatory<br><br>(Puel, 1995; Le Prell, et al., 2001)<br><br>& neurotoxic in acoustic trauma & ischemic injury (Janssen, et al., 1991; Eybalin, 1993) |
| **Cochlear nucleus** | Glutamate, Aspartate Acetylcholine<br><br><br><br>GABA, Glycine | Excitatory<br><br>(Musiek & Hoffman, 1990)<br><br><br><br>Inhibitory<br><br>(Musiek & Hoffman, 1990) |
| **Superior olivary complex** | Glutamate, NMDA<br><br><br><br>GABA, Glycine | Excitatory<br><br>(Musiek & Hoffman, 1990)<br><br>Inhibitory<br><br>(Musiek & Hoffman, 1990) |
| **Lateral leminiscus** | GABA | Possibly inhibitory<br><br>(Moore & Moore, 1987) |
| **Inferior colliculus** | Glutamate<br><br><br><br>Glycine, GABA | Excitatory<br><br>(Faingold, et al., 1989; Musiek & Hoffman, 1990)<br><br>Inhibitory<br><br>(Faingold, et al., 1989; Musiek & Hoffman, 1990) |
| **Medial geniculate body** | ? | ? |
| **Auditory cortex** | Acetylcholine, Opioids | Not clear<br><br>(Musiek & Hoffman, 1990) |

?: not known, NMDA: N-methyl-D-aspartate

## 1.1.4 The efferent auditory pathway

The efferent auditory pathway arises in the auditory cortex and descends into the brainstem to reach the cochlea (Suga, et al., 2000). The anatomy of the higher efferent auditory system is still not clearly defined (Musiek & Oxholm, 2003), but

it is thought to run in parallel to the ascending auditory pathway (Figure 1.1.6) . The best described part of the efferent system is the olivocochlear pathway that projects from the superior olivary complex to the cochlea (reviewed by Warr, 1992), and has two main pathways (Figure 1.1.3 and 1.1.4):

- Medial olivocochlear system (MOC) that projects mainly to the contralateral cochlea, and connect to the OHC, and to a lesser extent type II ganglion cells.
- Lateral olivocochlear system (LOC) that projects mainly to the ipsilateral cochlea, and ends on the type I afferent dendrites that connect to the IHC.

Image removed for copyright reasons

**Figure 1.1.6:** Efferent auditory pathway that arises from the auditory cortex descending into the auditory brainstem to reach the cochlea from Noback & Demarest, 1981).

Knowledge about the efferent system function is still very limited. Several neurotransmitters have been identified in the efferent auditory system (see Table 1.1-B) along with possible functions, which provide insight into the role of the efferent auditory system in hearing.

**Table 1.1-B:** Neurotransmitters of the efferent auditory system.

| Level | Efferent Auditory System | |
|---|---|---|
| | **Neurotransmitter** | **Possible Function** |
| **Outer hair cells** | Acetylcholine | Mainly Inhibitory (Eybalin, 1993; Dallos, et al., 1997; Le Prell, et al., 2001) |
| | GABA | Inhibitory (Eybalin, 1993; Le Prell, et al., 2001) |
| **Inner hair cells** | Acetylcholine | Excitatory (Felix & Ehrenberger, 1992) |
| | Dynorphin | Excitatory (Sahley & Nodar, 1994; Sahley, et al., 1999) |
| | GABA | Inhibitory (Eybalin, 1993; Le Prell, et al., 2001) |
| | Dopamine, Enkephalin | Inhibitory (Pujol, 1994; Gil-Loyzaga, 1995; Le Prell, et al., 2001) |
| **Cochlear nucleus** | Glutamate | Excitatory (Thompson & Schofield, 2000) |
| | GABA | Inhibitory (Thompson & Schofield, 2000) |
| **Superior olivary complex** | Glutamate | Excitatory (Thompson & Schofield, 2000) |
| **Lateral leminiscus** | ? | ? |
| **Inferior colliculus** | Glutamate | Excitatory (Thompson & Schofield, 2000) |
| | GABA, Glycine | Inhibitory (Huffman & Henson, 1990) |
| **Medial geniculate body** | Glutamate | Excitatory (Thompson & Schofield, 2000) |
| **Auditory cortex** | Possibly Glutamate | Excitatory (Thompson & Schofield, 2000) |

?: not known

The function of the olivocochlear system on hearing is still not fully understood. Activation of the MOC neurons leads to a release of acetylcholine, which activates the acetylcholine receptors of the OHC that leads to both a fast and slow inhibitory effect on OHC activity (Cooper & Guinan, 2003). The reduction of the motile action of the OHC is thought to be the fast effect, while the slow effect is thought to be due to the reduced stiffness of the OHC brought about by acetylcholine (reviewed by Pickles, 2008). The inhibitory effect on the OHC dampens the vibration of the basilar membrane and thus decreases the gain of the cochlear amplifier (Dallos, et al., 1997). However, the activation of the MOC may in some circumstances enhance the vibration of the basilar membrane, but the mechanism is still not fully understood (Cooper & Guinan, 2006). An enhancement in the transient stimulus by recording the compound action potential has been observed in the presence of ipsilateral noise and attributed to an effect of the efferent system (Dolan & Nuttall, 1988; Kawase et al., 1993). These findings reflect the complexity of the efferent system and suggests its importance in processing complex sounds in noise (a complex filter system) with an anti-masking role (reviewed by Guinan, 2006).

The efferent auditory system could also have a protective effect on the cochlear hair cells. Sectioning of the olivocochlear bundle, increases the susceptibility to sound induced damage of hair cells (Le Prell, et al., 2001; Rajan, 2001). In addition some neurotransmitters of the lateral olivocochlear efferent system (such as enkephalin and GABA), are thought to be involved in postsynaptic inhibitory modulation of the glutamatergic afferent synapse at the IHCs (Table 1.1-A and 1.1-B), and thus may protect the auditory nerve from glutamate excitotoxicity (Thompson & Schofield, 2000; Gáborján, 2001).

Thus, the efferent system seems to act as an auto regulatory feedback mechanism, that is mainly inhibitory, but may also be excitatory at different levels and so adjust and improve the processing of the auditory signal (Suga, et al., 2000).

### 1.1.5 Summary of auditory system

In summary, the structure of the auditory system is quite complex with interactions between the ascending (afferent) and descending (efferent ) pathways as demonstrated in Figure 1.1.7. The neurotransmitter receptors of the afferent and efferent auditory system are a potential target for hormonal modulation of auditory function, along with the cochlear fluid homeostasis and blood flow (see section 2.2).

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**Figure 1.1.7:** Schematic illustration of the afferent and efferent auditory system.

The OHC feeds mechanical

oscillation to the IHC that transform the mechanical signal to a neural one that is conveyed to the higher auditory nuclei. The efferent system arises from the auditory cortex and runs parallel to the afferent system towards the cochlea providing multiple feedback loops with greater detail known about the OCB (Ceranic & Luxon, 2008).

(CN: cochlear nucleus, IC: inferior colliculus, IHC: inner hair cell, LB: lateral bundle, LL: lateral lemniscus, LN: lateral nucleus,  MB: medial bundle, MGB: medial geniculate body, MN: medial bundle, OCB: olivocochlear bundle, OHC: outer hair cells, SOC: superior olivary complex)

### 1.1.6 Links between the auditory and other parts of the central nervous system

The auditory system has connections with other structures of the CNS that may modulate auditory function. These extra-auditory structures are targets of certain hormones and, thus, indirectly these hormones may influence auditory function.

The main structures of the CNS with connections with the auditory system are:

- The **limbic system** which regulates instinctive behaviour and emotions, has its main connection with the auditory system via the medial geniculate body and is thought to be important in attaching emotional significance to acoustic stimuli (LeDoux, et al., 1984; LeDoux, 1993). The limbic system expresses hormone receptors that include receptors for stress related hormones and reproductive hormones (Gray & Bingaman, 1996; Jennes & Langub, 2000).

- The **hypothalamus**, is the integrator centre for the endocrine and autonomic systems and is linked with the auditory system through the inferior colliculus (Adams, 1980), although, its effect on the auditory function is unclear. The hypothalamus contains the supra-chiasmatic nucleus, which is thought to regulate the circadian rhythm (Halasz, 2000; Levine, 2000), and expresses almost all types of hormone receptors (reviewed by Jennes & Langub, 2000).

- The **reticular system** is concerned with the behavioural state of arousal and alertness and projects serotonergic fibers to almost all levels of the auditory system from the cochlea (Gil-Loyzaga, et al., 2000) to the auditory cortex (Juckel, et al., 1997). The ascending reticular system reacts more to "important" than to "unimportant" stimuli, and this may be related to hearing in noise and selective attention (Chermak & Musiek, 1997). The reticular formation is involved in the stress response and expresses adrenal steroid receptors (Jennes & Langub, 2000). The presence of noise, or other stressful stimuli was found to modulate the serotonergic system, by

increasing the release of serotonin (Singewald, et al., 1998). Serotonin was also found to modulate the neural responses in the inferior colliculus depending on the type of auditory stimuli, thus influencing auditory processing (reviewed by Hurley, et al., 2002). Animal and human studies have found that the serotonergic system is sexually dimorphic (structurally and functionally different between males and females). For example, there is increased serotonin activity in female rat brain compared to males (Carlsson & Carlsson, 1988) and a decrease in whole brain serotonin synthesis in women compared to men (Nishizawa, et al., 1997). It seems that oestrogen contributes to this dimorphism, either enhancing or decreasing serotonin binding depending on the site of the receptor in the brain, length of oestrogen treatment and species (reviewed by Rubinow, et al., 1998).

## 1.2 Functional assessment of the auditory system

There are a number of clinical tests used to evaluate the auditory function at different levels of the auditory system. Figure 1.2.1 gives a topographic representation of the main afferent auditory tests and the single efferent auditory test used in clinical practice and research.
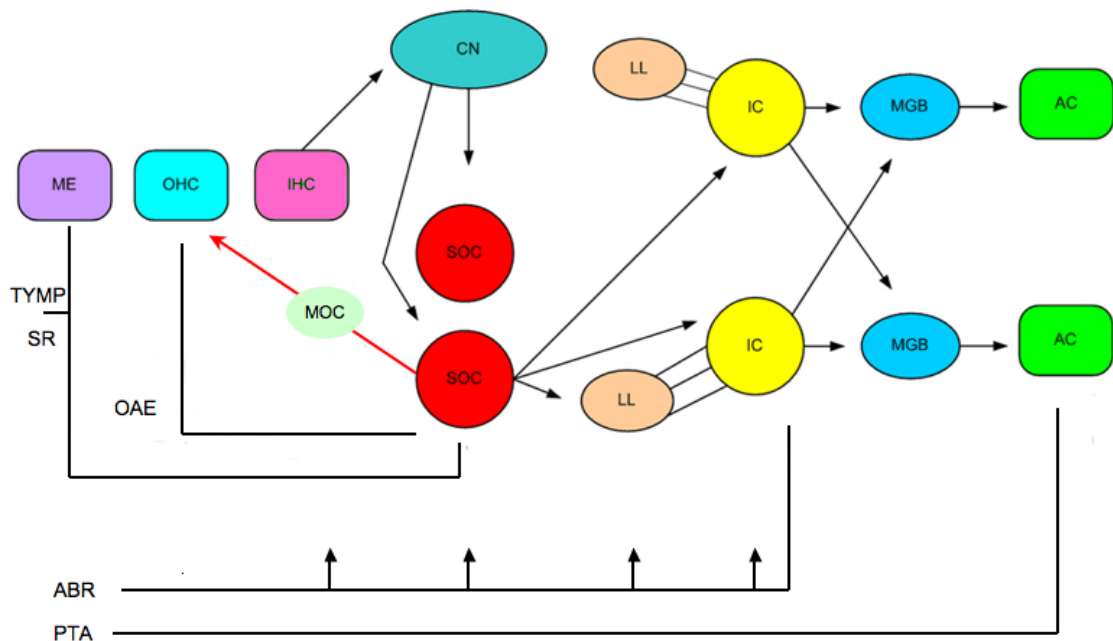


**Figure 1.2.1:** Topographic representation of auditory tests (adapted from Ceranic, et al., 2002).

The efferent test is represented by the red arrow.
(TYMP: tympanometry, SR: stapedial reflexes, OAE: otoacoustic emissions, ABR: auditory brainstem response, PTA: pure tone audiometry, ME: middle ear, OHC: outer hair cells, IHC: inner hair cells, OAE: otoacoustic emissions, MOC: medial olivocochlear system, CN: cochlear nucleus, SOC: superior olivary complex, LL: lateral lemniscus, IC: inferior colliculus, MGB: medial geniculate body, AC: auditory cortex)

### 1.2.1 Pure tone audiometry

Pure tone audiometry (PTA) is a basic audiometric test that reflects overall auditory sensitivity across a range of frequencies. The test is used to ascertain normal hearing thresholds, which are less or equal to 25 dB HL at each of the tested frequencies (WHO, 2006). The testing method for air conducted thresholds

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involves presenting tone pulses from a commercial audiometer through earphones at 6 frequencies from 0.25 kHz to 8 kHz in octave steps (British Society of Audiology, 2004). The test is subjective and limited both in term of topographic value and because it only evaluates listening in quiet.

### 1.2.2 Tympanometry

Tympanometry is an objective test that evaluates the middle ear. It reflects the changes in the physical properties of the middle ear system as the air pressure in the ear canal is varied (Hall III & Chandler, 1994). This is achieved with single frequency stimulation of 226 Hz at 85 dB SPL, to measure ear canal volume, middle ear pressure and tympanic membrane compliance (British Society of Audiology, 1992) and the response is recorded as demonstrated in Figure 1.2.2. A normal middle ear pressure ranges between -50 and +50 daPa in adults with the mean being 0 daPa, and normal compliance ranges between 0.3 and 1.6 ml  with a mean of 0.7 ml (British Society of Audiology, 1992).
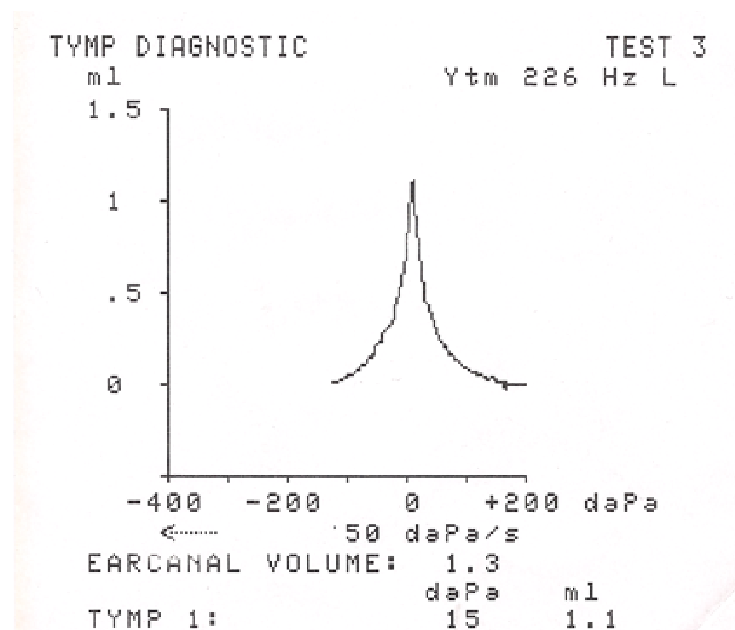


**Figure 1.2.2:** The trace of a normal tympanogram.

The peak represent the middle ear pressure (15 daPa) and the height of the peak represent the tympanic membrane compliance (1.1 ml).

Normal middle ear function is needed to record valid otoacoustic emissions.

### 1.2.3 Otoacoustic emissions

Otoacoustic emissions (OAE) were defined by Kemp in 1978 (Kemp, 1978) and are signals recorded in the ear canal. They are considered to reflect the integrity and function of the outer hair cells (Kemp, et al., 1990). The generation of OAE is related to the active motility of the OHC (see section 1.1.2.2), and they reflect the nonlinear, biomechanical function of the OHC that is responsible for the sensitivity and sharp frequency selectivity of the cochlea (Kemp & Chum, 1980; Kemp, 1986).

There are two basic types of OAE, those recorded in absence of acoustic stimulation, known as spontaneous OAE, and those recorded following an acoustic stimuli, known as evoked OAE. Evoked OAE are divided according to the type of acoustic stimuli applied into: transient evoked OAE (TEOAE), distortion product OAE (DPOAE) and stimulus frequency OAE (SFOAE). The most commonly used in clinical practice are SOAE, TEOAE and DPOAE.
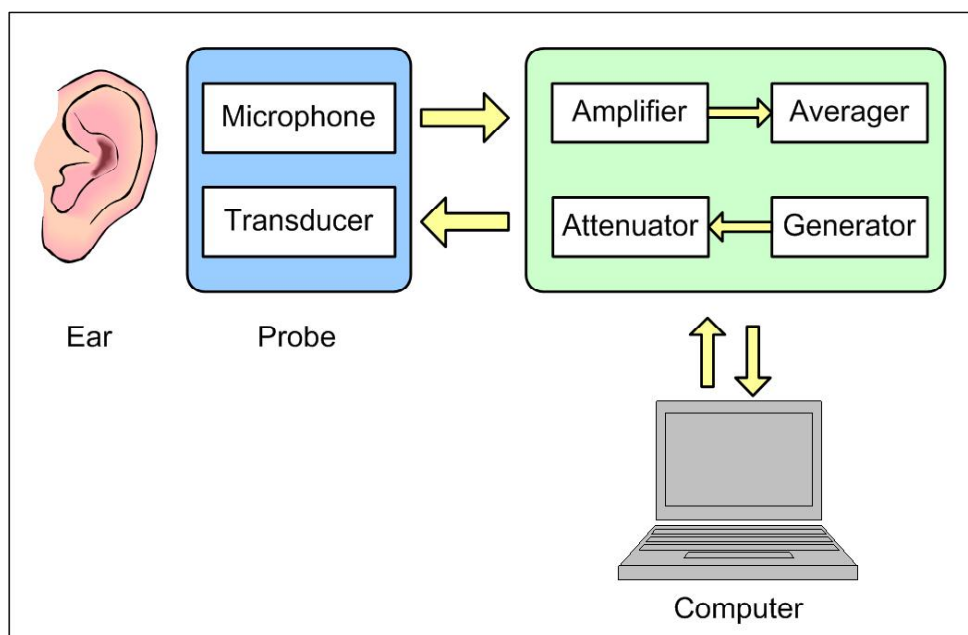


**Figure 1.2.3:** Schematic diagram of the standard setup for otoacoustic emission recording.

The standard recording setup for OAE includes a probe that contains a microphone and a transducer that delivers the stimulus from the stimulus

generator. The signal from the ear is picked up by the microphone and delivered to the signal averager and the display system as illustrated in Figure 1.2.3.

It is important to note that only a fraction of the acoustic energy from the cochlea can be recorded in the ear canal, due in part to loss of up to 15 dB of OAE energy through the retrograde transmission via the middle ear (Hall, 2000a). Therefore, the status and function of the middle ear has to be taken in account when recording OAE.

### 1.2.3.1 *Spontaneous otoacoustic emissions*

Spontaneous otoacoustic emissions (SOAE) are narrow band signals emitted by the cochlea in the absence of any acoustic stimulation. They result from the micromechanical activity of the outer hair cells (Kemp, 1979). They can be recorded from 40-70 % of the normal hearing population, and are more prevalent in females, up to 75% of females compared to 58% of males (Penner & Zhang, 1997).

*Clinical significance*

The presence of SOAE is associated with functionally intact outer hair cells (OHCs) and exquisite hearing sensitivity with audiometric thresholds better than 15 dB HL at the homologous frequency (Probst, et al., 1987; Bonfils, 1989). They show intra-session as well as inter-session frequency stability with variations being less than 1-2%, however the SOAE amplitude show a wider range of variations (Ceranic, 2003).

The SOAE reveal some cyclic physiological variations, circadian (Bell, 1992; Haggerty, et al., 1993) and menstrual (Bell, 1992; Haggerty et al, 1993; Penner, 1995) that are thought to be due to hormonal changes; however circulatory changes may also play a role. These fluctuations in SOAE may not only reflect cochlear function, but also the higher auditory and neural centres that regulate cochlear function (reviewed by Ceranic, et al., 1998a).

*Method of recording*

- The SOAE are recorded following a weak (about 75 dB SPL) synchronizing click (details in section 4.3.1.3). This method is used in the ILO 88/92 Otodynamic equipment, and is the most commonly used method of recording SOAE clinically. Figure 1.2.4 is an example of a trace.

- The SOAE are recorded using a sensitive microphone placed in the ear canal with no stimulus and the signal is averaged in the frequency domain.
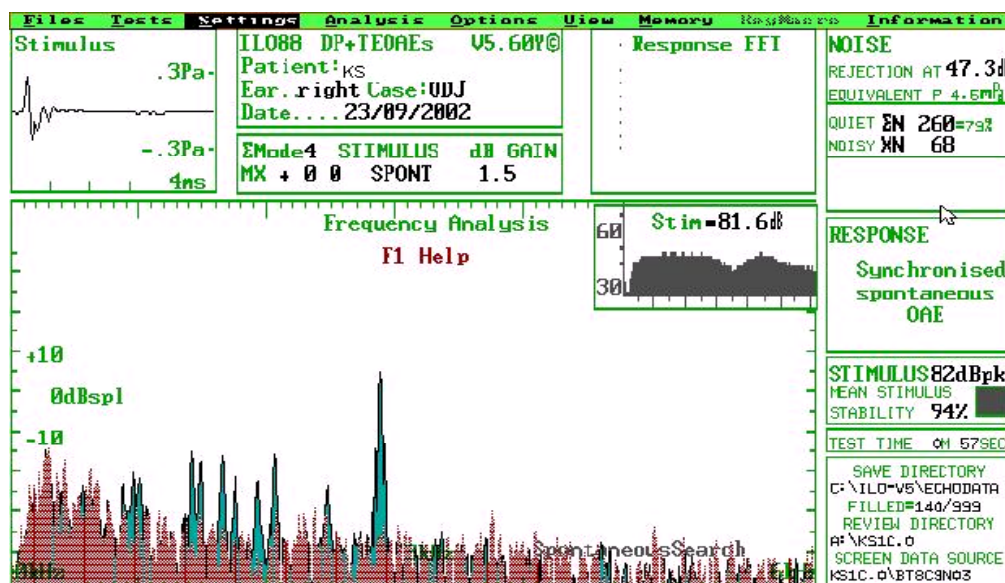


**Figure 1.2.4:** SOAE trace recorded by ILO 88/92 showing multiple peaks seen in blue.

### 1.2.3.2 *Transient evoked otoacousic emissions*

Transient evoked otoacoustic emissions (TEOAE) are sound signals recorded in the sealed ear canal in response to clicks. TEOAE are associated with functioning OHC and are present in about 96-100% of normal hearing ears but are commonly absent if hearing thresholds are greater than 35 dB HL (Probst, et al., 1991). The TEOAE is frequency dispersive, with high frequencies having shorter latencies than low frequencies. Another characteristic of TEOAE, is that their amplitude exhibit compressive non linearity as a function of the stimulus intensity. The

maximum gain in TEOAE amplitude is recorded near hearing threshold levels
(Kemp, 1978; Ceranic, 2003).

*Clinical significance*

The TEOAE is a reliable indicator of OHC structural integrity, and has excellent
test-retest stability and an intra-subject variability in amplitude of less than 1 dB.
However, there is great inter-subject variability (Harris, et al., 1991; Franklin, et
al., 1992; Marshall & Heller, 1996). The absence of TEOAE suggest hearing loss
of at least 25 dB due to cochlear or middle ear pathology, and in general OAEs
cannot be recorded from ears with hearing loss greater than 35 dB HL (Bonfils &
Uziel, 1989; Probst & Harris, 1993).

*Method of recording*

The recording technique most commonly used is the differential non-linear
method (details in section 4.3.1.3). Click stimuli are delivered through a probe in
the ear canal. The fast Fourier transform (FFT) spectrum analysis and average
waveform are calculated automatically by the ILO 88/92 Otodynamic equipment
commonly used for the test.

## 1.2.4 Medial olivocochlear reflex (MOC suppression)

The function of the MOC in humans can be evaluated by recording OAE with and
without contralateral white-noise stimulation. The difference in responses is
considered to be the medial olivocochlear effect. The contralateral stimulation
reduces the amplitude of TEOAE and thus indicates the inhibitory action of the
MOC.

*Clinical significance*

The magnitude of the suppression of the TEOAE depends on the intensity of both
contra- and ipsilateral stimuli (Collet, et al., 1990; Veuillet, et al., 1996). The
amount of suppression is normally more than 1 dB and usually between 1 to 3 dB
using the ILO 88/92 Otodynamics otoacoustic analyser, and is more effective in
the right ear (Hall, 2000b). There is a inter-subject variability in the level of

suppression, with good intra-subject stability (Ceranic, et al., 1998b; De Ceulaer, et al., 2001).

*Method of recording*

Ipsilateral TEOAE are recorded using linear clicks, while broad band "white" noise is used for the contralateral stimulation. The contralateral noise can be delivered through a dual channel OAE analyzer (Ceranic, et al., 1998b) or from an audiometer (Coelho, et al., 2007). Usually a number of TEOAE recordings are undertaken with and without contralateral stimulation to reject any artifacts and the average responses of the TEOAE with and without contralateral stimulation are calculated and the difference between the means represents the suppression effect (details in section 5.3.1.4).

### 1.2.5  Auditory brainstem evoked responses

The auditory brainstem evoked responses (ABR) are short latency potentials recorded from the scalp during a brief acoustic stimulation. The ABR consists of a series of seven positive waves which are labelled by Roman numerals (I-VII) and recorded within 10 ms of the stimulus onset (Figure 1.2.5). The wave latencies reflect the time lapse between the stimulus onset and the time of the highest synchronous activity in the generator site of the wave (Pratt, 2003). The origins of the ABR waveforms are still the subject of controversy, but current consensus is that Wave I is generated from the spiral ganglion of the cochlea (distal portion of the eighth nerve) and wave III is thought to originate from both the cochlear nucleus and the superior olivary complex in the lower pons. Wave V is thought to be generated from the upper pons with contribution from the superior olivary complex, lateral lemniscus and possibly also from the inferior colliculus (Pratt, 2003).

The evaluation of the ABR waveform provides objective information on the integrity of the lower auditory brainstem pathways. Wave I, III and V are usually examined in clinical practice, and the absolute wave latencies, interpeak intervals, and interaural differences are the most commonly evaluated parameters.

*Method of recording*

The evoked potentials are recorded using conventional EEG electrodes placed on the vertex (Cz) and each mastoid (A1and A2) during acoustic stimulation. The subject is asked to lie down and the acoustic click stimulus is delivered via earphones (details in section 5.3.1.5).

Image removed for copyright reasons

**Figure 1.2.5:**Auditory brainstem response. Waves I-VII and the possible generator sites (adapted from Duane, 1977).

# Chapter 2 : The Endocrine System and Potential Effects of Hormones on the Auditory System

Body homeostasis is controlled both by the nervous and endocrine systems, which regulate and control metabolism, growth, reproduction, and behavioural responses to the external environment.

The nervous system provides rapid electrical responses to stimuli via neurotransmitters that transmit the signal from one nerve fiber to another. On the other hand, the action of the endocrine system is slower and provides long term responses to stimuli which are initiated by blood borne hormones to their target organs.

## 2.1 Endocrine system structure

The endocrine system is a collection of ductless glands that produce hormones, which target other endocrine glands or non-endocrine tissues. The target cells usually have specific receptors for specific hormones.

The following is a short description of some of the major glands of the endocrine system that may influence the auditory system. Other endocrine glands not described, such as the thyroid and parathyroid gland may also have some affect auditory function, but were not reviewed in this thesis.

### 2.1.1 The hypothalamus

The hypothalamus is the major neurohormonal control centre and is connected to other parts of the central nervous system including the auditory system via the inferior colliculus (see section 1.1.5). It is responsible in regulating sleep, hunger, thirst, mood, body temperature, reproductive behaviour and the release of hormones from other glands especially the pituitary gland.

The hypothalamus secretes trophic hormone-releasing and release-inhibiting hormones into a portal venous system that targets the anterior pituitary to control the release of the anterior pituitary hormones. The cell bodies that produce these hormones are present in the paraventricular, preoptic, periventricular and arcuate nuclei (Halasz, 2000). They include gonadotophin releasing hormone (GnRH), corticotrophin releasing hormone (CRH), thyrotrophin releasing hormone (TRH), somatostatin, somatotrophin releasing hormone and a prolactin inhibiting factor, which is possibly dopamine (Clemens, et al., 1980). The hypothalamus also contains the cell bodies of the magnocellular neurons in the supraoptic and paraventricular nuclei that produce the posterior pituitary hormones vasopressin and oxytocin (Halasz, 2000).

### 2.1.2   The pituitary gland

The pituitary gland is a small gland (weighing about 1g in humans) located in the base of the brain and controls all the major endocrine glands in the body. It is composed of two parts, the neurohypophysis (posterior pituitary) and adenohypophysis (anterior pituitary).

*The posterior pituitary*

Contains the axons of the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus that release vasopressin and oxytocin into the blood stream (Fink, 2000).

*The anterior pituitary*

Produces four glandotropic hormones (i.e. hormones targeting specific peripheral endocrine glands) and two aglandotropic hormones (i.e. not gland specific). These hormones are under the control of the hypothalamus, which either stimulates or inhibits their release through hormonal and neural feedback loops (Fink, 2000).

The glandotropic hormones are:
- Adrenocorticotrophic hormone (ACTH) which stimulates the release of hormones from the adrenal gland.

- Thyroid stimulating hormone (TSH) which stimulates the release of hormones from the thyroid.
- Follicle stimulating hormone (FSH) and lutenizing hormone (LH) which both target the testis to release testosterone or the ovaries to release oestrogen and progesterone.

The aglandotropic hormones are:
- Growth hormone (GH) which stimulates protein synthesis and skeletal growth with the aid of growth factors produced in the liver.
- Prolactin which stimulates breast enlargement during pregnancy and milk production after delivery. The release of prolactin is also stimulated by stress in humans (Delitala, et al., 1987).

### 2.1.3   Pineal body (gland)

The pineal gland is a small reddish-brown structure almost in the centre of the brain and upper part of the midbrain overlying the cerebral aqueduct. It contains many bioactive peptides including ACTH and vasopressin, but the most studied compound secreted from the pineal gland is melatonin.

### 2.1.4   The adrenal glands

The adrenal glands are located above each kidney. The main function of the adrenal gland is the release of hormones in response to stress and the control of fluid electrolyte balance of the body.
It has two distinctive sections:
- The inner medulla, which produces catecholamines as well as endogenous opioids.
- The outer cortex, which produces adrenal steroids.

### 2.1.5   The ovaries and testicles

The ovary in females is responsible for the production of the ovarian steroids (oestrogen and progesterone), while the testis in males produce androgens such as

testosterone. The gonads along with the nervous system control the reproductive function and behaviour of the individual.

## 2.2 Potential effects of hormones on the auditory system

Other hormones may also be involved in affecting the auditory function but those of importance to this work are outlined below:

- Ovarian steroid hormones (oestrogen and progesterone).
- Stress related hormones (glucocorticoids , catecholamines and opioids).
- Fluid and electrolyte regulating hormones (aldosterone and vasopressin).
- Melatonin.

### 2.2.1 Ovarian steroid hormones

Oestrogen and progesterone are the main hormones that regulate reproductive behaviour in females and also play an important role in modulating the activity of several CNS structures.

#### 2.2.1.1 *Oestrogen*

There are three types of oestrogens produced naturally in women, oestradiol (E2), oestrone (E1) and oestriol (E3). Oestradiol is the primary oestrogen produced from the ovaries in premenopausal women with the highest affinity to oestrogen receptors. Oestrone is a metabolite of oestradiol, and is also produced by the conversion of androstenedione (an adrenal steroid) in the adipose tissue, and is the predominant oestrogen in postmenopausal women. Oestriol is the principal oestrogen synthesised from the placenta during pregnancy and is also a metabolite of oestradiol (Ruggiero & Likis, 2002).

The majority of oestradiol that circulate in the bloodstream is protein bound (Anderson, 1974). About 69% of oestradiol is bound to sex hormone binding globulin (SHBG), and to a lesser extent to serum albumin (30%), and only a small amount (about 1%) is unbound (Speroff & Fritz, 2005). The free and albumin bound oestradiol are biologically available and able to enter the cell and bind to

the nuclear receptors (Pardridge, 1986). Two types of oestrogen receptors have been identified, and are known as oestrogen receptor alpha and beta, (ERα and ERβ), and are widely distributed in the body (Kuiper, et al., 1998; McEwen & Alves, 1999; Nilsson, et al., 2001) including the auditory system (see below).

Oestrogens influence physiological functions in a variety of organs and systems in both females (Nilsson, et al., 2001) and males (Sharpe, 1998; Lombardi, et al., 2001), including the skeletal, cardiovascular and the nervous systems and the male urogenital tract, mammary glands and female reproductive organs. Many of the physiological effects attributed to testosterone in males have been found to be due to oestrogen as a result of the conversion of testosterone to oestrogen by the enzyme aromatase, which is present in the CNS (Sharpe, 1998).

*General effect on the CNS:*
The effect of oestrogens on the CNS has been studied extensively (Kuiper, et al., 1998; McEwen & Alves, 1999; Behl & Manthey, 2000; Garcia-Segura, et al., 2001). They act as central neuroactive and neuromodulator molecules by their influence on other neurohormones and neurotransmitters. They are mainly excitatory to neurons (Smith, et al., 2002). Oestrogens are also thought to be neuroprotective and a decrease in oestrogen levels after the menopause, or in Turner's syndrome, is associated with an increased frequency of neurodegenerative disorders. This protective effect is brought about by its genomic action on alpha and beta oestrogen receptors (ERα and ERβ), possible membrane receptors and/or its antioxidant effect (Behl & Manthey, 2000; Garcia-Segura, et al., 2001).

Oestrogen regulates the serotonergic and GABA-ergic systems (McEwen & Alves, 1999), although it seems to have a dual effect on the GABA system. During the female reproductive cycle, the preoptic area of the hypothalamus may control gonadotropin-releasing hormone production. In this site, oestrogen was found to increase the level of GABA by attenuating the GABA-B receptor autoinhibition of GABA-ergic neurons as a result of a negative feed back function, but when oestrogen reached a higher level it decreased the levels of glutamic acid decarboxylase (GAD) so attenuating GABA production (Wagner, et

al., 2001). This attenuation of GAD occurs transiently in other areas of the CNS, especially the hippocampus and is important in synaptogenesis (McEwen, et al., 2001). The modulation of specific mRNA subunits of GABA-A receptor by progesterone requires the presence of oestrogen (Weiland & Orchinik, 1995). Oestrogen also facilitates glutamate mediated neural activity in the cortex (Woolley, et al., 1997), and acts as an antioxidant, which protects neurons from glutamate excitotoxicity (Behl & Manthey, 2000).

*Potential effect on auditory system:*

Theoretically, oestrogen may influence the auditory function at different levels of the CNS, through its known actions as a modulator of the GABA-ergic, serotonergic, and glutamatergic systems (Woolley, et al., 1997). Oestrogen may have an excitatory action on auditory nerve fibres, as it has been found to be mainly excitatory to neurons in other areas of the CNS (Smith, et al., 2002). On the other hand, oestrogen may exert a neuroprotective effect on the auditory system, as it does in other areas of the CNS (Behl & Manthey, 2000; Garcia-Segura, et al., 2001).

Originally, the messenger RNA (mRNA) that encodes oestrogen receptors in the rat cochlea were not identified (Nathan, et al., 1999). However, recent research has confirmed the presence of ERα and ERβ, mainly by imunohistochemistry, in the inner ear (including outer and inner hair cells, spiral ganglion type I cells, the stria vascularis and cochlear blood vessels) in both humans (inner ears of normal adult and foetal tissue and those with Turner syndrome) (Stenberg, et al., 2001), and animal models such as mice and rats (Stenberg, et al., 1999; Meltser, et al., 2008; Simonoska, et al., 2009a), vocal fish (Forlano, et al., 2005), and zebra finches (Noirot, et al., 2009). Oestrogen receptors have also been identified in almost all the major auditory nuclei in the brain stem as well as the auditory cortex except for the medial geniculate body in mice (Charitidi & Canlon, 2010). Previous contradictory findings between studies that looked at the rat cochlea, may be the result of the small number of receptors and the low stability of the mRNA.

The presence of the receptors in the spiral ganglion and outer and inner hair cells, suggest that oestrogen may influence auditory transmission, while the receptors in the stria vascularis may affect cochlear fluid electrolyte balance (Lee & Marcus, 2001) and are of importance in the development of cells that produce the endolymph in the cochlea (Chen & Nathans, 2007). Additionally, the oestrogen receptors in the cochlear blood vessels may influence auditory function, by modulating cochlear blood flow (Laugel, et al., 1987). The absence of ERβ has been associated with hearing loss and susceptibility to acoustic trauma in mice (Meltser, et al., 2008; Simonoska, et al., 2009b), and a mutation in the gene that encodes the ERβ was found to be the cause of one form of autosomal-recessive nonsyndromic hearing loss known as DFNB35 (Collin, et al., 2008). The previous studies suggest the importance of oestrogen in the normal function of the cochlea and the auditory system.

The use of anti-oestrogen treatment (tamoxifin) for three days in ovarectomized rats did not effect the oestrogen receptor content of the cochlea (Stenberg, et al., 2003). However, a study by Thompson, et al., (2006) demonstrated that blocking oestrogen receptors for three months in young adult female CBA mice leads to a decline in contralateral suppression of distortion product otoacoustic emissions (DPOAEs); which is attributed to the function of the MOC. The decline seen was similar to that which occurs with aging and precedes the onset of age related hearing loss (Guimaraes, et al., 2004). The differences between the studies suggest that prolonged anti-oestrogen treatment may be needed to show a difference. Thompson and coworkers' (2006) findings support the role of oestrogen in influencing the auditory system both in the cochlea and the more proximal parts of the auditory system.

### 2.2.1.2 *Progesterone*

Progesterone is the main ovarian hormone produced by the luteinised granulosa cells within the ovarian follicles, following an LH surge, and is secreted by the corpus luteum during the luteal phase of the ovarian cycle (section 3.1.4). It is important in preparing the female genital tract for fertilization and then in maintaining pregnancy. Progesterone is also produced in the adrenal glands and

the CNS, and is a precursor to other steroid hormones and acts as a neurosteroid (Baulieu, 1998).

*General effect on the CNS:*
Progesterone and its metabolites are important in the regulation of GABA-A receptors (Follesa, et al., 2001), but require the presence of oestrogen as mentioned above. They also bind well with GABA-A receptors and act like benzodiazepines, displaying anaesthetic, antiepileptic, and sedative-hypnotic actions (McEwen & Alves, 1999). Progesterone and its metabolites tend to increase serotonin turnover (Genazzani, et al., 2000) and thus may contribute to the mood disturbances related to the female reproductive cycle. This action of progesterone implies that it has a mainly inhibitory function on the CNS, which would balance the mainly excitatory action of oestrogen (Katzenellenbogen, 2000; Smith, et al., 2002).

*Potential effect on auditory system:*
Specific progesterone receptors have not been identified in the cochlea, but progesterone may cross react with other steroid receptors (such as glucocorticoid and mineralocorticoid receptors) present in the cochlea or more proximal areas of the auditory system (Lang, et al., 1990; Nathan, et al., 1999). Progesterone and its metabolites also interact with the steroid binding sites on GABA-A receptors (Follesa et al., 2001), which are present throughout the auditory system (see Table 1.1-A and 1.1-B).

In the proximal auditory system, it is unclear if progesterone receptors are present in specific auditory structures, although they have been identified in other areas of the CNS, which have links to the auditory pathways, including the hypothalamus, limbic system and the reticular formation. However, such receptors may also be present in the auditory pathways, although not reported in the literature to date.

### 2.2.2   Stress related hormones

#### 2.2.2.1   *Glucocorticoids*

Cortisol (a steroid-based hormone) is the main glucocorticoid secreted from the adrenal cortex in response to stress, and acts through glucocorticoid receptors, which are widely distributed in the CNS (Jennes & Langub, 2000) and other organs. Cortisol affects carbohydrate, fat and protein metabolism, being anabolic in the liver, and catabolic in skeletal muscles (Hadley & Levine, 2006a), which is important in adaptation to a stressful stimulus.

Glucocorticoid receptors have been identified in the inner ear of animals (Rarey, et al., 1993; Zuo, et al., 1995; Shimazaki, et al., 2002), and humans (Rarey & Curtis, 1996) more in the cochlear than the vestibular tissue. Within the cochlea they are present in the sensory (the organ of Corti's hair cells and supporting cells) and non-sensory (spiral ligament and stria vascularis) tissues, suggesting both a possible role in homeostasis of inner ear fluids and signal transduction (reviewed by Horner, 2003). Glucocorticoids may also influence the auditory function by interacting with receptors found in the brainstem nuclei, including the mesencephalic raphe nuclei and locus coeruleus, which contain serotonergic and noradrenergic neurons (Jennes & Langub, 2000).

#### 2.2.2.2   *Catecholamines and endogenous opioids*

Catecholamines (adrenaline and nor-adrenaline) and endogenous opioids (such as β-endorphin, enkephalins, dynorphins) are released in response to stress from the adrenal medulla and act mainly through the nervous system. Catecholamines are the main neurotransmitters of the sympathetic nervous system, which is activated during stress (Brook & Marshall, 2001) while, opioids are also released from the pituitary and the limbic system during stress (Sapolsky, 2002).

Endorphins and enkephalins act as analgesics and produce a euphoric state (Brook & Marshall, 2001). Opioid binding of mu (μ) and delta (δ) opioid receptors commonly lead to neural inhibition (Crain & Shen, 1998), but may also lead to

neural excitation especially through dynorphin sensitive kappa (κ) opioid receptors (Mains & Eipper, 1999).

Opioid receptors have been identified in the mammalian cochlea (Jongkamonwiwat, et al., 2003; Jongkamonwiwat, et al., 2006) and enkephalins and dynorphins are thought to act as neurotransmitters in the auditory system (see section 1.1.4). Nor-adrenaline fibres of the sympathetic innervation of the cochlea surround the labyrinthine artery and the modiolar branches and control cochlear blood flow (Brown, 2001). Adrenaline and nor-adrenaline were found to influence the function of the stria vascularis by regulating the secretion of K+ and Cl- ions, activity of Na+, K+-ATPase, and general metabolism of the cells (reviewed by Ciuman, 2009). Sympathetic innervation is also seen in other auditory structures including the cochlear nucleus (Thompson, 2003) and the superior olivary complex (Mulders & Robertson, 2001) and thus has a potential to modulate higher levels of auditory function.

## 2.2.3   Fluid and electrolyte regulating hormones

### 2.2.3.1   *Aldosterone*

Aldosterone (a steroid based hormone) is the main mineralocorticoid secreted from the adrenal cortex and is mainly involved in regulating sodium homeostasis, and indirectly volume homeostasis. It is also released during stress. It raises blood pressure by increasing plasma volume, and increasing arteriolar sensitivity to vasoconstrictor agents (Brook & Marshall, 2001). Aldosterone acts on the distal renal tubules and collecting ducts of the kidney by increasing sodium reabsorption mainly by regulating the enzyme Na+, K+-ATPase (Brook & Marshall, 2001). The action of aldosterone is through mineralocorticoid receptors that are also distributed in the CNS, but to a lesser degree than glucocorticoid receptors (Jennes & Langub, 2000).

There is a functional similarity between the glomeruli of the kidney and the stria vascularis of the cochlea, as both are involved in ion exchange and are separated

from circulation by a basement membrane. This analogy may suggest that hormones have a role in inner ear fluid homeostasis, as seen in the kidney (Meyer, et al., 2002). The enzyme Na+, K+-ATPase, which is regulated by aldosterone, has been identified in the inner ear epithelium suggesting the hormonal modulation of endolymph secretion and fluid homeostasis (Ferrary & Sterkers, 1998).

Mineralocorticoid receptors have been identified within the cochlea (Rarey & Luttge, 1989) in the marginal cells of the stria vascularis and spiral ganglion cells (Furuta, et al., 1994). Thus aldosterone may be involved in endolymph homeostasis, although the function of the mineralocorticoid receptors on the spiral ganglion cells remains unknown. The absence of circulating adrenal hormones do not lead to electrophysiological changes in inner ear fluids (Ferrary, et al., 1996) but to a decrease in endolymph volume (Lohuis, et al., 2000).

Aldosterone treatment in mice with autoimmune hearing loss seems to have a similar effect to prednisolone in improving hearing thresholds and reversing the pathology in the stria vascularis due to autoimmune disease (Trune, et al., 2000). This suggests that aldosterone may play a protective role in the cochlea and may even have a positive effect on hearing function in the elderly (Tadros, et al., 2005). The latter authors found that aged men and women with lower levels of aldosterone (but still in the clinically normal range) had worse hearing thresholds than subjects with aldosterone levels in the upper middle of the normal range.

### 2.2.3.2 *Vasopressin*

Vasopressin, also known as antidiuretic hormone (ADH) is produced from the paraventricular neurons of the hypothalamus. It is involved in fluid homeostasis in a similar way to aldosterone, and is also released during stress and stimulates the release of adrenocorticotropic hormone (ACTH) (Halasz, 2000). Vasopressin acts as a neurotransmitter in the CNS and affects brain development, memory, learning, and body temperature (Halasz, 2000; Jennes & Langub, 2000).

The vasopressin membrane receptors include V1a, which is expressed widely in the CNS, in the frontal and piriform cortex, olfactory system, hippocampus, and

throughout the midbrain, pons and medulla (Jennes & Langub, 2000) and may therefore, directly or indirectly modulate the auditory system. ADH may also play a role in regulating cochlear fluid. Adenylate cyclase (an enzyme regulated by ADH), which is found in the kidney, has also been identified in the inner ear epithelium (Ferrary & Sterkers, 1998). Vasopressin receptors V1a and V2 have been identified throughout the developing rat cochlea, but only in the spiral ganglion and spiral ligament of the adult cochlea. Thus, this hormone is currently thought to be important in cochlear development (Furuta, et al., 1998).

### 2.2.4   Melatonin

Melatonin is a neuroactive substance derived from serotonin by the melatonin forming enzyme serotonin N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT). It has multiple effects on the CNS, but these are mainly related to the sleep/wake cycle possibly by its action on high affinity binding sites on the supra-chiasmatic nucleus and other areas of the CNS (Urbanski, 2000).

Melatonin was found to be synthesised in the guinea pig cochlea by melatonin-forming enzymes, NAT and HIOMT, and is detectable in the organ of Corti, the basilar membrane and to a lesser degree in the cochlear nerve and stria vascularis, including the spiral ligament (Biesalski, et al., 1988). The concentration of melatonin in the cochlea is affected by light, and correlates with the peripheral concentration of melatonin (Lopez-Gonzalez, et al., 1997). The function of melatonin in the cochlea is unknown, but may be protective, as it was found to prolong both the post-mortem activity of OHC (Lopez-Gonzalez, et al., 1999) and ameliorate the ototoxicity of aminoglycosides and cisplatnum in rats (Lopez-Gonzalez, et al., 2000a; Lopez-Gonzalez, et al., 2000b).
Melatonin has an anticonvulsant and anxiolytic action by enhancing GABA (Golombek, et al., 1996) and benzodiazepine function (Guardiola-Lemaitre, et al., 1992) and may theoretically have an effect on GABA-ergic fibers of the auditory system.

## 2.3 Hormone Measurement

Hormone levels can be measured in bodily fluids, such as serum, urine and saliva. The fluid sample used depends on the hormone being tested and the clinical utility. For example salivary cortisol gives a better measure of adrenal gland function than serum levels (Vining, et al., 1983; Gozansky, et al., 2005; Lewis, 2006), while the salivary levels of oestrogen are lower than serum oestrogen levels (Lu, et al., 1999), so may not give a reliable measurement if the oestrogen level is too low in the tested subject. Serum samples are commonly used in clinical practice to test most hormone levels. The method of analysis is by immunoassay, either using radioactive or chemilumenescent labelled antibodies (Neal, 2000). One or two antibodies may be used depending on the molecular size of the hormone. The two antibodies method are used for larger sized hormones (such as the growth hormone) in a non-competitive assay where the bound labelled antibodies are measured, while the one antibody method is used for the smaller sized hormones (such as steroid hormones) in a competitive assay where the unbound labelled antibodies are measured (Ekins, 2002). The competitive assay technique was used in the study to measure serum oestradiol and progesterone (section 5.3.2).

# Chapter 3 : Physiological Variations in Hormones and Auditory Function

## 3.1 Physiological variations in hormones that may affect auditory function

The levels of hormones vary in response to internal and external stimuli, and many vary in a cyclic fashion. These changes are important for the control of organism homeostasis. The hypothalamus acts as the neural control centre of the endocrine system and regulates the physiological variation in hormones (Halasz, 2000).
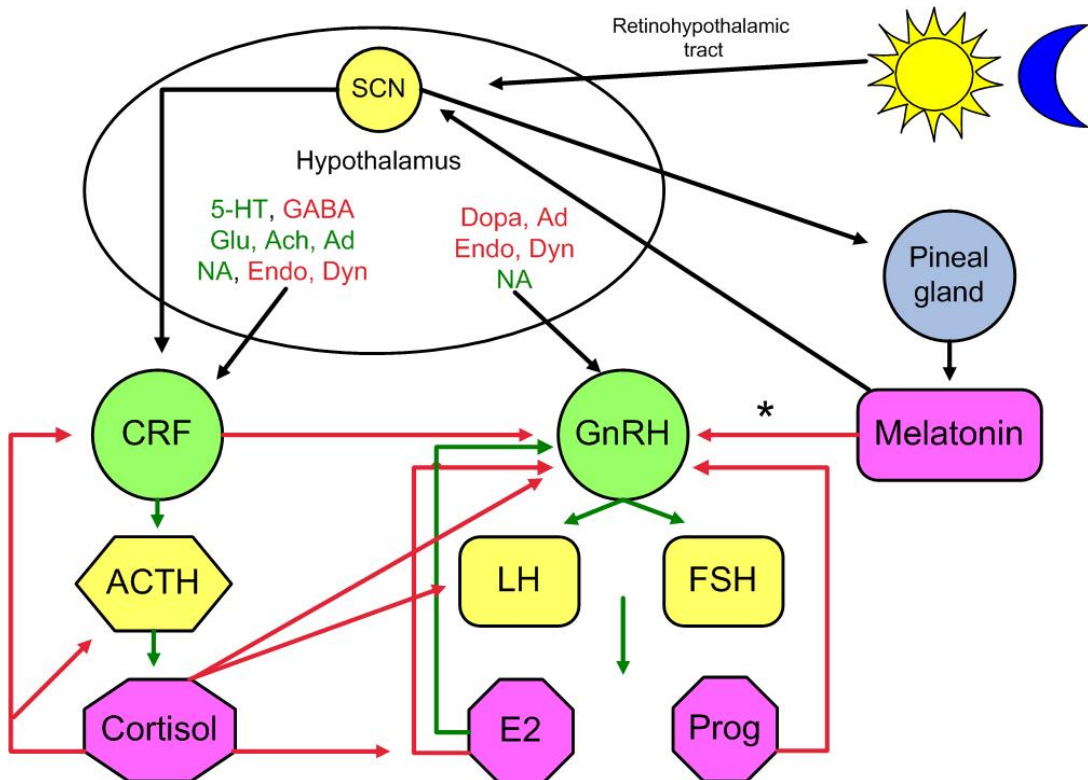


**Figure 3.1.1:** Hypothalamic pituitary adrenal and gonadal axis and the circadian cycle.

Light effect on the SCN leads to inhibition of melatonin synthesis from the pineal gland. * The inhibitory effect of melatonin on GnRH is only seen before puberty and in certain pathological conditions in humans (Silman, 1991; Kadva, et al., 1998).

Green lines and text: Excitatory effect, Red lines and text: inhibitory effect, ACh: acetylcholine, ACTH: adrenocorticotrophic hormone, Ad: adrenaline, CRF: corticotrophin releasing factor, Dopa: dopamine, Dyn: dynorphin, E2: oestrogen, Endo: β-endorphin, FSH: follicular stimulating hormone, GABA: γ-aminobutaric acid, GnRH: gonadotrophin releasing hormone, LH: lutenizing hormone, NA: nor adrenaline, Prog: progesterone, SCN: suprachiasmatic nuclei, 5-HT: serotonin.

The endocrine changes related to reproductive function (ovarian cycle, pregnancy, and menopause), daily rhythm (circadian) and exposure to stressful stimuli could in turn affect auditory function. However, the hormones involved in these physiological changes also influence each other (Figure 3.1.1).

Melatonin plays an important role in the control of reproductive behaviour in seasonal breeding mammals (reviewed by Arendt, 1998) possibly through an inhibitory effect on the gonadotrophin releasing hormone (GnRH) (Silman, 1991). The effect of melatonin on human reproduction is not clear, but low levels of melatonin have been associated with precocious puberty and high levels with delayed puberty (Hadley & Levine, 2006b). Abnormally high melatonin levels have been found in females with functional hypothalamic amenorrhea and males with hypogonadotrophic hypogonadism, but it is not clear if melatonin contributes to the disorder or is the result of it. The deficiency in GnRH due to Kallman's syndrome or functional hypothalamic amenorrhea is associated with high nocturnal levels of melatonin that may be due to the lower levels of oestrogen that affect melatonin secretion (Kadva, et al., 1998).

Acute or chronic stress tends to have a negative effect on reproductive function, which occurs at multiple levels (reviewed by Kalantaridou, et al., 2004). GnRH release is suppressed by corticotrophin releasing factor (Chrousos, et al., 1998), cortisol (Saketos, et al., 1993) and dynorphin and β-endorphin through μ and κ receptors in the hypothalamus (Petraglia, et al., 1986) which reduces plasma levels of lutenizing hormone (LH). Glucocorticoids also suppress LH levels directly through their receptors (McGivern & Redei, 1994) and inhibit oestrogen action (Rabin, et al., 1990). This suppression of the hypothalamic pituitary gonadal axis leads to lower levels of oestrogen and progesterone, which, in turn may affect auditory function.

The interaction between the different hormones enhances the possible multidirectional effects of hormones on the auditory system.

### 3.1.1   The circadian cycle and auditory function

The circadian pacemaker in mammals is found in the paired supra chiasmatic nuclei of the hypothalamus, which also control the rhythm of melatonin synthesis through a multi synaptic pathway (Urbanski, 2000). Melatonin, cortisol and vasopressin show a clear circadian pattern. Melatonin levels are highest at night and lowest during the day (Arendt, 1998). Cortisol levels are highest at dawn and low at dusk (Despopoulos & Silbernagl, 1991), while vasopressin levels are higher during the night (Kostoglou-Athanassiou, et al., 1998b). This means that the circadian cycle influences almost all aspects of human physiology and psychology, and therefore the auditory system may also be under this influence.

The rhythm of the suprachiasmatic nuclei is entrained by light through input from the eye via the retinohypothalamic tract (Block, et al., 2000). In subterranean animals, such as the mole, the inferior colliculus (with auditory information) projects to the suprachiasmatic nuclei, and may have a similar function as the retino-hypothalamic tract in other mammals so it can entrain to the environment (Kudo, et al., 1997). In humans, a recent study has found that auditory stimuli lead to a phase shift in circadian rhythms (Goel, 2005). This finding implies that a connection between the auditory pathways and the supra chiasmatic nuclei also exists in humans, which may lead to the direct effect of circadian cycle on auditory function and vice versa.

The possible influence of the circadian cycle on auditory tests has been examined in humans. Diurnal changes have been reported in ***otoacoustic emissions*** that reflect cochlear function. Wit (1985) measured SOAE in two volunteers on ten different days, twice daily for the female volunteer and three times daily in the male volunteer. He reported that the SOAE frequency significantly shifted to a lower frequency in the afternoon sessions compared to the morning measurements. Bell (1992) demonstrated a similar finding by monitoring his own SOAE and those in two female volunteers. Bell and one of the female subjects displayed clear circadian variation in frequency of the SOAE. The SOAE frequency was highest in the morning and shifted toward a lower frequency during the waking hours and returned during sleep to early morning levels. The

changes corresponded to 1% change in frequency, with no amplitude fluctuation. Haggerty and co-workers (1993) also reported a significant 24-hour variability in SOAE frequency in a male and female subject, which was less than 1 %. The pattern of change was not identical in the two subjects; however, it was similar in that SOAE frequency decreased during late evening and early morning hours. These changes suggest that the SOAE generator is synchronised with the biological circadian rhythm. The peripheral auditory structures may be less inhibited along with other subcortical structures during sleep (Lancel, 1993). The increase in SOAE levels during sleep may be due to the lower inhibition during sleep

Diurnal changes were not found in the ***auditory evoked potentials*** arising from the brain stem (Romani, et al., 2000). However, the auditory evoked potentials which arise from cortical structures such as the P300 seem to be affected by the time of day (reviewed by Polich & Kok, 1995). This may be attributed to circadian changes in cognitive function that contribute to the P300 (Wesensten, et al., 1990).

### 3.1.2   The response to stress and auditory function

The presence of emotional or physical stress initiates the endocrine stress response by the release of corticotrophin releasing factor (CRF). This activates the hypothalamic pituitary adrenal axis, and leads to the release of cortisol and adrenaline from the adrenal gland (Figure 3.1.1) and activation of the sympathetic nervous system.

Auditory stimuli, such as noise, can initiate the endocrine response to stress (Spreng, 2000; Michaud, et al., 2003). The underlying pathway may be through the medial geniculate body, which acts as the interface between the auditory system and the stress responsive limbic system. The non-primary area of the auditory cortex and the medial geniculate body was found to have more CRF mRNA than other areas of the rat's central auditory system (Imaki, et al., 1991). On the other hand, the hormones involved in the response to stress may affect auditory function directly by an excitatory effect, which may lead to damage

through glutamate induced neurotoxicity (Pujol, et al., 1993). The possible interaction between stress and the auditory system is demonstrated in Figure 3.1.2.
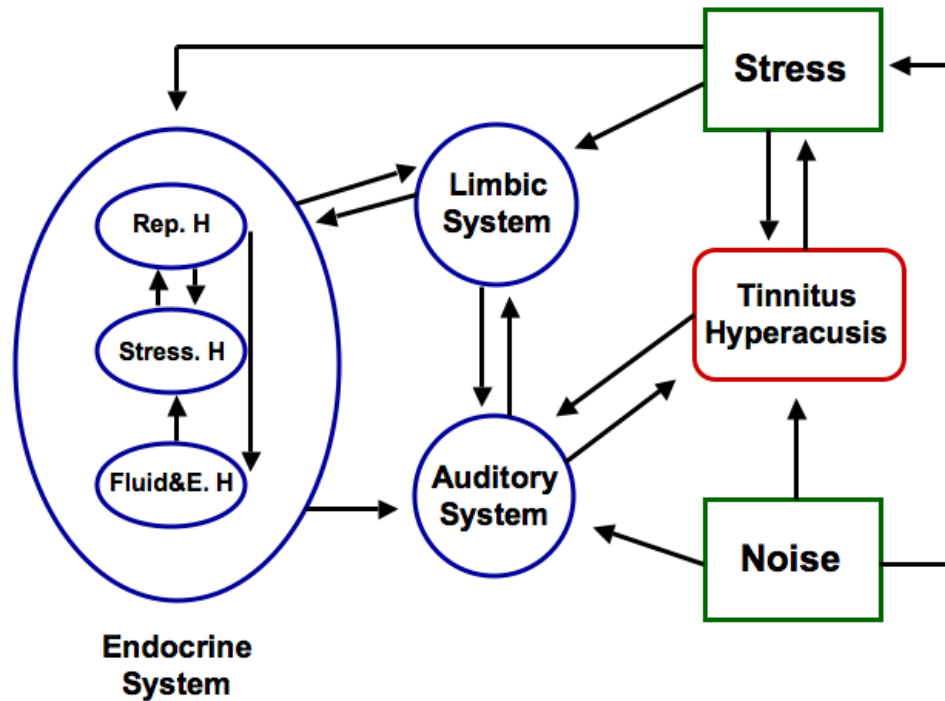


**Figure 3.1.2:** Possible interaction between stress, endocrine system and auditory system.

The stress response can be initiated by noise that may lead to auditory symptoms (tinnitus and/or hyperacusis) that in turn can cause stress and can be aggravated by it.

Fluid & E. H = Fluid and electrolyte hormones, aldosterone and vasopressin, Rep. H = Reproductive hormones, Stress. H = Stress hormones, cortisol, catecholamines and opioids.

### 3.1.3   Gender differences in auditory function

Reproductive hormones have been implicated in gender differences in sensorimotor (Becker, 2002) and cognitive (Hampson, 2002) functions in animals and humans. The underlying cause for these gender differences may be due to sexual dimorphism in CNS structures from the exposure to reproductive hormones during development (de Courten-Myers, 1999; Rhodes & Rubin, 1999) which may also affect the auditory system. Indeed, sexual dimorphism has been noted in auditory structures. The cochlea in females is shorter than the cochlea in males (Sato, et al., 1991), and may have a larger number of OHC (Wright, et al., 1987)

which are stiffer and thus more sensitive to the acoustic stimulus (Morlet, et al., 1996). The sexually dimorphic structure of the serotonergic system (Rubinow, et al., 1998) as mentioned above (section 1.1.6) may also modulate neural transmission in the auditory brain stem and cortical structures. The differences in reproductive hormones during development and in adulthood may explain the gender effect in the auditory function, which may theoretically be "better" in females due to the excitatory and protective effects of oestrogen.

A summary of the major gender differences in auditory function:

- Several studies have reported that adult females have more sensitive hearing in higher frequencies (measured by *pure tone audiometry*) compared to males (Jerger & Hall, 1980; McFadden, 1993; Davis, 1995; McFadden, 1998). This has also been noted in carefully screened populations for noise exposure (Johansson & Arlinger, 2002). In school aged children, girls tend to have lower audiometric threshold compared to boys, however the difference is not statistically significant (Roberts & Huber, 1970; Haapaniemi, 1996).

- *Otoacoustic emissions* (OAE) are associated with good hearing sensitivity (Probst, et al., 1991). Women tend to have OAE with larger amplitudes compared to men (McFadden, 1993; Hall, 2000b; McFadden, et al., 2009a) and are more likely to have recordable spontaneous otoacoustic emissions (SOAE) (75% of females compared to 58% of males (Penner & Zhang, 1997). The gender difference is also seen in both neonates (Kei, et al., 1997) and older children (Lamprecht-Dinnesen, et al., 1998; O'Rourke, et al., 2002) and may be due to prenatal hormonal exposure. These sex differences in OAE have also been reported in some animals including monkeys (Lonsbury-Martin & Martin, 1988; McFadden, et al., 2006), mice (Guimaraes, et al., 2004), and sheep (McFadden, et al., 2009b).

- The excitatory effect of oestrogen and sexual dimorphism in the CNS and auditory system may affect the neural transmission in the auditory brain stem leading to gender differences in *auditory brain stem evoked*

*responses* (ABR). Female adults were found to have shorter ABR wave latencies and larger wave V amplitude compared to males (Jerger & Hall, 1980; Jerger & Johnson, 1988; Trune, et al., 1988; Dehan & Jerger, 1990). The same findings were also noted in neonates (Chiarenza, et al., 1988; Stuart & Yang, 2001), but to a lesser extent in older children (Trune, et al., 1988; Hall III, 1992). Shorter ABR latencies were also reported in female rats compared to male rats (Church, et al., 1984).

The effect of reproductive hormones on the auditory function may be more clearly noted in the endocrine changes that occur during the ovarian cycle.

### 3.1.4   Ovarian cycle and auditory function

The average female ovarian cycle lasts for 28 days (normal range 21-35 days), with day one being the first day of menses (Figure 3.1.3).
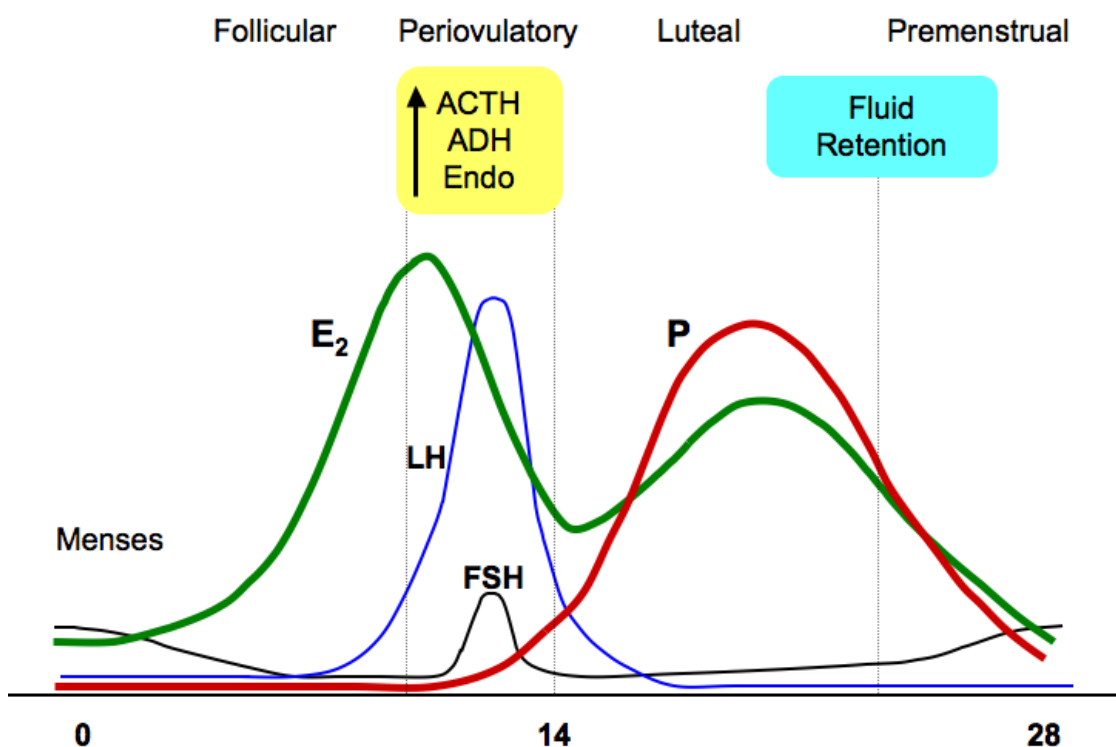


**Figure 3.1.3:** Schematic representation of the changes in reproductive hormones during the average menstrual cycle, and the associated rise of other hormones in the periovulatory phase.

Oestrogen is secreted during the proliferative (follicular) phase of the cycle, in response to FSH. On reaching its maximum level, oestrogen simulates release of GnRH from the hypothalamus, which, in turn, initiates the LH surge. Ovulation occurs 38-42 hours after the beginning of the LH surge (Djahanbakhch, et al., 1981). Progesterone secretion starts to rise following luteinisation of the granulosa cells in the luteal phase of the cycle. If fertilization does not occur the level of both hormones decline and the next cycle begins.

These changes in reproductive hormones levels have an impact on the hypothalamic pituitary adrenal axis (Bloch, et al., 1998). The higher level of oestrogen during the follicular phase is associated with a rise in other hormone levels. The basal plasma level of adrenocorticotrophic hormone (ACTH) was found to rise in the late follicular phase (Genazzani, et al., 1975; Mauri, et al., 1990) possibly due to the enhancing effect of oestrogen on corticotrophin releasing factor gene transcription in the hypothalamus (Kirschbaum, et al., 1999). The rise of ACTH during the late follicular phase is not associated with higher free cortisol, due to oestrogen induced changes in corticosteroid-binding protein levels (Kirschbaum, et al., 1999; Altemus, et al., 2001). The lower level of free cortisol may affect the physiological response to stress during this phase of the menstrual cycle.

Vasopressin levels were also reported to be higher during the follicular phase of the cycle compared to the mid-luteal phase (Kostoglou-Athanassiou, et al., 1998a). The enhancement of vasopressin secretion is possibly due to oestrogen, which was found to increase vasopressin levels in females, who had undergone oopherectomy (Forsling, et al., 1996). This may lead to fluid retention or redistribution, which occurs in some women in the pre-menstrual period of the menstrual cycle (Tollan, et al., 1993) and may also affect the fluid balance in the cochlea and thus affect auditory function.

The level of β-endorphin peaks two to four days before ovulation followed by a dip in levels post ovulation and then there is a gradual rise again during the late luteal phase, about 24 hours before the next menses (Vrbicky, et al., 1982; Tang, et al., 1987; Ferrer, et al., 1997). Females with polycystic ovarian disease and

amenorrhea have levels of β-endorphin, which are lower than in normal females (Martinez-Guisasola, et al., 1999). There is also evidence that oestrogen stimulates opioid receptor expression and stabilises the levels of β-endorphins that tend to decrease after menopause (surgical or spontaneous). This may be associated with mood changes that can be helped by oestrogen treatment, which increases β-endorphin levels in plasma (Genazzani, et al., 2000). Oestrogen also affects mood by facilitating the function of enkephalin that is also important for reproductive behaviour (Pfaff, et al., 2000). It is not clear if the changes in β-endorphin is intrinsic in the regulation of the ovarian cycle or is due to the effect of oestrogen.

Progesterone alone had no effect on β-endorphin levels in the hypothalamus and pituitary of oopherectemised (OVX) rats. However, treatment with both oestrogen and progesterone reverses the effect of oestrogen alone on β-endorphin levels in the pituitary, and increases the levels of β-endorphin in the hypothalamus (Genazzani, et al., 2000). Thus the effect of progesterone on β-endorphin levels requires the presence of oestrogen.

The changes seen in other hormones other than the reproductive hormones during the ovarian cycle may also potentially contribute to the changes reported in auditory function during the ovarian cycle (see section 3.1.4.1).

### 3.1.4.1  *Auditory tests during the ovarian cycle*

The fluctuation of hormones during the ovarian cycle may potentially lead to fluctuation in auditory function and other sensory processes such as vision, olfaction and touch (reviewed by Parlee, 1983). The optimal function of the auditory system may occur during the peak of oestrogen circulation due to its excitatory and protective effect in the central nervous system. Correspondingly, the low levels of hormones during the premenstrual phase may relate to less than optimal auditory function.

Sisneros and Bass (2003) found that the auditory nerve fibers of a midshipman fish (a vocal seasonally breeding fish) are more responsive to male matting calls

during the breeding season and not at other times. A later study by the same group (Sisneros, et al., 2004) reported that oestradiol treatment of female midshipman fish during the non-breeding season makes their auditory nerves respond more to male mating calls. The auditory system of female songbirds becomes more sensitive to male birdsong during the breeding season and processes it in a way that initiates their reproductive behaviour. This is dependent on the presence of oestrogen (Maney, et al., 2006; LeBlanc, et al., 2007; Maney, et al., 2008), and a similar finding was also reported in the auditory system of female frogs (Lynch & Wilczynski, 2008). No studies have reported these changes in mammals, but McFadden and his colleagues (2006) reported that female rhesus monkeys had larger OAE amplitudes during the Autumn breeding season which coincide with higher levels of estrogen compared to the Summer (non breeding) months. The male monkeys in their study also demonstrated smaller OAE amplitudes during the Autumn breeding season compared to the summer months that coincided with higher testosterone levels. These findings suggest auditory modulation related to fluctuation in reproductive steroids may also be found in mammals.

Auditory function in women during the ovarian cycle has been previously investigated, with conflicting findings (Table 3.1-A). Fluctuation in auditory function during different stages of the cycle has been demonstrated, but the studies lacked precise timing of the cycle, together with exact correlation between hormonal levels and auditory function. Only a few studies monitored ovarian steroid levels during the cycle, and these examined only auditory brainstem-evoked responses.

**Table 3.1-A:** Studies of auditory function and ovarian cycle and the effect of reproductive hormones.

| Study | Subjects | Auditory test & Methodology | Documentation of ovulatory cycle | Findings |
|---|---|---|---|---|
| (Baker & Weiler, 1977) | 4 ♀<br><br>4 ♀ OC<br><br>4 ♂ | **PTA** (audiometric) at 0.25, 0.5, 1, 2, 4 & 8 kHz | **History** | • OC group had lower thresholds than the normal control groups ♀ and ♂<br><br>• ♀ had lower thresholds during the first half of the cycle than the second half<br><br>• No change in thresholds in OC group and ♂ during repeated testing |
| (Cox, 1980) | 12 ♀<br><br>13 ♀ OC | **PTA** (Bekesy) at 0.5, 1 & 2 kHz | Daily **BBT** | • No significant change in auditory sensitivity between groups<br><br>• Poorer thresholds in the menstrual phase for all<br><br>• Negative middle ear pressure during the menstrual phase in all |
| (Petiot & Parrot, 1984) | 12 ♀<br><br>11 ♀ OC<br><br>8 ♂ | **PTA** (Bekesy) at 4 & 6 kHz | Daily **BBT** | • ♀ using OC hearing thresholds lower than ♀ in all test sessions<br><br>• No change in 4 kHz thresholds during cycle |

| | | | | |
|---|---|---|---|---|
| (Swanson & Dengerink, 1988) | 10 ♀<br><br>10 ♀ OC<br><br>12 ♂ | **PTA** (Bekesy) at 4 & 6 kHz | Daily **BBT**<br><br>Cervical mucus changes | • ♀ 4 kHz threshold was lowest during ovulation (cycle day 13-14) and highest during menses (cycle day 2-3)<br><br>• ♀ 4 kHz threshold during ovulation was significantly lower than OC group and ♂<br><br>• No significant change was found in 6 kHz threshold or difference between the groups |
| (Laws & Moon, 1986) | 10 ♀<br><br>10 ♂ | **Acoustic reflex threshold (ART)** | **History** | • ♀ had significantly higher ART thresholds during menses (cycle day 1-6) than during the rest of the cycle (day 7-26)<br><br>• No cyclic changes seen in ♂ during the same test period |
| (Bell, 1992) | 4 ♀ | **SOAE** | **History** & Daily BBT in 2 subjects only | • 3 out of 4 had a clear variation in SOAE frequency<br><br>• ↓in the frequency before menses and ↑time of ovulation (cycle day not mentioned) |
| (Haggerty, et al., 1993) | 8 ♀<br><br>2 ♂ | **SOAE** | **History** | • 6 females had monthly variation in SOAE<br><br>• had ↑ in frequency variation before menses<br><br>• 5 had ↓in the frequency before menses and ↑time of ovulation (cycle day not mentioned) |

70

| | | | | |
|---|---|---|---|---|
| (Penner, 1995) | 1♀ (NMC, FA , OC) | **SOAE** | Daily **BBT** | • During NMC ↓ in the frequency of SOAE before menses and ↑ near the time of ovulation<br><br>• Less fluctuation in SOAE frequency were noted during amenorrhea and the use of OC |
| (Yellin & Stillman, 1999) | 13 ♀ | **SOAE, DPOAE, TEOAE** | Daily **BBT** | • SOAE dominant early in the cycle, gradually decreased and least prevalent before menses<br><br>• No cyclic changes in TEOAE or DPOAE were found |
| (Amit & Animesh, 2004) | 15 ♀ | **TEOAE** | **History** | • TEOAE amplitude significantly higher during menses (cycle day 1-3) and lowest during the luteal phase (cycle day 22-25)<br><br>• TEOAE amplitude significantly lower during mid cycle (day 12-15) compared to during menses but higher than the luteal phase<br><br>• 3 subjects did not have TEOAE responses during mid cycle and luteal phase |
| (Arruda & Silva, 2008) | 21 ♀ | **TEOAE, DPOAE** | **History** | • No change in TEOAE amplitude or reproducibility during three tested phases of the cycle (follicular, ovulatory and luteal but days not specified)<br><br>• No change in DPOAE during the cycle |
| (Fagan & Church, 1986) | 10 ♀ | **ABR** at 50 dB nHL | Daily **BBT** | • No fluctuation in ABR latencies during ovarian cycle |

| | | | | |
|---|---|---|---|---|
| (Zani, 1989) | 4 ♀<br><br>4 ♀ OC | **ABR** at 65 dB SPL | Daily **BBT** | • Wave V latency significantly longer during mid-cycle (cycle day 14-15) but not in ♀ using OC<br><br>• Wave I and III latency tends to be longer during mid cycle and premenstrually (cycle day 23-24) but not in ♀ using OC |
| (Dehan & Jerger, 1990) | 10 ♀ | **ABR** at 80 dB nHL | **Blood** (E2, P, FSH and LH) | • Wave V latency longer just before ovulation and shorter in luteal and pre-menstrual period (cycle days not mentioned) |
| (Elkind-Hirsch, et al., 1992a) | 9 ♀<br><br>9 ♀ OC | **ABR** at 70 dB nHL | **Blood** (E2, P, FSH and LH) | • Significant ↑ in wave III ,V and I-V latency associated with ↑ E2 during mid-cycle phase (cycle day 12-15)<br><br>• No significant changes in ABR latencies in the OC group |
| (Elkind-Hirsch, et al., 1992b) | 5 ♀ POF on HRT | **ABR** at 70 dB nHL | **Blood** (E2, P, FSH & LH) | • HRT lead to fluctuation in ABR as seen in NMC<br><br>• Wave V and I-V latency significantly ↑during the E2 only replacement compared to P and E2 replacement phase |

| | | | | |
|---|---|---|---|---|
| (Elkind-Hirsch, et al., 1994) | 5 ♂<br><br>9 ♀<br><br>9 ♀ OC<br><br>5 ♀ POF<br><br>5 ♀ PCOD | **ABR** at 70 dB nHL | **Blood** (E2, P, FSH, LH, DHEAS & T) | • Wave V latency in descending order: ♂ , ♀PCOD (higher androgen levels), then ♀POF , ♀OC and ♀<br><br>• Significant ↑ in wave V latency during E2 only replacement for POF and mid cycle in ♀ |
| (Tasman, et al., 1999) | 19 ♀ | **ABR** at 70 dB | Daily **urine LH & BBT** | • Wave V and III-V latency ↑ in follicular phase (cycle day not mentioned) |
| (Resende, et al., 2000) | 15 ♀ | **ABR** at 85 dB | **History** of changes in vaginal secretion | • No significant difference in ABR latencies in the three testing sessions |
| (Yadav, et al., 2002) | 20 ♀ | **ABR** at 70 dB nHL | Daily **BBT** | • A trend of ↑ in ABR wave latencies and interpeak intervals during the mid-cycle phase (cycle day 11-15), but not significant. |
| (Serra, et al., 2003) | 94 ♀ | **ABR** at 100 dB SPL | **Ultrasonography & serum** P level | • ABR wave latencies and interpeak intervals significantly↓ in the periovulatory phase (cycle day not mentioned) |
| (Caruso, et al., 2003b) | 94 ♀ before & after OC | **ABR** at 100 dB SPL | **Ultrasonography & serum** P level | • ABR wave latencies and interpeak intervals significantly↓ in the periovulatory phase (cycle day 13-16).<br><br>• No significant difference in ABR wave latencies and interpeak intervals during OC |

| (Fleck & Polich, 1988) | 10 ♀<br>10 ♀ OC | **P300 ERP** auditory discrimination | **History** | • P300 amplitude smaller in beginning than in mid cycle but not significant for both groups<br><br>• P300 latency longer in ♀ using OC but not significant |
|---|---|---|---|---|
| (Yadav, et al., 2003) | 20 ♀<br>20 ♀ OC | **LLAEP** at 90 dB SPL | Daily **BBT** | • ♀ P2 and N2 wave latency longest during mid cycle (day 11-15) and pre menstrually (day 25-27) and shorter during menses (day 1-3) and luteal phase (day 17-22)<br><br>• ♀ using OC P2 and N2 wave latency shorter during menses (day 1-3) and longer when taking pills (day 7-28) |
| (Walpurger, et al., 2004) | 18 ♀ | **ERP** auditory discrimination | **History & salivary** E2 & P level | • No change in N1 and P2 latency or amplitude during cycle<br><br>• N2 latency longer in follicular (day15-22) and luteal ( 3-9 days before next cycle) phase than in menses (day 2-4 )<br><br>• No change in the P300 with cycle |

NMC= normal menstrual cycle, FA= functional amenorrhea OC= oral contraceptives, BBT= basal body temperature, POF= premature ovarian failure, PCOD= polycyctic ovarian disease, HRT= hormone replacement therapy, E2= oestrogen, P= progesterone, DHEAS= dehydroepiandroterone sulfate, T= testosterone, PP= progesterone phase ↑=increase, ↓=decrease, ♂= males, ♀= females with normal menstrual cycle, LLAEP= long latency auditory evoked potentials, ERP= event related potential.

The auditory and acoustic reflex *thresholds* seem to be less sensitive during the menstrual phase of the cycle as observed by Cox (1980), Petiot and Parrot (1984), Laws and Moon (1986), and Swanson and Dengerink (1988). Baker and Weiler (1977) on the other hand reported that audiometric thresholds were higher during the second half of the cycle, but it is not clear from the study if that part of the cycle included the menstrual phase. The higher thresholds during the menstrual phase is unexplained. However, the associated negative middle ear pressure observed during the menstrual phase in some subjects could contribute to this threshold elevation. An increase in interstitial fluids (possibly due to progesterone (Tollan, et al., 1993; Pechere-Bertschi, et al., 2002), affects the Eustachian tube function and, thus may lead to worse threshold during the menstrual phase of the cycle. Another possibility is that a fluctuation in hormones affects higher areas of auditory processing and, thus, leads to changes in auditory thresholds, similar to the findings documented in other sensorimotor and cognitive functions (Hampson, 2002).

Petiot and Parrot (1984), and Swanson and Dengerink (1988) observed that the 4 and 6 kHz thresholds were lower around the time of ovulation, while Cox (1980) did not find any change in thresholds of the frequencies tested during the ovarian cycle (see Table 2.1-A). These findings may suggest that the effect of hormone fluctuation during the cycle on hearing sensitivity is frequency specific. The fluctuation in *SOAE* frequency with the ovarian cycle may give more weight to the this latter hypothesis.

The greater frequency variation of SOAE near the time of ovulation, with a shift toward a higher frequency, implies an excitatory effect of oestrogen on the SOAE generator and the frequency shift toward a lower frequency and less frequency variation in SOAE near menstruation may be due to the low levels of oestrogen and progesterone. Oestrogen may be involved in this frequency change, because the oestrogen level is lowest in the late luteal phase (before menses) and peaks near ovulation, which has been associated with an increase in neuronal excitability and inhibition of GABA function (Wagner, et al., 2001).

Only three studies evaluated **TEOAE** during the ovarian cycle (Yellin & Stillman, 1999; Amit & Animesh, 2004; Arruda & Silva, 2008). Yellin and Stillman (1999) and Arruda and Silva, (2008) did not observe any change in TEOAE responses during the cycle. On the other hand, Amit and Animesh (2004) reported that the TEOAE responses tend to decrease during the cycle and that three of their subjects did not have recordable TEOAEs in two testing sessions (see Table 3.1-A). This may have confounded their results as it raises the question whether their subjects had a cochlear dysfunction. More studies are needed to clarify TEOAE results during the ovarian cycle.

The majority of studies that recorded **ABR** reported changes with the ovarian cycle (see Table 3.1-A). However, the studies that recorded hormone levels and ABR gave conflicting findings. The shorter ABR latencies reported by Caruso et al. (2003b) and Serra and colleagues (2003) during the periovulatory phase of the cycle suggest that a high oestrogen level is associated with shorter ABR latencies. This has also been found in oestrogen treatment of young ovariectomised rats (Coleman, et al., 1994). The higher level of oestrogen may alter the speed of sensory neurotransmission in the brain stem by modulating glutamate transmission (Behl & Manthey, 2000) or may result in an increase in neurosteriods (such as allopregnelone) which facilitate GABA inhibition in the auditory midbrain (Disney & Calford, 2001). The oestrogen treatment of ovariectomised rats increases the level of allopregnelone both centrally and peripherally in a dose dependant fashion (Stomati, et al., 2002). This possible effect on neurosteriods may explain the results of Dehan and Jerger (1990) and Elkind-Hirsch et al (1992a, 1992b and 1994) who demonstrated longer ABR latencies during the periovulatory phase. The difference between the ABR latencies in the periovulatory phase and luteal phase would be compatible with progesterone blunting the effect of oestrogen, by inhibiting its action in the auditory brainstem. This mechanism may also explain the larger *long latency auditory potentials* recorded during the periovulatory phase (Yadav, et al., 2003; Walpurger, et al., 2004). However, the fluctuation in hormones does not seem to affect the P300 component of the auditory evoked response (Fleck & Polich, 1988; Walpurger, et al., 2004) that reflects higher cognitive auditory processing (Polich & Kok, 1995).

### 3.1.4.2 Auditory symptoms during the ovarian cycle

No studies have reported auditory symptoms during the ovarian cycle. However a few case reports in the literature have described women who had fluctuating hearing loss, that occurred in the late luteal phase and improved after the onset of menses (Miller & Gould, 1967; Andreyko & Jaffe, 1989), or that corresponded with the onset of menses (Souaid & Rappaport, 2000) suggesting variations with the menstrual cycle. Miller and Gould (1967) described two women with this condition one of whom had worse symptoms when given progesterone. A similar case was reported by Andreyko and Jaffe (1989). Their patient's symptoms improved during pregnancy, while she was breastfeeding (she was amenorheic) and with the use of nafarelin (GnRH analogue that leads to ablation of sex steroids by down-regulation of GnRH receptors and decreases secretion of FSH and LH leading to anovulation and amenorrhea). On the other hand, Souaid and Rappaport (2000) described a case of a 45-year old woman, who had bilateral hearing loss , right ear blockage and tinnitus with the onset of menses that improved later during the cycle. They also reported that she had an abnormal ABR recorded in the middle of her ovarian cycle (prolonged III-V interpeak interval on the left, and delayed wave V with prolonged I-V interpeak interval on the right). She was treated with diuretics, which improved her symptoms, and the ABR results of the right ear but the ABR results of the left ear remained unchanged, and her hormonal profile was within normal limits.

These cases suggest that in some women the auditory system is more sensitive to the fluctuation of hormones during the ovarian cycle. The effect of diuretics in improving the symptoms of one of the cases raises the possibility of the effect of fluctuation in reproductive hormones on inner ear fluids. Another possibility is that the diuretics lessen the effect of possible fluid retention that can lead to oedema in the Eustachian tube and the middle ear (as mentioned above in section 3.1.4.1).

### 3.1.5 Pregnancy and auditory function

During pregnancy there is a higher level of both ovarian hormones compared to non pregnant women, as well as other complex changes in the normal female

physiology (Hadley, 2000). These changes lead to fluid retention and a hyperdynamic circulation, which may impact upon the circulation in the cochlea and cochlear fluid homeostasis.

Tsunoda and colleagues (1999), reported no changes in **pure tone** or **impedance** audiometry comparing pregnant and non-pregnant women. However a study by Sennaroglu and Belgin (2001) found that pregnant women had higher pure tone thresholds at 125, 250 and 500 Hz, than post-partum and non-pregnant women, but were still within the normal range (less than 20 dB HL), and pregnant women in the third trimester had significantly lower uncomfortable loudness level compared with post-partum and non-pregnant subjects. These findings suggest that the physiological changes in pregnancy may affect auditory function mimicking the auditory dysfunction seen in Menière disease, i.e. low frequency involvement with lowering of uncomfortable loudness levels.

Burns (2009) studied the ***SOAE*** in two pregnant women during their pregnancy and post partum. He reported that the SOAE frequency shifted to a lower frequency in the measurements taken before giving birth and then shifted to a highest frequency in the measurement recorded after giving birth, and was relatively stable during pregnancy. The shift in SOAE frequency corresponded to the drop in the levels of oestrogen and progesterone between late prepartum to postpartum (Tulchinsky, et al., 1972).

The higher levels of ovarian hormones in pregnancy may shorten ***ABR*** wave latencies as seen in previous studies (section 3.1.4.1), but the higher level of progesterone may blunt this effect. Neural conduction in the brain stem may be slower during pregnancy due to the higher levels of steroids that facilitate GABA inhibition (Disney & Calford, 2001). Egeli and Gurel (1997) compared the ABR in 62 women in different stages of pregnancy, and 58 non pregnant women, and found that only wave I latency was significantly longer in the pregnant women. On the other hand, Tandon and co-workers (1990) found that the absolute wave latencies were slightly shorter, but did not reach significance in eight pregnant women in the third trimester compared to aged matched non-pregnant women. However, the I-III, III-V, and I-V inter peak latencies, which indicate neural

conduction were significantly longer in pregnant women. An ABR study of 38 females, 20 in different stages of pregnancy (including post-partum) and 18 non-pregnant women by Sennaroglu and Belgin (2001) did not find any difference. This contradiction may be attributed to difference in methodology and sample sizes.

*Auditory symptoms* such as aural fullness, changes in auditory sensitivity or tinnitus have been reported in pregnancy (Gurr, et al., 1993; Tsunoda, et al., 1999). There has also been a case of a pregnant woman who had severe tinnitus and right conductive hearing loss who demanded immediate delivery because she could not cope with the tinnitus, which resolved after cesarean delivery (Mukhophadhyay, et al., 2007). No specific pathology was found to have caused these symptoms, which were attributed to "pregnancy". The results of a postal questionnaire by Gurr and co-workers (1993) found that the prevalence of tinnitus in pregnant women (25%) was higher than non-pregnant women (11%) and this was statistically significant. Tsunoda and colleagues (1999) noted that 25% of pregnant women in their survey reported ear problems, including tinnitus and aural fullness that resolved after giving birth which was also significantly higher than in non-pregnant women. The underlying cause is not known, but hormones or the fluid retention that occurs during pregnancy may be involved.

*Sudden hearing loss* has also been reported during pregnancy (Lavy, 1998; Wang & Young, 2006; Pawlak-Osinska, et al., 2008). Lavy (1998) described two cases of sudden hearing loss that occurred during the third trimester. The first case presented with bilateral hearing loss with tinnitus and was treated with carbogen therapy and bed rest and the hearing improved. The second case complained of deafness and a blocked feeling in the right ear, but only had a hearing test three weeks post partum that revealed bilateral sensorineural hearing loss and was not offered any treatment because the hearing loss was felt to be permanent. The author did not describe the investigations the patients had, so an underlying cause cannot be dismissed. Wang and Young (2006) described twelve cases of unilateral sudden hearing loss, which they have observed during a ten year period in different stages of pregnancy. They accounted for 3% of all cases of sudden hearing loss seen during that period. The underlying cause of the sudden hearing

loss was unknown in 11 of the pregnant women, while one was diagnosed with an acoustic neuroma. All cases were in investigated for underlying medical problems including clotting deficits and autoimmune diseases. Six of the eleven pregnant women received treatment (intravenous dextran to enhance blood flow) while the remaining five refused to take any treatment. Hearing improved significantly in the women who received treatment (83%) compared to those who did not take treatment (20%). Pawlak-Osinska and colleagues (2008), described a women that presented with unilateral sudden hearing loss during two consecutive pregnancies. The first time she presented she was 27 weeks pregnant and the second time was two years later when she was 4 weeks pregnant. The hearing loss was in opposite ears in the two pregnancies. She was fully evaluated in both presentations including detailed blood chemistry and no specific cause was found. She received treatment in her first presentation (vasodilators, steroids and vitamin B) and her hearing recovered to normal after two days. However, she refused treatment during her second pregnancy and her hearing was found to be normal after 5 months.

It is not clear if the sudden hearing loss during pregnancy is due to the physiological changes seen in pregnant women (for example the high level of oestrogen may predispose pregnant women to thromboembolic diseases that may affect the cochlea) or is just a coincidental finding. However, from these case reports it seems that early diagnosis and treatment is associated with better hearing recovery.

### 3.1.6 Menopause and auditory function

Menopause is defined as the last menstrual period a woman experiences and can only be determined after twelve months of amenorrhea (Soules, et al., 2001). During the menopausal transition women experience several physiological changes which are mainly attributed to the decreasing levels of ovarian steroids especially oestrogen (reviewed by Hammond, 1996). The decreasing levels of hormones in the post menopausal period has been associated with changes in mood and cognitive function (Genazzani, et al., 2007) and may be helped by hormone replacement therapy (Sherwin, 1996; van Amelsvoort, et al., 2001).

Changes in auditory function have been noted in postmenopausal women attributed in part to the lower levels of ovarian hormones.

A few studies explored the possible association of hearing loss assessed by ***pure tone audiometry*** in postmenopausal women. Kim and co-workers (2002) studied a large group of postmenopausal women (n=1830) and 10 % of them were described to have a hearing loss by screening audiometry, and the rest acted as a control group. The women with hearing loss were significantly older than the control group and had lower serum oestradiol levels. The presence of hearing loss was significantly associated with age and serum oestradiol levels. Kilicdag and colleagues (2004) reported that postmenopausal women using oestrogen therapy had lower pure tone thresholds compared to those not using oestrogen therapy. A study by Hederstierna, et al. (2007) evaluated the hearing thresholds in a group of women (n=143) around the time of menopause and found that 40% were defined as having hearing loss. They reported that the group of women who were not using hormone replacement therapy (HRT) had a tendency to poorer hearing thresholds compared to pre- and perimenopausal and post menopausal women, who were using HRT. These studies suggests that HRT and in particular oestrogen therapy may have a beneficial effect on hearing sensitivity. A follow up hearing study by Hederstierna and her colleagues (2010) on a group of their previous cohort of women (n=104), found that there was a rapid decline in hearing thresholds that seemed to be triggered by the menopause.

The study by Guimaraes and co-workers (2006) explored the hearing function in more detail in a group of 124 postmenopausal women. They found that postmenopausal women who were taking the combined oestrogen and progesterone HRT had higher pure tone auditory ***thresholds***, lower levels of high frequency ***DPOAE***, and poorer performance in the ***hearing in noise test*** compared to postmenopausal women, who were either on oestrogen only replacement therapy or not taking any HRT. Their results suggests that progestin (a synthetic progesterone found in combined HRT) has a negative effect on both peripheral and central auditory function and this further supports the positive role of oestrogen on hearing function.

The possible effect of menopause and HRT on ***auditory evoked potentials*** has also been studied. The low levels of oestrogen and progesterone may alter nerve conduction times (Pascual, et al., 1991) and, thus, affect ABR latencies. ABR latencies tend to increase with age, however postmenopausal women have prolonged ABR wave latencies and inter peak latencies compared to younger women of a significantly greater degree than that seen in men of matched age groups (Jerger & Hall, 1980; Wharton & Church, 1990). These findings in women cannot be solely age related changes and hormones may therefore be of importance. Indeed, HRT, in post menopausal women, brings ABR latencies closer to the values seen in premenopausal women (Caruso, et al., 2000). ABR latencies were also found to be significantly shorter in post menopausal women after the use of different forms of HRT; tibolone (a synthetic steroid with combined oestrogenic, progestogenic and androgenic action) (Sator, et al., 1999), combined estrogen and progesterone (Khaliq, et al., 2003), trasdermal oestrogen therapy (Caruso, et al., 2003a), and oral oestrogen therapy (Khaliq, et al., 2005). Moreover, the effect of HRT in post menopausal women on ABR latencies is similar to what is seen in females with premature ovarian failure (POF) after treatment (Elkind-Hirsch, et al., 1992b) and oestrogen treatment in animal studies (Coleman, et al., 1994; Cooper, et al., 1999). These findings suggest that ovarian hormones have an effect on synaptic transmission in the auditory brainstem. However, the use of HRT does not seem to have an effect on the more central auditory evoked potentials, such as the slow vertex response (Khaliq, et al., 2003, 2005) or the auditory event related potentials (Walpurger, et al., 2005). This is possibly due to that these potentials reflect higher and more complex auditory processing (Polich & Kok, 1995).

The onset of ***age related hearing loss*** is later in women compared to men (Pearson, et al., 1995) and seems to coincide with the menopause (Murphy & Gates, 1997) suggesting a possible role for reproductive hormones in its pathogenesis. The sex difference in age related hearing loss is also seen in mice models of age related hearing loss (CBA mice) (Guimaraes, et al., 2004). The age related decline in distortion product otoacoustic emissions (DPOAE) amplitudes occurred earlier in the male CBA mice, while in female CBA mice the greatest decline in DPOAE amplitudes occurred in older age mice following the mouse

menopause. This finding suggests that oestrogen my have a protective role on the cochlea and outer hair cell function.

## 3.2   Auditory pathology and hormones

Hormones may play a role in the development of pathological conditions of the auditory system, including:

- Tinnitus and hyperacusis

- Menière disease

- Auditory dysfunction in pre-menstrual syndrome

### 3.2.1   Tinnitus and hyperacusis

Tinnitus can be defined as an auditory perception in the absence of any external auditory stimulation, while hyperacusis has been described as an intolerance to ordinary environmental sounds, often associated with tinnitus (Anari, et al., 1999; Andersson, et al., 2001). Both tinnitus and hyperacusis may result from a raised rate and/or altered pattern of spontaneous neural activity (Eggermont, 2003). Hence, the mechanisms which alter spontaneous auditory activity may generate both symptoms.

Tinnitus occurs with abnormalities/dysfunction at any level of the auditory system. The tinnitus-related activity, as any auditory signal, is probably, subject to the normal process of habituation/adaptation, which protect the auditory system from over stimulation through the attenuation of repetitive signals. This is supported by the observation that the intrusiveness of tinnitus declines over time (Tyler & Baker, 1983; Andersson, et al., 2001).

The process of habituation involves complex neuronal circuits and multiple transmitter system (Mesulam, 1990; Kandel, 2001), including acetylcholinergic, dopaminergic, GABA-ergic, nitric oxide and serotonergic systems. The serotonergic system is thought to play a role in modulating neuronal responses to repetitive stimulation and to act as a "gain-control" between facilitating and inhibitory mechanisms (Hegerl & Juckel, 1993). There is also evidence for the

involvement of the serotonergic system in the process of habituation of sensory stimuli (Gottfries, et al., 1976; Hegerl & Juckel, 1993). Dysfunction in these neurotransmitter systems may lead to a dysfunction of the process of habituation. Indeed, their disregulation has been associated with the pathophysiology of depression and mood disorders (Owens & Nemeroff, 1998; Ressler & Nemeroff, 2000; Meyer, et al., 2001). Serotonin has also been suggested to play a role in the generation of tinnitus and hyperacusis (Marriage & Barnes, 1995; Gopal, et al., 2000; Simpson & Davies, 2000), which may explain the well recognized co-morbidity of severe tinnitus/hyperacusis and psychological disorders (Forsling, et al., 1996; Zoger, et al., 2001).

The contribution of stress, which activates various biological functions, including the above neurotransmitter systems, may influence the auditory system through different pathways, particularly in individuals with impaired mechanisms of habituation, e.g. in individuals with psychological/psychiatric disorders, as outlined above. The link between stress and tinnitus is well recognised (Erlandsson & Hallberg, 2000; Holgers, et al., 2000), but, in addition dysfunction of adrenal stress hormones, such as in Addison's disease (Henkin, et al., 1967; Henkin & Daly, 1968) may lead to hyperacusis.

The reproductive hormones may also have a role in the occurrence of tinnitus and hyperacusis, and both oestrogen and progesterone could influence the auditory system, as described earlier. Oestrogen has been generally considered to have an excitatory role and to have a neuroprotective effect, and there is a strong relationship between oestrogen and serotoninergic pathways (Rubinow, et al., 1998). On the other hand, progesterone and its metabolites are known to have a potent inhibitory effect, through the interaction with the GABA receptors. Therefore, in some women the alterations in these hormones, both physiological and pathological, may lead to increased susceptibility to developing tinnitus. The reported finding of tinnitus being more common in women under the age of 40-45 (i.e. during reproductive years) than in men (Davis, 1983) may also support the possible role of the reproductive hormones in the perception of tinnitus.

### 3.2.2 Menière disease and endocrine system

Menière disease is a disease of poorly understood aetio-pathophysiology. The assumption that hormones could play a role in pathogenesis of the disease may be supported by the following data from the literature:

- The female to male ratio shows a slight female preponderance (1.3:1) (Schessel, et al., 1998) while a recent study reported that the female to male ratio (1.89:1) was significant (Harris & Alexander, 2010). The peak incidence for the disease is in the fourth to sixth decade of life (Paparella, 1991), which spans the menopause. This raises the possibility that the alterations in reproductive hormone levels may aggravate or initiate the disease.

- The symptoms of Menière disease often become manifest during periods of stress (American Psychiatric Association, 1994). The disorder is more common in professionals and management occupations (Watanabe, et al., 1995) raising the suspicion that the endocrine and neural changes associated with the stress response may be involved in pathogenesis of the disease.

- Endolymphatic hydrops underlying Menière disease may involve hormones maintaining fluid and electrolyte balance (Juhn, et al., 1991; Naftalin, 1994).

A few studies have tried to explore the relationship between the endocrine system and Menière disease especially the possible role of reproductive hormones and stress and fluid and electrolyte related hormones.

### 3.2.2.1 *Reproductive hormones*

The possible role of a sharp fall in the levels of reproductive hormones *pre-menstrually* with exacerbation of Menière disease has been noted in some studies. Andrews and colleagues (1992) found that only 6 out of 109 women reported this relationship, while Morse and House ( 2001) noted that in 11 out of the 13 women described more symptoms pre-menstrually. A further study by Price and co-

workers (1994) reported a case in which the patient's symptoms were exacerbated pre-menstrually but disappeared with leuprolide (a GnRH analogue that leads to ablation of sex steroids by downregulation of GnRH receptors and decreases secretion of FSH and LH leading to anovulation and amenorrhea) treatment. The symptoms recurred when she was prescribed progesterone, but not when given oestrogen. The fluid retention or redistribution that occurs in some women in the pre-menstrual period (Tollan, et al., 1993) may also play a role in exacerbating symptoms. The fluctuation of symptoms during the menstrual cycle in some patients with Menière disease may imply that variation in either the reproductive or stress-related hormones might be involved in the pathophysiology of this disease

Pregnancy may also affect Menière disease and may be related to the changes in fluid electrolyte balance and higher levels of progesterone. More attacks have been reported during early pregnancy, when the serum osmolality is low in a case report by Uchide and colleagues (1997), which is possibly related to the sharp rise of hormones during the first trimester.

The specific influence of the menopause, when there is a sharp fall in reproductive hormones, on the onset or worsening Menière disease has not been reported in the literature, but as noted above the peak incidence of the disease spans the age range of the menopause and thus suggests a possible link.

### 3.2.2.2 *Stress-related hormones*

Exacerbation of Menière disease tends to occur in relation to stress (Hinchcliffe, 1967; Soderman, et al., 2004; Takahashi, et al., 2005),  and adrenal hormones are thought to be involved (Mateijsen, et al., 2001). Patients with Menière disease were found to have higher levels of cortisol compared to healthy controls (van Cruijsen, et al., 2005). However, the higher levels of cortisol are possibly the result of the disease not the cause because those with a longer history of Menière disease had the highest levels of cortisol. Another possibility is that patients with Menière disease may have an altered response to stress compared to patients with

facial spasm and thus contribute to the pathophysiology of the disease (Horner & Cazals, 2005).

### 3.2.2.3   *Fluid and electrolyte related hormones*

Altered inner ear fluid homeostasis may be the underlying mechanism, as seen in animal models of endolymphatic hydrops (Juhn, et al., 1991; Naftalin, 1994; Juhn, et al., 1999).

The possible effect of ADH (vasopressin) in Menière disease has also been studied. An increase in the level of plasma ADH in patients with Menière disease has been related to the vertiginous attacks (Takeda, et al., 1995; Aoki, et al., 2005). The levels of ADH were significantly higher in patients with Menière disease compared to patients with chronic otitis media before surgery, and  the levels of vasopressin type-2 receptors were higher in the inner ears of patients with Menière disease compared to patients with acoustic neuromas (Kitahara, et al., 2008). However, patients with Menière were not found to have abnormal levels of aldosterone compared with controls (Mateijsen, et al., 2001). Aldosterone levels may however be abnormally elevated before or after an attack, which may be difficult to measure clinically.

### 3.2.3   Pre-menstrual syndrome and auditory function

Females who suffer from premenstrual syndrome (PMS) also known as Premenstrual Dysphoric Disorder (PMDD) may have changes in their auditory function. PMDD is a syndrome occurring during the late luteal phase of the ovarian cycle and is characterized by moderate to severe alteration in mood, behaviour and physical well being that impairs the personal, professional and/or social function in 5-8% of women during their reproductive years (American Psychiatric Association, 1994; Yonkers, 1997).

The underlying pathophysiology is poorly understood, but may be related to progesterone in view of the cyclic nature of the symptoms, although no consistent finding has been documented (Rubinow & Schmidt, 1995; Yonkers, et al., 2008).

Dysregulation of the stress response and stress related hormones such as ACTH, β-endorphin, and cortisol play a role in mood disorders, such as depression, so they have also been implicated in the pathogenesis of PMS. Differences in basal levels of these hormones between control females and those with PMS have not been firmly established (Bloch, et al., 1998). However, a recent study has found that women with PMS have dysregulation of the stress response (activation of the HPA axis in response to a external stressor) compared to those without PMS (Roca, et al., 2003).

Allopregnenolone, a metabolite of progesterone and a potent GABA-A agonist, is also associated with stress (Baulieu, 1998), and has a profound anxiolytic effect (Brot, et al., 1997). Females with PMS may have altered GABA (Epperson, et al., 2002) and/or allopregnenolone (Monteleone, et al., 2000; Girdler, et al., 2001) functions, and this may contribute to the mood disorder.

These hormone alterations in females with PMS have a potential effect on the auditory system, possibly leading to greater inhibition of auditory function. Changes in auditory function have been documented in a study that compared ABR latencies between females with and without PMS (Howard, et al., 1992). Females with severe PMS had longer wave III and wave V latencies, while those with moderate PMS had longer wave III latencies only, compared with females without PMS. Howard and colleagues (1992) also noted that ABR latencies did not change after treating PMS with fluoxetine (a selective serotonin reuptake inhibitor) suggesting that the underlying pathophysiology in females with PMS might not be functional but structural. Also, Ehlers and collegues (1996) reported that females diagnosed with PMS had longer P3 latency of the auditory event related potentials compared to normal controls.  The presence of the difference in ABR and auditory event related potentials between females with and without PMS may help in identifying women predisposed to PMS.
Further studies examining auditory function in larger samples of females with PMS are needed to clarify these findings.

## 3.3  Summary

The above text highlights the complexity of the effects of hormones on the auditory system, with multidirectional and multidimensional interactions.

The same hormone may exert its action on the auditory system through multiple pathways (modulating blood supply, inner ear fluids, sensory neurotransmission in the cochlea, brain stem and cortex or through extra-auditory connections), as demonstrated in Figure 3.3.1. For example, oestrogen could lead to neural excitation and thus facilitate auditory transmission, but the possible increase in neurosteriods in the brainstem may counteract this effect.
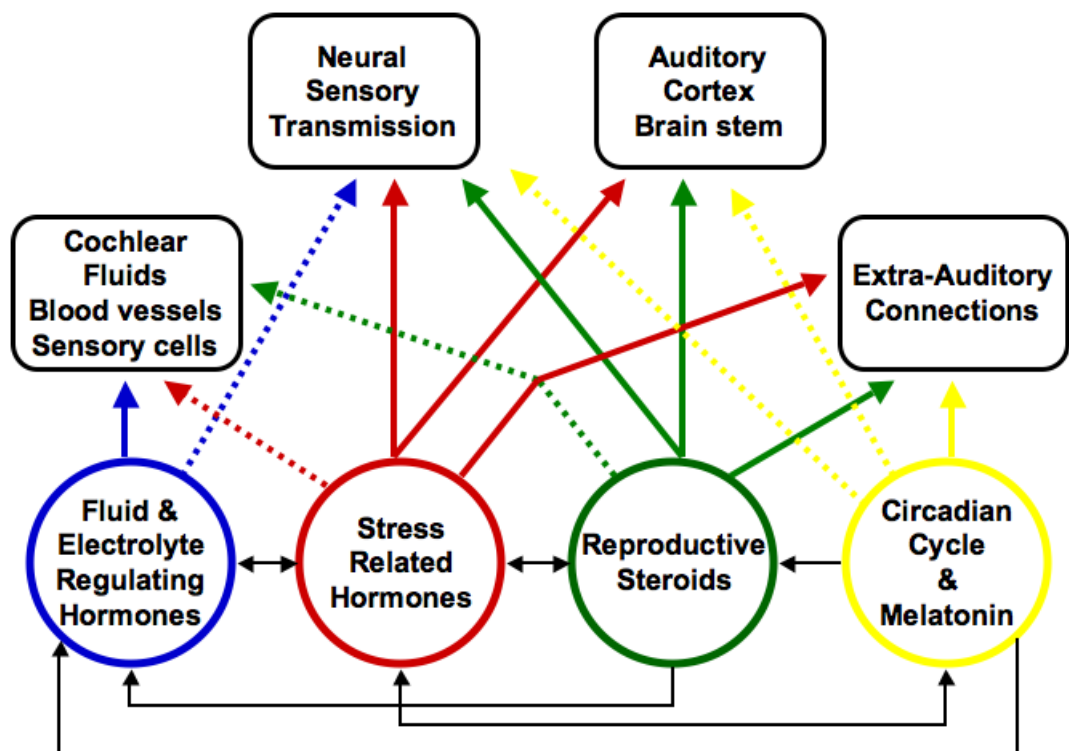


**Figure 3.3.1:** Hormone action on auditory system. The main effects are denoted with solid lines

The difficulty in pinpointing the exact effect of a hormone on the auditory system arises from the complex anatomy of the auditory system and the interactions between the various hormones that occur as the result of dynamic nature of the

physiological processes. However, some general effects can be observed. *The reproductive hormones*, in general, facilitate auditory function, with a protective effect on the auditory system. *Aldosterone* and *vasopressin* are mainly involved in maintaining cochlear fluid balance. *Melatonin* possibly has a protective effect on auditory system. The hormones involved in the response to *stress* are, generally, excitatory, but in the long run may lead to damage and may play an important role in auditory pathology.

# Chapter 4 : Thesis Project, Aims and Hypotheses

## 4.1  Background

Circumstantial evidence and previous studies suggest that ovarian steroid hormones may influence the auditory system (section 2.2.1, 3.1.4, 3.1.5, and 3.1.6).

Auditory function in women during the menstrual cycle has been previously investigated, with inconsistent or conflicting findings (see Table 3.1-A). Although fluctuation in auditory function during different stages of the cycle has been demonstrated in some studies, they lack the precise timing of the cycle and the correlation between the hormonal levels and auditory function. Only a few studies have measured ovarian steroid levels and these explored auditory brainstem responses.

## 4.2  Thesis Project

The project included two case studies and three research studies.
The case studies were performed before starting the main research studies to assess the sensitivity of the techniques and the test protocol.

  **Case 1:** Otoacoustic emissions in a woman during the menstrual cycle.

  **Case 2:** Otoacoustic emissions in woman with premature menopause treated with hormone replacement therapy.

The research studies were:

  **Study I:** Auditory function in women during the ovarian cycle.

  **Study II:** Auditory function in women during the ovarian cycle compared with men during a similar period of time.

  **Study III:** Auditory function in women undergoing assisted conception treatment.

## 4.3   The hypotheses

1.   Variation in auditory function at the cochlear and brainstem levels is associated with fluctuation of ovarian steroids during the ovarian cycle.

2.   There is no variation in auditory function in men tested over a similar period of time compared to women during the ovarian cycle.

3.   The variation in auditory function in women undergoing assisted conception treatment is greater than in women during the ovarian cycle due to greater difference in ovarian hormones levels.

## 4.4   Aims of the thesis

1.   To investigate whether there is variation in different aspects of auditory function (from the cochlea to the inferior colliculus) using sensitive techniques, with simultaneous measurement of hormone levels during the ovarian cycle, to document the normal physiological variations that occurs during the ovarian cycle.

2.   To compare the auditory function between men and women over an identical time period.

3.   To monitor and compare auditory function in two groups of women:
     I.   Women during a single normal ovarian cycle.
     II.   Women undergoing standard assisted conception treatment.

# Chapter 5 : Materials and Methods

## 5.1 Subjects

Two groups of healthy women of reproductive age, between 20 and 50 years old, with normal hearing and a group of aged matched men:

I.  Healthy female subjects during a single normal ovarian cycle (defined in section 3.1.4).

II.  A group of healthy men matched for age, to assess auditory function in the absence of hormonal variation characteristic of the ovarian cycle.

III.  Women undergoing standard assisted conception treatment, as defined below (section 9.1)

### 5.1.1 Inclusion Criteria

The inclusion criteria for all subjects were the presence of normal hearing and middle ear function assessed by pure tone audiometry and tympanometry (defined in section 1.2.1 and 1.2.2).

Additional criteria for women were:
*  A history of a regular menstrual cycle with a cycle length between 26 and 30 days.
*  No use of hormonal contraception for at least 3 months prior to participation in the study.

The subjects were excluded for the following reasons:
o  endocrinological pathology
o  chronic medical illness (with the exception of allergies)
o  regular drug treatment
o  otological conditions

### 5.1.2 Subject recruitment

The group of healthy women and men (group I and II) were recruited from the staff and students at the National Hospital for Neurology and Neurosurgery, Great Ormond Street Hospital, and the Institute of Child Health.

The women undergoing assisted conception treatment (group III) were recruited from the Academic Department of Reproductive Medicine, Newham University Hospital and the Fertility Centre, The Barts and The London School of Medicine and Dentistry.

## 5.2 General Protocol

All subjects underwent a protocol that included:

- An oral interview to provide information on auditory function and to establish that the inclusion criteria were fulfilled (see Appendix I).

- Otoscopy, to exclude any obvious otological abnormality of the ear canal and tympanic membrane.

- Tympanometry, to ascertain normal middle ear function (defined in section 1.2.2), which is necessary to record valid OAE.

- Standard pure tone audiometry (PTA), to determine that the subject had normal hearing sensitivity (defined in section 1.2.1).

- Otoacoustic emission recording to assess cochlear function, and included:
  - Spontaneous otoacoustic emission
  - Transient evoked otoacoustic emissions

- Medial olivocochlear function test to assess the efferent auditory pathway including and distal to the MOC (section 1.2.4).

- Auditory brainstem responses to assess the eighth nerve and auditory brainstem function (section 1.2.5).

The auditory tests (except PTA) were repeated four or three times in each subject according to the specific research study protocol (section number 7.2, 8.2, and 9.2), to ensure continuing normality of the middle ear function and to monitor auditory function during different hormonal profiles in the same subject and between subjects.

In addition to the auditory tests, at each testing session the female subjects had a sample of blood taken to document their serum oestrogen and/or progesterone levels.

## 5.3   Procedures

### 5.3.1   Auditory tests

All tests were performed in a sound treated booth which met international standards (ISO 8253-1, 1989). The booth had double doors and walls covered with low reflective, absorbent materials.

The auditory tests took about 45-60 min to perform, according to the study protocol as outlined above (section 5.2).

#### 5.3.1.1   *Pure tone audiometry*

*Equipment:*  GSI 61 audiometer (Grayson Stadler Inc. Model 61) audiometer and TDH-50 headphones.

*Test method:*
The hearing thresholds for pure tone stimuli at frequencies from 0.25 Hz to 8 kHz in octave steps were measured following the standard method of the British Society of Audiology (2004). Tone pulses of 1-2 second duration were used to avoid adaptation and influence of temporal integration with ascending sound levels in steps of 5 dB.

### 5.3.1.2  Tympanometry

*Equipment:* GSI 33 Tympanometer (Grayson Stadler Inc. Model 33).

*Test method:*

Single frequency tympanometry was performed with a continuous probe tone of 85 dB SPL at 226 Hz, as recommended by the British Society of Audiology (1992).

*Test reproducibility:*

The within subject variability in tympanometry is low. Porter and Winston (1973) reported that the middle ear pressure may change by 20 daPa. Similar findings were found by latter authors (Wiley & Barrett, 1991; Gaihede & Ovesen, 1997). The change in the tympanic membrane compliance usually does not exceed 0.1 ml (Wiley & Barrett, 1991; Gaihede & Ovesen, 1997).

### 5.3.1.3  Otoacoustic emission recording

Equipment: ILO 88/92 Otodynamics otoacoustic analyser hardware and software, version 5, connected to a Windows 96 compatible computer to record spontaneous and transient evoked otoacoustic emissions. A SGS-type general purpose probe was used, which contained a miniature microphone and transducer. The probe was fitted in the ear with a rubber tip.

The subjects were tested in a sound treated room with the test equipment outside the room to minimise noise contamination.

### a) Spontaneous evoked emissions (SOAE)

Click-synchronised SOAE was recorded using the Otodynamics ILO88/92 in "SOAE search" mode. In this mode, a weak (approximately 75 dB SPL) synchronizing 80 µs clicks were presented over a period of 80 ms through a probe fitted in the external ear canal. As most of the evoked responses last less than 20 ms, the signals over a 20-80 msec post-stimulus, i.e. silent period that primarily represents spontaneous cochlear activity between the stimuli, were analysed. Typically, 260 responses were averaged and FFT analysis was performed in the

spectral band from 0 to 6250 Hz, with a resolution of 12.2 Hz. The frequency and amplitude of SOAE corresponding to the maximum level of the narrow-band signal (a spectral peak), in the entire available SOAE spectrum, from 0 to 6250 Hz, was determined using a cursor.

The SOAE spectral peak amplitude was required to be more than 5dB above the noise level and repeatable in two consecutive recordings to be considered present. SOAE at frequencies < 500 Hz were not considered due to the higher susceptibility to noise contamination in this frequency region.

The following variables were analysed:
- Number of SOAE peaks.
- SOAE peak amplitude (dB SPL).
- SOAE inter-session frequency shift: calculated as a percentage of the difference in the frequency of the SOAE peak in each testing session (fx) compared to the average SOAE peak frequency (F).

$$\frac{(fx - F)}{F} \times 100$$

### b) Transient evoked emissions (TEOAE)

TEOAE was recorded using the method described by Kemp, et al. (1990). The stimuli were unfiltered rectangular clicks (bandwidth $\approx$ 5 kHz), duration of 80 $\mu$s, presented at a repetition rate 50/s, with peak reception level 80 dB $\pm$ 3 dB SPL. They were presented in the non-linear differential mode: 4 clicks, with 3 clicks at the same level and polarity and fourth click three times greater in level and reverse polarity, and 10 dB increase in amplitude. This paradigm cancels the linear portion of the stimulus and response, including meatal and middle ear echo, so that nonlinear cochlear emissions can be extracted. The number of sweeps, during the period of collection, was 260 and they were recorded and averaged alternately in two separate, A and B, buffers, using a synchronous time-domain averaging technique. The post-stimulus analysis time was 2.5 – 20 ms and the passband 0.5 - 6 kHz with the noise rejection level set at 47.3 dB SPL.

The TEOAE responses were considered for analysis if they were above 3 dB and more than 50 % reproducibility in at least 3 of the frequency bands.

The following variables were assessed:

- Total TEOAE response (dB SPL).
- The TEOAE signal-to-noise ratio in five frequency bands centered at 1, 2, 3, 4, and 5 kHz.
- The TEOAE inter-session differences: TEOAE response in each session was compared to the other testing sessions using the compare function in the ILO software provided under the analysis menu. This function subtracts any two TEOAE waveforms and provides information about the overall difference between the two responses. This sensitive analysis also compares the click stimuli and noise levels present in the TEOAE recording, so provides greater validity as it compares whether the testing situation was similar in the repeated testing sessions and, thus, it identifies true differences between repeated TEOAE responses as displayed in Figure 5.3.1a and 5.3.1b.
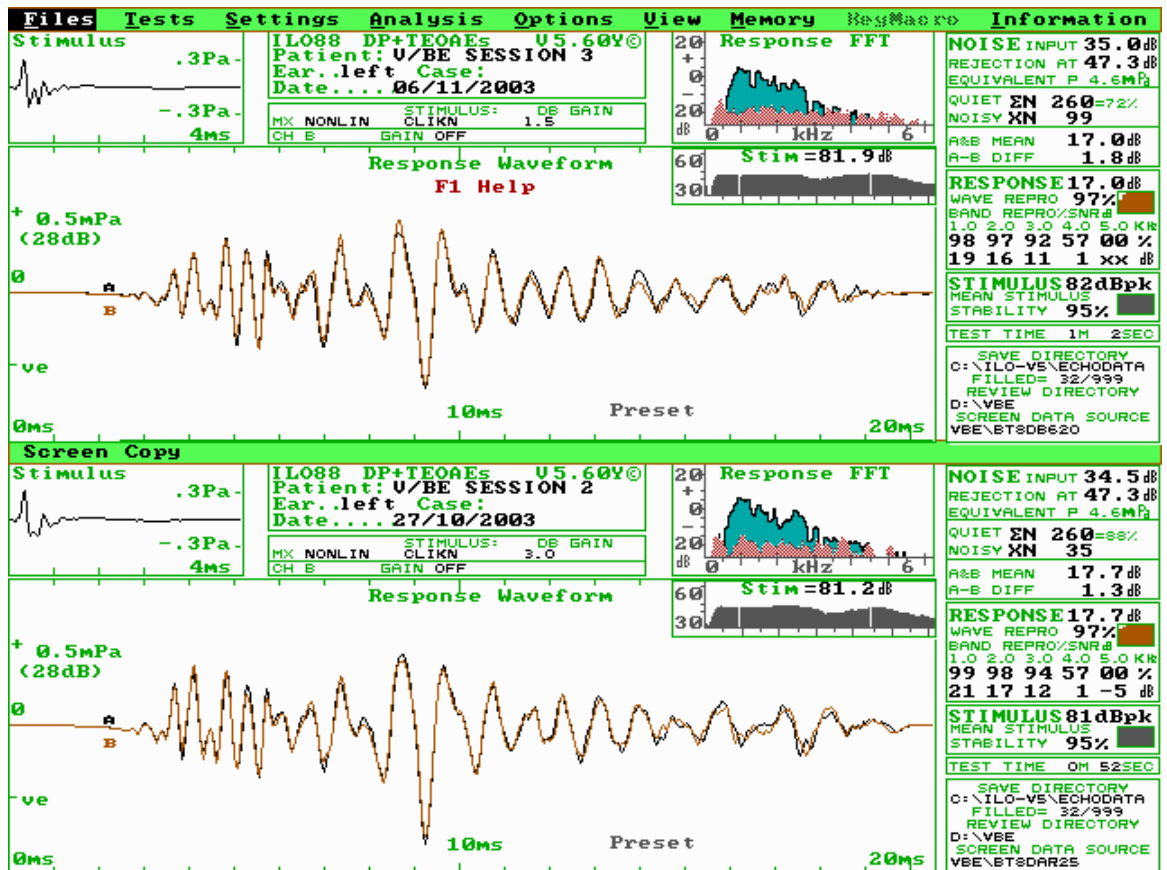
**Figure 5.3.1a:** An example of a TEOAE trace from the left ear of one of the subjects on two different testing sessions, ten days apart.

The over all TEOAE response in the top panel is 17 dB SPL and 17.7 dB SPL in the lower panel. The reproducibility of the TEOAE in both sessions was high, 97%, and is seen graphically by the almost identical waveforms recorded in the A and B buffers.
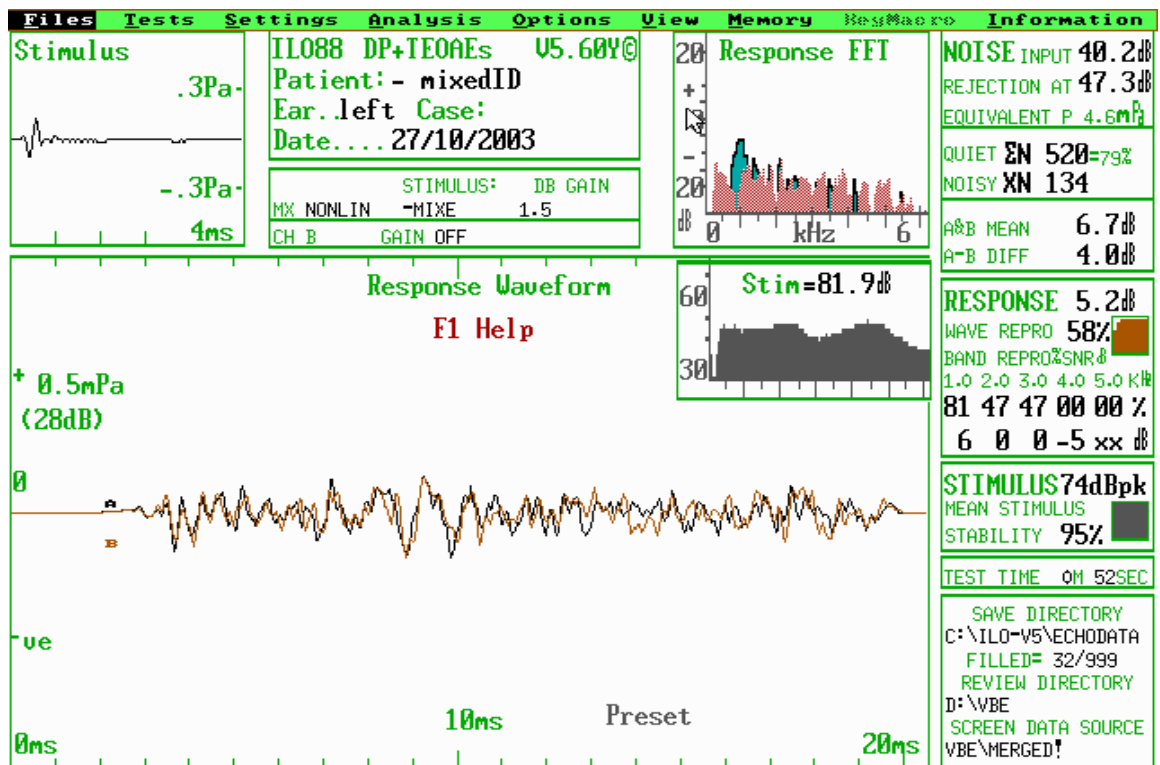
**Figure 5.3.1b:** The subtracted trace from the above subject (Figure 5.3.1a) as performed by the compare function of the ILO software. The overall difference in the TEOAE response was 5.2 dB.

The response waveform represents the difference between the two above traces (Figure 5.3.1a) from both the A and B buffers.

*Test reproducibility:*

The SOAE has well documented frequency stability with variations ranging between 1-2% (reviewed by Ceranic, 2003), however the SOAE amplitude is more variable, and can vary by 10 dB (van Dijk & Wit, 1990; Wit, 1993). The TEOAE has been found to be highly reliable in the same subject (reviewed by Hall, 2000c). The within subject variability of the TEOAE response has been documented to be on average 1 dB (Harris, et al., 1991; Marshall & Heller, 1996) with a high test retest correlation (0.99) (Vedantam & Musiek, 1991).

### 5.3.1.4   Medial olivocochlear function test

The medial olivocochlear (MOC) function was evaluated using a method similar to that described by Ceranic, et al. (1998b). A schematic description of the test method is seen in Figure 5.3.2.

A dual channel OAE analyzer was used (ILO 88/92), one channel (A) for ipsi-
and the other (B) for contralateral acoustic stimulation. Both, ipsi- and
contralateral, stimuli were delivered through identical probes (SGS-type general-
purpose probe). The ipsilateral stimulation was a linear click with a peak stimulus
level of 60±3 dB SPL. The contralateral stimulation was a 5ms burst of white
noise (0.5 - 6 kHz) at 40 dB sensation level. An alternating technique, a
"Difference B on/off" mode, from the ILO92 software was used. This mode
allows alternating recording of TEOAE responses with and without contralateral
stimulation. A total of 600 sweeps were recorded, in 10 groups of 60 sweeps. The
average responses were directly computed and the difference obtained by
subtraction, represented the suppression effect. According to the departmental
normative database, a normal MOC suppression was considered ≥ 1 dB. An
example is displayed in Figure 5.3.3.
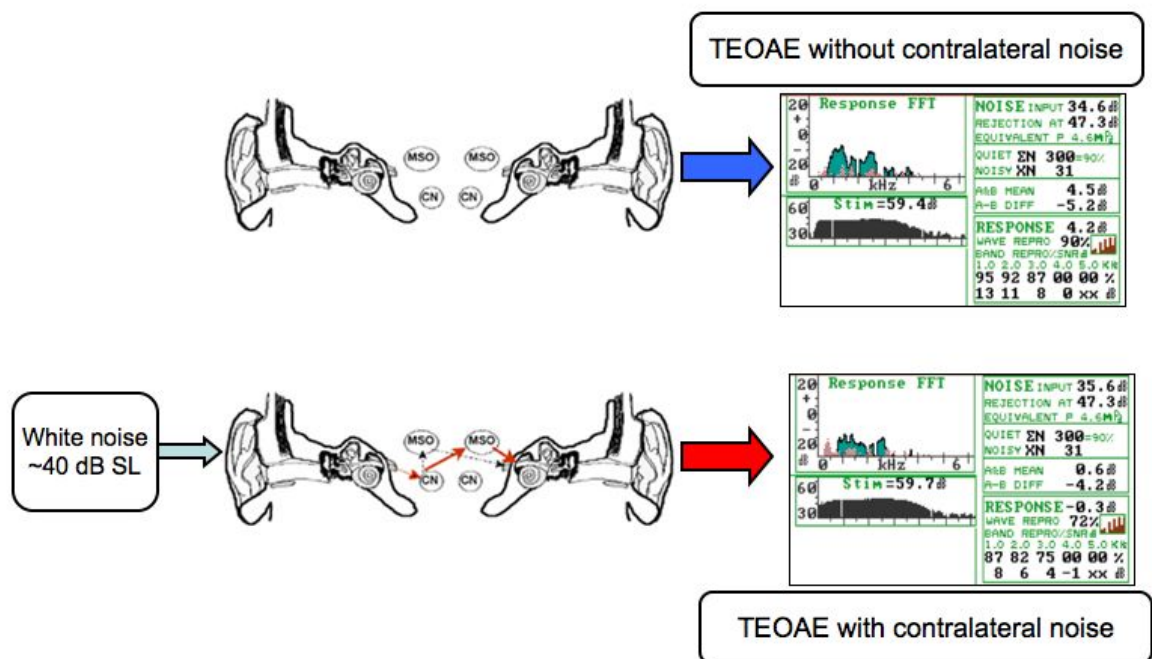


**Figure 5.3.2:** Schematic description for the MOC suppression test and the neural
pathways being activated (adapted from Lalaki, 2005, permission to reproduce granted
kindly by S Hatzopoulos).

The overall TEOAE amplitude recorded was 4.2 dB SPL without contralateral noise, and
-0.3 dB SPL with contralateral noise. The overall suppression was 4.5 dB in this example.
(CN: cochlear nucleus, MSO: medial superior olive, SL: sensation level).
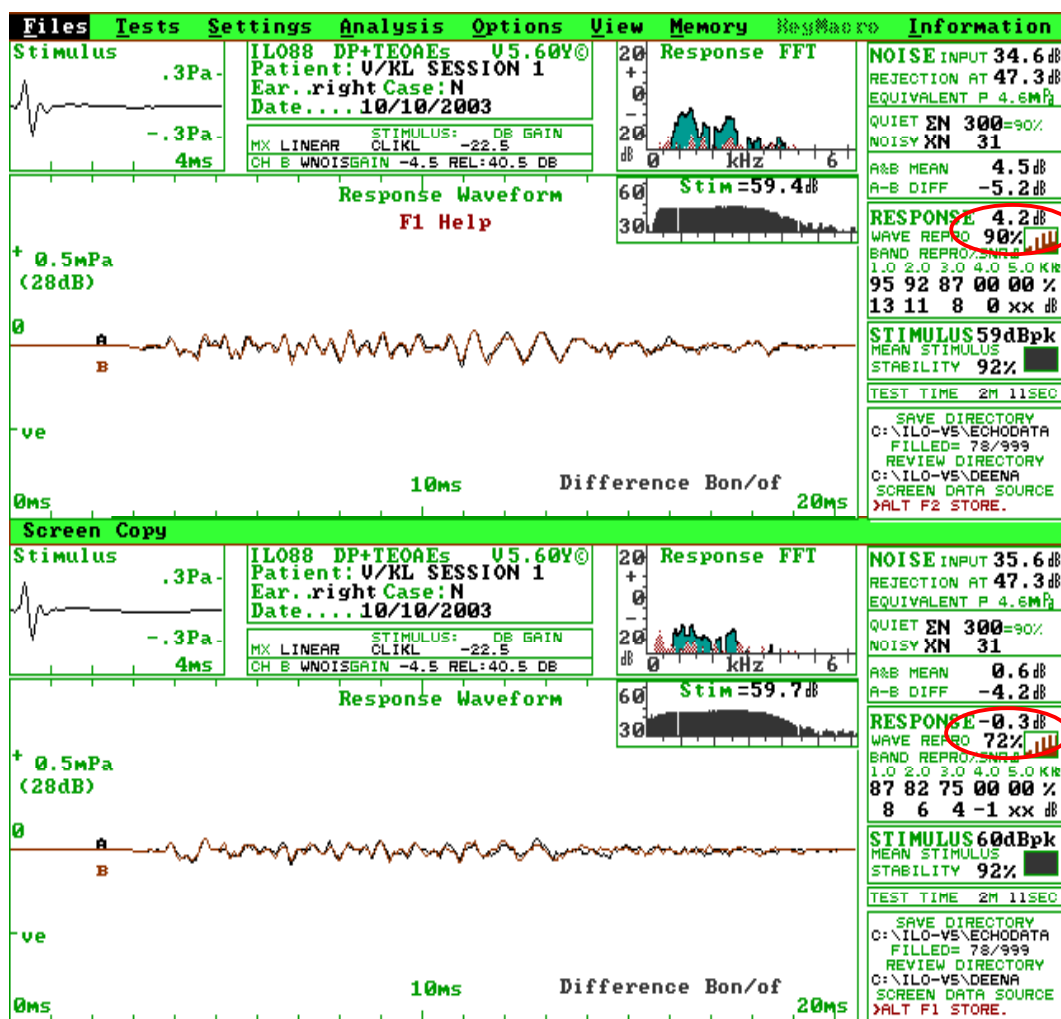
**Figure 5.3.3:** TEOAE trace recoded with contralateral stimulation (lower panel) and without contralateral stimulation (upper panel).

The difference between the responses of the two traces is the amount of suppression, which is 4.5 dB in this example.

*Test reproducibility:*

The variability in MOC suppression within a subject is low. The test retest comparison was performed by the author three times a week for one month, and the suppression remained stable, within ±1dB.

### 5.3.1.5  *Auditory brainstem response (ABR)*

*Equipment:* ABRs were recorded using the Nicolet Biomedical Spirit 2000 applying the standard clinical methods of the Neuro-otology Department at the

National Hospital for Neurology and Neurosurgery (similar to the methods described by Musiek, et al., 1994).

*Test Method:*

The evoked potentials were recorded using conventional EEG electrodes placed on the vertex (Cz) and each mastoid (A1and A2) during acoustic stimulation. The subjects were asked to lie down and close their eyes and the acoustic stimulation was delivered via THD-39 headphones. The stimuli used were 100μs clicks at 80 dB HL. The repetition rate was less than 20/s to assess all waveforms and measure the wave and inter-wave latencies. The responses were band-pass filtered from 150-3000 Hz and 1024 sweeps were averaged to obtain one recoding in an analysis time of 12 ms. The recording was repeated twice from each ear to verify the waves and reject any artifacts.

The following variables were assessed:
- Wave I, III and V absolute latencies.
- I-III, III-V and I-V inter-peak intervals.

*Test reproducibility:*

The ABR latencies are stable within a subject over several sessions. The difference in the absolute latencies between sessions is less than 0.08 ms (Oyler, 1989).

### 5.3.2   Serum hormone levels

Serum hormone levels were measured to monitor:
- The physiological changes in oestrogen and progesterone levels during the ovarian cycle in the women from group I
- The changes in oestrogen and progesterone levels as a result of assisted conception treatment in the women from group III

Serum oestradiol and progesterone levels were measured at the Chemical Pathology Laboratory, Newham University Hospital, London, using direct chemiluminescence, oestradiol and progesterone, by a competitive immunoassay.

The inter- and intra-assay coefficients of variation for the hormone assays were respectively: oestradiol 8.5 and 4.0%; progesterone 8.1 and 3.9%.

## 5.4 Calibration

The test equipment is regularly calibrated according to the Neuro-otology department protocol.

Calibration of the ILO88/92 system was performed using a 1 $cm^3$ test cavity supplied by the manufacturer, on average once every two months. Synthetic stimuli, three tones at different frequencies, provided by the ILO88/92 software, were delivered through the probe tip inserted into the test cavity. Additional, "biological", calibration was carried out by the author, on average once a month. The inter-session performance of the probe remained stable (within ±1dB) throughout the project execution.

## 5.5 Data Analysis

### 5.5.1 Statistical power analysis

The difference to be detected in the OAE response is calculated to be 1.4 dB from previous studies reported in the literature, and the number of subjects in each group was calculated to be eight setting the p value at 0.05 and statistical power at 80% (Machin, et al., 1997).

### 5.5.2 Statistical tests

The statistical tests were performed using SPSS (statistical package for social scientists) version 17.0 (SPSS Inc., 2008) and included the following:

– linear mixed-effect modeling (LMM) was used for a more reliable detection of changes in auditory function measures between the test sessions. The LMM method takes into account that the subjects in the model leads to variations in the variable and that the repeated measures are correlated and not independent. The independence of the observations is a key assumption in ANOVA analysis which is violated in repeated measures designs. Another major

advantage to the use of LMM instead of repeated measures ANOVA, is that LMM does not require that each subject has the same number of observations and thus accommodates for any missing observation and unbalanced designs (McCulloch & Searle, 2001; Garson, 2009). The auditory function measure was the dependent variable and the test session was both the fixed and repeated factor with pairwise comparison of the estimated marginal means between the different test sessions. The between subject factors was either gender or study group.

– Paired sample t-test was used to analyse the TEOAE inter-session differences in each group of subjects.

– Correlation between auditory function measures and serum hormone levels were analysed using linear regression for the two groups of women.

– Independent sample t-test was used to analyse age and auditory tests results between the groups.

# Chapter 6 : Case Studies

The case studies were performed before starting the main research studies to assess and evaluate the sensitivity of the techniques and the test protocol.

## 6.1 Case 1: Otoacoustic emissions in a woman during the menstrual cycle

### 6.1.1 Subject

The subject was a 30 year old healthy woman with a regular normal menstrual cycle (28-32 days).

The medical history was unremarkable, and she reported that her hearing was normal, without any auditory symptoms.

### 6.1.2 Procedures

The procedures included otoscopy, tympanometry, PTA, otoacoustic emission recording and the medial olivocochlear (MOC) suppression test, as outlined above in the "Methods" section (section 5.3.1). They only difference was that the probe used to record the OAEs was a B type probe which records lower levels of noise and responses than a SGS type probe.

### 6.1.3 Protocol

Initial baseline recordings of TEOAE, SOAE and MOC suppression were performed. This was followed by repeated recording of TEOAEs and MOC on average three times per week during two consecutive months.

The test sessions were all undertaken at the same time of day to avoid the physiological diurnal fluctuations in OAEs (section 3.1.1).

The sessions corresponded to two consecutive menstrual cycles. The suspected day of ovulation was assessed by counting back 14 days from the menstrual onset of the following cycle. This corresponded to day 15 of the first cycle and day 18 of the second cycle.

### 6.1.4 Results

During the initial session, SOAE were not recordable from either ear in the subject, so the further sessions only involved TEOAE recording and MOC suppression.

The average TEOAE response was 10.6 dB SPL in both ears and the average MOC suppression was 2.2 dB in the right ear and 2.3 dB in the left ear during the two months of testing (Table 6.1-A). The average TEOAE response and the level of MOC suppression were similar in both ears.

**Table 6.1-A:** Mean (± SD) values of TEOAE and MOC suppression during the repeated testing

|  | Right Ear | Left Ear | Mean of both ears |
|---|---|---|---|
| **TEOAE Response (dB SPL)** | 10.6 ± 0.6 | 10.6 ± 0.5 | 10.6 ± 0.5 |
| **MOC Suppression (dB)** | 2.2 ± 0.3 | 2.3 ± 0.3 | 2.3 ± 0.2 |

#### 6.1.4.1 *Transient evoked otoacoustic emissions*

The range of the TEOAE responses and highest and lowest levels during the two studied cycles are presented in Table 6.1-B. The highest responses were recorded on day 12 and day 17 in the first and second cycle respectively. The lowest response recorded from both ears was on day 3 in the first cycle, and day 22 from the right ear, and day 15 and 19 from the left ear in the second cycle.

**Table 6.1-B:** TEOAE response during the two tested cycles

| TEOAE Response (dB SPL) | Right ear | | Left ear | | Both ears | |
|---|---|---|---|---|---|---|
| | 1st cycle | 2nd cycle | 1st cycle | 2nd cycle | 1st cycle | 2nd cycle |
| Range (mean±sd) | 9.1-11.2 (10.3±0.6) | 10-11.7 (10.7±0.5) | 9.4-11.1 (10.3±0.5) | 10.3-11.7 (10.9±0.4) | 9.2-11.1 (10.3±0.5) | 10.3-11.7 (10.8±0.4) |
| Highest response | Day 12 | Day 17 | Day 12 | Day 17 | Day 12 | Day 17 |
| Lowest response | Day 3 | Day 22 | Day 3 | Day 15 and 19 | Day 3 | Day 8 |

The TEOAE responses recorded from both ears are plotted in Figure 6.1.1. The highest response amplitudes were recorded near the time of suspected ovulation in both cycles, and lower levels of the TEOAE were recorded in the beginning of the cycle as well as after the suspected day of ovulation.
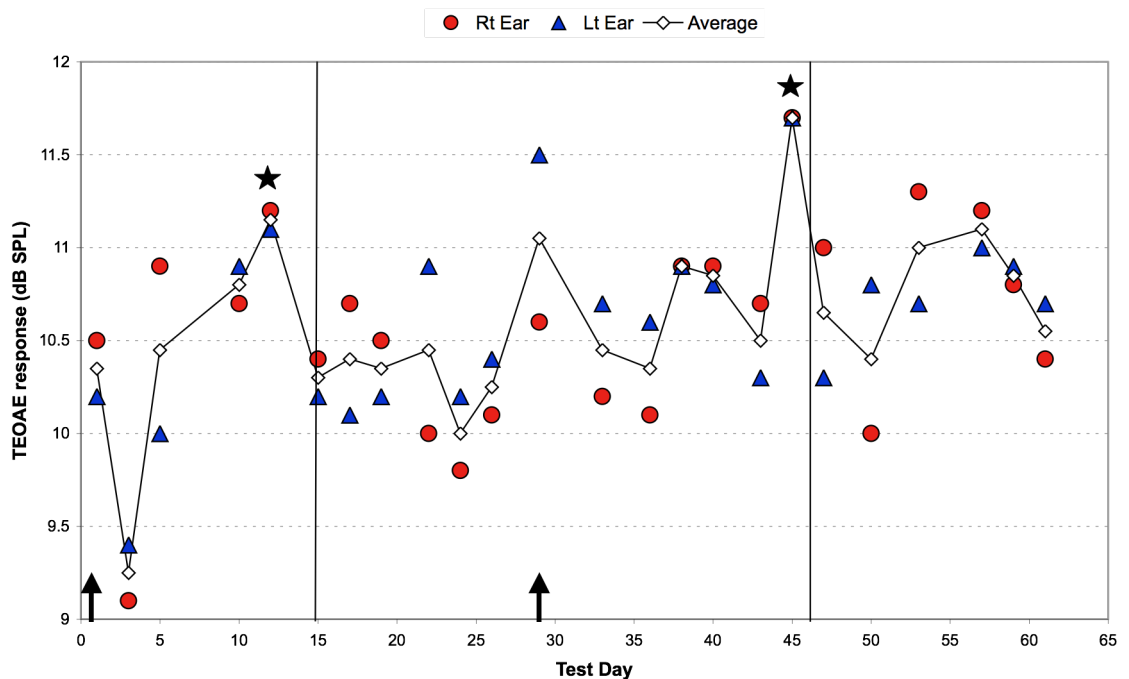


**Figure 6.1.1:** The TEOAE response during two consecutive menstrual cycles. The arrows indicate the first day of the cycle and the lines indicate the suspected day of ovulation.
★highest response

### 6.1.4.2 *Medial olivary cochlear suppression test*

The range of the MOC suppression was 1.8-3 dB for the right ear and 1.6-2.9 dB for the left ear. Lower MOC suppression was recorded in the first part of the cycles, while higher MOC suppression was recorded in the second part of the cycles in both ears as presented in Table 6.1-C.

**Table 6.1-C:** MOC suppression during the two tested cycles

| MOC Suppression (dB) | Right ear | | Left ear | | Both ears | |
|---|---|---|---|---|---|---|
| | 1st cycle | 2nd cycle | 1st cycle | 2nd cycle | 1st cycle | 2nd cycle |
| **Range** | 1.8-3 | 1.8-2.9 | 2-2.8 | 1.6-2.9 | 2-2.7 | 1.8-2.5 |
| **(mean±sd)** | (2.3±0.3) | (2.2±0.3) | (2.4±0.3) | (2.2±0.4) | (2.4±0.2) | (2.2±0.2) |
| **Greatest suppression** | Day 24 | Day 23 | Day 15 and 22 | Day 1 | Day 15 | Day 23 |
| **Lowest suppression** | Day 3 | Day 10 | Day 5 and 10 | Day 17 | Day 3 | Day 13 |

A lower level of suppression was noted before the suspected day of ovulation in both tested cycles as displayed in Figure 6.1.2 and the MOC suppression was greater in the second part of the cycles after the suspected day of ovulation.
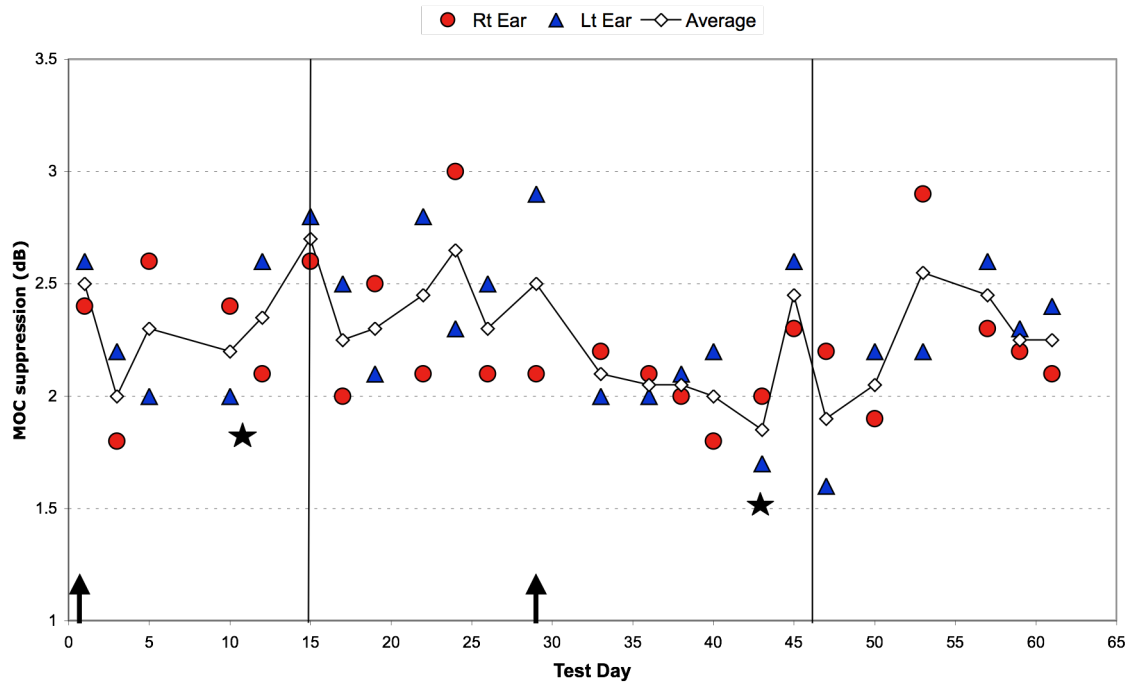
**Figure 6.1.2:** The MOC suppression results of the left and right ear. The arrows indicate the first day of the cycle and the lines indicate the suspected day of ovulation. ★lower MOC suppression

### 6.1.5 Discussion

The overall TEOAE response variation between the test sessions in this case study was up 2.3 dB in the right ear and 1.7 dB in the left, suggesting higher variation than previously reported variability (Harris, et al., 1991; Franklin, et al., 1992; Marshall & Heller, 1996). There was a variation in the values of the MOC suppression during the repeated testing that was up to 1.2 dB in the right ear and 1.3 dB in the left ear.

During the first and second cycle in this subject, the highest TEOAE amplitude response (Figure 6.1.1) and lower MOC suppression (Figure 6.1.2) were recorded before the suspected day of ovulation, and lower TEOAE amplitudes along with greater MOC suppression were observed after the suspected day of ovulation in the second part of the cycles. Previous studies, either did not report any significant changes in TEOAE responses during the cycle (Yellin & Stillman, 1999; Arruda & Silva, 2008) or that the responses decrease during the cycle (Amit & Animesh, 2004). This could be due to different methodologies (reviewed in section 3.1.4.1).

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MOC suppression during the ovarian cycle had not previously been reported in the literature.

The higher TEOAE responses and lower MOC suppression seem to have corresponded with the LH surge and the higher level of oestrogen that occurs during the ovarian cycle before ovulation (Djahanbakhch, et al., 1984). The lower TEOAE responses and greater MOC suppression were observed in the luteal phase of the cycle, when progesterone is the dominant hormone (section 3.1.4).

As reviewed in the Introduction (section 2.1), oestrogen is mainly excitatory to neurons (Smith, et al., 2002), while progesterone has an inhibitory effect and acts as a GABA agonist (McEwen & Alves, 1999). The effect of oestrogen on the GABA-ergic or cholinergic fibers of the MOC may lead to inhibition of the MOC fibers so that they are less inhibitory to the OHC leading to higher TEOAE responses. Progesterone, on the other hand, may facilitate the inhibitory effect of the MOC fibers, and thus dampen OHC action leading to lower levels of TEOAE responses.

The finding of the higher TEOAE response and lower level of MOC suppression in the periovulatary phase of the cycle is consistent with the greater frequency shifts seen with the SOAE in previous studies (Bell, 1992; Haggerty, et al., 1993; Penner, 1995). The higher oestrogen levels during this phase of the ovarian cycle may lead to greater "excitation" in the cochlea. The presence of higher levels of progesterone in the luteal phase of the cycle may balance the excitatory effect of oestrogen (Smith et al., 2002) and this may lead to an "inhibitory" effect of the cochlea reflected by lower TEOAE responses and a greater level of MOC suppression observed in the luteal phase of the cycle.

The limitation of this study and the previous reported studies is that the hormonal changes were not documented along with the auditory tests. Therefore, a proposed larger study of the auditory function in normal subjects with normal ovarian cycle with hormone level documentation, and subjects with controlled hormonal profile may reveal more conclusive results.

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## 6.2 Case 2: Otoacoustic emissions in a woman with premature menopause treated with hormone replacement therapy

### 6.2.1 Subject

A 36 year old patient with premature menopause was referred for auditory investigation because of difficulty in hearing and intermittent tinnitus for the last five years.

She was seen before and during treatment with combined cyclic hormone replacement therapy (HRT) (Prempak®-C, Wyeth laboratories). During the first 16 days of the 28 day cycle, the subject was taking conjugated oestrogens (0.625 mg) followed by 12 days (day 17-28) of 0.625 mg conjugated oestrogens plus 0.15 mg of norgestrel (acts as progesterone) tablets.

Her past history was unremarkable except for episodes of migraine.

### 6.2.2 Procedures

She underwent auditory investigations at different times during three consecutive cycles of HRT treatment.

The test procedures were the same as the previous case (section 6.1.2), apart from recording of the auditory brainstem responses (ABR) (method described in section 5.3.1.5), to rule out retrocochlear pathology.

### 6.2.3 Protocol

The patient underwent auditory investigations before and at different times during three cycles of HRT.

The auditory tests were performed as follows:
- Baseline auditory tests before starting HRT.

- Monitoring of cochlear function with OAEs during three cycles of HRT treatment as outlined above:
    - 7th, 17th and 24th day of the first HRT cycle.
    - 18th day of the second HRT cycle.
    - 7th and 27th day of the third cycle.

### 6.2.4   Results

The baseline auditory investigations revealed that the subject had normal hearing thresholds levels on PTA and normal middle ear function assessed by tympanometry. Her ABR latencies were within the normal range, and TEOAE and SOAE were recorded from both ears. The level of MOC suppression was normal ( $\geq 1$ dB) according to the departmental database.

#### *6.2.4.1   Spontaneous otoacoustic emissions*

Multiple SOAE were recorded from both ears. The right ear had a greater number of spectral peaks (11) compared to the left ear (3).

The repeated test sessions revealed variability in SOAE spectral components in both ears as illustrated in Figure 6.2.1. The variability was more obvious in the right ear than in the left ear. The variations observed included disappearance of some of the frequency components during the second part of the HRT cycle, spectral frequency shifts that ranged from 0.4 - 4.3%, and appearance of new spectral peaks that were not recorded before HRT treatment.

In the right ear a greater number of SOAE spectral peaks were recorded in the first part of the HRT cycle compared to the second part. A similar change was seen in the left ear but to a lesser extent as outlined in Table 6.2-A.

**Table 6.2-A:** The number of spectral peaks recorded before and during HRT treatment.

|  |  | Right Ear | Left Ear | Total SOAE Peaks |
|---|---|---|---|---|
| **Baseline** |  | 11 | 3 | 14 |
| **Cycle 1** | **Day 7** | 12 | 3 | 15 |
|  | **Day 17** | 6 | 2 | 8 |
|  | **Day 24** | 10 | 1 | 11 |
| **Cycle 2** | **Day 18** | 10 | 2 | 12 |
| **Cycle 3** | **Day 7** | 12 | 3 | 15 |
|  | **Day 27** | 10 | 5 | 15 |
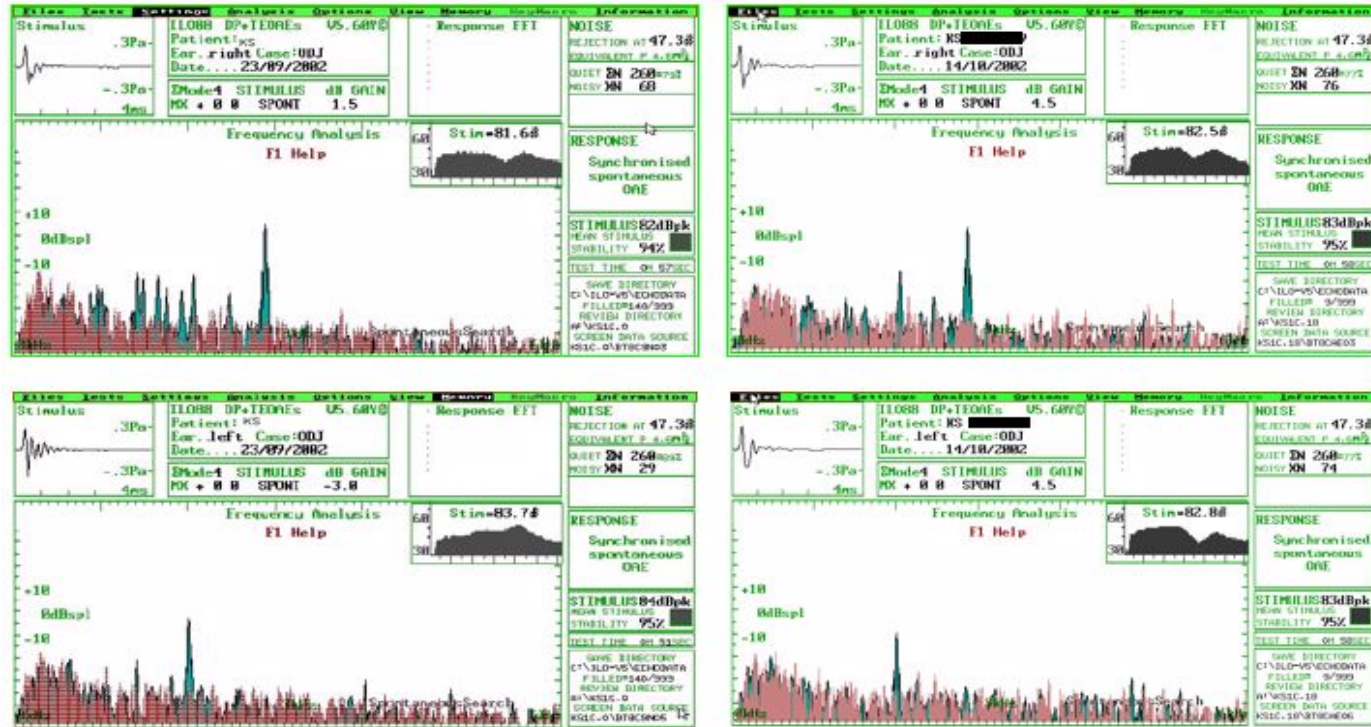
**Figure 6.2.1:** Example of SOAEs from the right and left ear during the first tested cycle.

### 6.2.4.2 *Transient evoked otoacoustic emissions*

The overall TEOAE responses revealed the following changes during the test sessions, as summarized in Table 6.2-B and displayed graphically in Figure 6.2.2.

**Table 6.2-B:** TEOAE responses (dB SPL) during HRT treatment.

|  |  | Right Ear | Left Ear | Mean |
|---|---|---|---|---|
| **Baseline** |  | 18.5 | 17.8 | 18.1 |
| **Cycle 1** | **Day 7** | 17.2 | 18.4 | 17.8 |
|  | **Day 17** | 13.8 | 19.5 | 16.6 |
|  | **Day 24** | 15.4 | 18.5 | 16.9 |
| **Cycle 2** | **Day 18** | 18.4 | 17.7 | 18.05 |
| **Cycle 3** | **Day 7** | 18.5 | 19.9 | 19.2 |
|  | **Day 27** | 15.6 | 19.8 | 17.7 |

There was a greater variation in the TEOAE response in the right ear compared to the left ear, with a marked reduction in the total TEOAE responses from the baseline by 4.7 dB in the first tested cycle, 0.1 in the second cycle, and by 2.9 dB in the third tested cycle. This reduction coincided with the combined progesterone and oestrogen replacement.
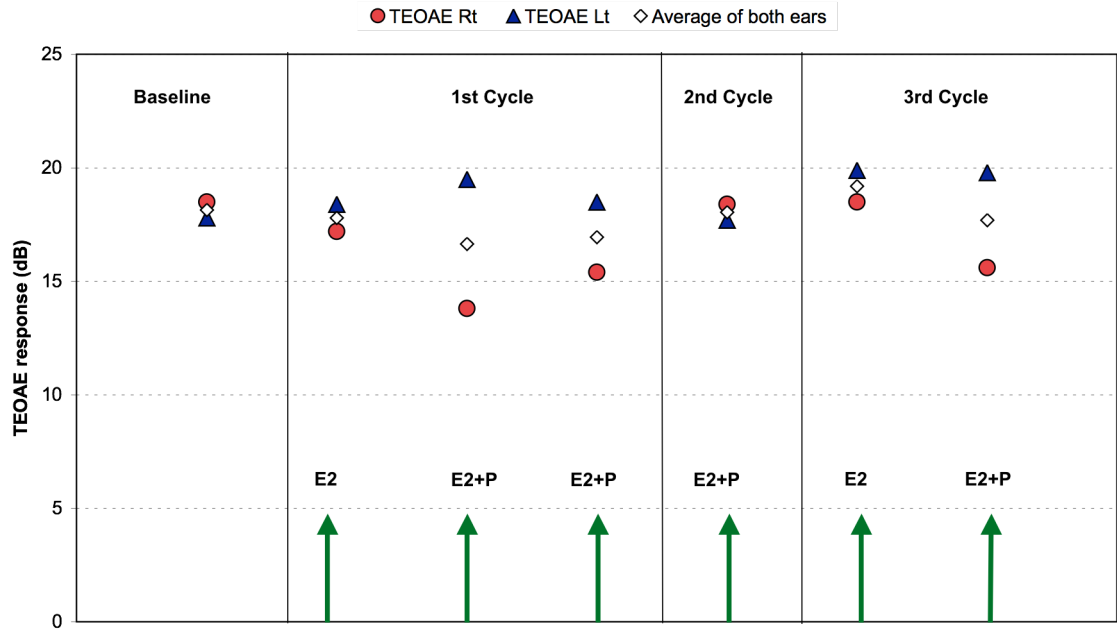
**Figure 6.2.2:** The TEOAE responses of the subject with premature menopause at different days during HRT treatment. The arrows point to the corresponding hormone or hormones dominant at time of testing. (E2 = oestrogen, P= progesterone)

### 6.2.4.3 *The medial olivary cochlear suppression test*

The MOC suppression was normal in both ears, which is $\geq 1$ dB according to the departmental database. The MOC suppression recorded during the HRT treatment was, on average, lower in both ears compared to the pretreatment recordings, but still within normal limits as seen in Table 6.2-C.

**Table 6.2-C:** The MOC suppression (dB) during HRT treatment.

|  |  | Right Ear | Left Ear | Mean |
|---|---|---|---|---|
| **Baseline** |  | 2.6 | 2.4 | 2.5 |
| **Cycle 1** | **Day 7** | missing | missing | missing |
|  | **Day 17** | 2.5 | 1.5 | 2 |
|  | **Day 24** | 2.3 | 1.4 | 1.85 |
| **Cycle 2** | **Day 18** | 2 | 2.6 | 2.3 |
| **Cycle 3** | **Day 7** | 2 | 2.1 | 2.05 |
|  | **Day 27** | 2.6 | 1.7 | 2.15 |

### 6.2.5  Discussion

There was a reduction in the number of SOAE spectral peaks and TEOAE levels in the second part of the HRT cycle, which coincided with the combined progesterone and oestrogen administration. Lower levels of TEOAE responses were also noted in Case 1 during the luteal phase in which progesterone is the dominant hormone.

Progesterone may have an inhibitory effect on cochlear function. Lower levels of TEOAE responses were noted in postmenopausal women who were taking HRT that contained progesterone, compared to postmenopausal women who were taking oestrogen only HRT (Guimaraes, et al., 2006). A similar finding of lower amplitudes of distortion products OAEs were observed in mice treated with oestrogen and progesterone, compared to mice receiving only oestrogen or placebo (Price, et al., 2009). The inhibitory effect of progesterone on cochlear function could be explained by enhancement of the GABA-ergic system (Follesa, et al., 2001), as well as counteracting and blunting the excitatory effect of oestrogen (Smith, et al., 2002).

The marked reduction noted in TEOAE responses and the greater variability in the SOAE seen in the right ear, compared to the left, may be due to the fact that, in general, the right ear is considered more sensitive than the left (reviewed by McFadden, 1993). This ear asymmetry and greater sensitivity in the right ear may suggest that the right ear is more susceptible to hormone changes.

The monitoring of cochlear function by OAE at different stages before and during treatment in a larger sample together with the evaluation of hormone levels will help to clarify these findings.

## 6.3   Conclusion

The results of these two cases suggest that cochlear function, as reflected by otoacoustic emissions, may be influenced by reproductive hormones.

The higher TEOAE responses in the periovulatary phase of the cycle in Case 1 and in the oestrogen only phase of the HRT in the second subject with premature menopause is possibly attributed to the "excitatory" action of oestrogen on the cochlea. On the other hand, the significant reduction in the TEOAE response and changes in the SOAE spectral peaks that corresponded to the introduction of progesterone in the HRT treatment of the Case 2 and the lower levels of TEOAE observed in Case 1 during the luteal phase of the cycle, is possibly due to the "inhibitory" action of progesterone on cochlear function. The effect of progesterone was not as clear in Case 1, while in the Case 2 the hormonal state was more controlled and the cochlear changes were more noticeable.

Case 1 also exhibited a lower level of MOC suppression during the periovulatary phase of the cycle, which may also be due to the "excitatory" action of oestrogen on olivocochlear bundle and more proximal levels of the auditory system.

The changes observed in the OAE were not always consistent if observing the right and left ear separately. However, the mean results of both ears show a

clearer pattern (see Figure 6.1.1 and 6.2.1). This trend suggests some synergy between the two ears, and, thus, reflects the overall change in cochlear function.

The monitoring of OAE at different stages of the normal ovarian cycle and in women with controlled hormonal profiles, with the recording of hormonal levels will help in clarifying these changes.

# Chapter 7 : Auditory function in women during the ovarian cycle

## 7.1 Introduction

The ovarian cycle in humans is the basis for reproduction, which involves different process in different systems to create the optimal condition for conception. Oestrogen and progesterone fluctuate during the ovarian cycle (described in section 3.1.4). Oestrogen is the main hormone during the follicular phase and reaches a peak just before the lutenising hormone (LH) surge, while progesterone levels are very low (Owen, 1975). Ovulation occurs after the LH surge and the progesterone level starts to rise (Djahanbakhch et al., 1981; Bakos, et al., 1994). The corpus luteum continues secreting progesterone and, to a lesser extent oestrogen which rises to the second peak near the mid-luteal phase. The lengths of the follicular and luteal phases are not consistent in women with normal cycles (Lenton, et al., 1984a; Lenton, et al., 1984b; Fehring, et al., 2006). This variability inherently leads to difficulty in studying sensory changes precisely with respect to the ovarian cycle.

Previous studies suggest that fluctuation in ovarian steroids, oestrogen and progesterone, during the ovarian cycle may influence some sensory processes, such as greater visual sensitivity (Eisner, et al., 2004), better colour discrimination (Giuffre, et al., 2007), a more sensitive sense of smell (Grillo, et al., 2001) and lower pain thresholds (Bajaj, et al., 2001) around the time of ovulation. Changes in auditory function during the ovarian cycle may also occur as described previously (section 3.1.4.1). Due to the diversity in reported methodologies and varying numbers of subjects (Table 3.1-A), no consistent and reliable conclusion can be drawn. With the exception of a few studies, which examined only auditory brainstem-evoked responses (Elkind-Hirsch et al., 1992; Serra et al., 2003), or auditory event related  potentials (Walpurger, et al, 2004), no other studies  have correlated changes in auditory function with levels of the ovarian hormones during the cycle.

In this study, several aspects of the afferent and efferent auditory function were evaluated with simultaneous measurements of hormone levels at four points in time during the ovarian cycle.

## 7.2   Study protocol

Normal hearing sensitivity (defined in section 1.2.1) was determined by PTA before participating in the study or during the first testing session.

Auditory tests (described in section 5.3.1 except for PTA) were performed four times during one ovarian cycle with day one being the first day of menses (Figure 3.1.3 ) as follows:

> **Session 1:** (5th – 8th day of the cycle) to correspond with the early follicular phase.

> **Session 2:** (10th – 14th day of the cycle) to correspond with the late follicular phase.

> **Session 3:** (20th – 23rd day of the cycle) to correspond with the early luteal phase.

> **Session 4:** (25th – 28th day of the cycle) to correspond with the late luteal phase.

Blood samples were taken at each test session to measure serum hormone levels: serum oestradiol in sessions 1-4 and progesterone in sessions 3 and 4. The method for the serum hormone level estimation is described in section 4.3.2.

***Documenting ovulation:***
The timing of the ovulation was determined by measurement of the LH surge using a commercial ovulatory kit (Clear Blue ovulation test, Unipath, UK) given to each volunteer in the study. The subject checked her urinary LH daily from day 10 of the cycle using the provided dipsticks until a positive result indicated the LH

surge (Nielsen, et al., 2001). The early luteal progesterone level was also used to confirm ovulation.

### 7.2.1 Statistical analysis

The statistical tests were performed using SPSS version 17.0 (SPSS Inc., 2008) and included the following:

– *Linear mixed-effect modeling (LMM):* was used for a more reliable detection of changes in auditory function measures or serum hormone levels between the test sessions (McCulloch & Searle, 2001; Garson, 2009). The serum hormone level or auditory function measure was the dependent variable and the test session was both the fixed factor and repeated factor. To examine the effect of both the test session and hormone levels on auditory function, auditory test was the dependent variable and the test session was the fixed and repeated factor and either oestradiol or progesterone was a covariate. Pairwise comparison of the estimated marginal means between the different test sessions was also performed.

– *Paired sample t-test:* was used to analyse the TEOAE inter-session differences calculated by the ILO software (section 5.3.1.3).

– *Linear regression:* was used to examine the correlation between auditory function measures and serum hormone levels (oestrogen or progesterone).

## 7.3 Subjects

Twenty three women who fitted the inclusion criteria (section 5.1.1) volunteered to take part in the study. However, seven of them did not complete the full study protocol (four withdrew after the first test session, one completed only the first two sessions, and the remaining two had only completed three of the four test sessions).

The positive LH surge, using the ovulatory kit (Clear Blue ovulation test, Unipath, UK), was documented in the 18 volunteers who had completed a minimum of three sessions, and the results of hormone tests (section 8.4.1) indicated that all 18 subjects had an ovulatory cycle.

The average age of the 18 subjects was 32.3 (± 8) years old (median 30, range 22-49 years). The median cycle length was 28 days and average cycle length 28.8 (± 1.7) days. Their cycles ranged between 26-33 days in length as demonstrated in Table 7.3-A.

**Table 7.3-A:** The age and cycle length of the 18 volunteers.

| Subject | Age (years) | Cycle length (days) |
| --- | --- | --- |
| 1 | 39 | 26 |
| 2 | 30 | 28 |
| 3 | 30 | 28 |
| 4 | 30 | 30 |
| 5 | 30 | 28 |
| 6 | 24 | 28 |
| 7 | 31 | 30 |
| 8 | 35 | 28 |
| 9 | 40 | 28 |
| 10 | 27 | 28 |
| 11 | 23 | 28 |
| 12 | 44 | 28 |
| 13 | 49 | 27 |
| 14 | 23 | 30 |
| 15 | 26 | 33 |
| 16 | 22 | 28 |
| 17 | 43 | 30 |
| 18 | 36 | 32 |

The following results are of the 18 subjects (36 ears) that had at least 3 test sessions.

## 7.4 Results

### 7.4.1 Serum hormone levels

The serum oestradiol levels changed significantly during the cycle [LMM, $F_{(3, 35.8)}$ = 17.4; $p < 0.001$], as demonstrated in Table 7.4-A and Figure 7.4.1. Oestradiol levels were significantly higher in session 2 and 3 compared to session 1 ($p < 0.001$) and session 4 ($p = 0.002$, and $p = 0.008$ respectively). There was no significant difference in oestradiol levels between sessions 2 and 3 ($p = 0.32$). The progesterone levels significantly changed between session 3 and session 4 [LMM, $F_{(1, 15.1)}$ = 43.8; $p < 0.001$] and was significantly higher in session 3 ($p < 0.001$) compared to session 4 (Table 7.4-A), which indicated that ovulation had occurred.

**Table 7.4-A:** Serum hormone levels (estimated mean ± SE) during the ovarian cycle.

| Time of cycle | Oestradiol (pmol/L) | Progesterone (nmol/L) |
|---|---|---|
| **Session 1 (early follicular)** (cycle day 5-8) | 168.6 ± 17.3 | N/T |
| **Session 2 (late follicular)** (cycle day 10-14) | 484 ± 62.5 | N/T |
| **Session 3 (early luteal)** (cycle day 20-23) | 407.3 ± 46.4 | 46.2 ± 5.6 |
| **Session 4 (late luteal)** (cycle day 25-28) | 249.2 ± 34.6 | 22.4 ± 5.1 |

N/T: not tested
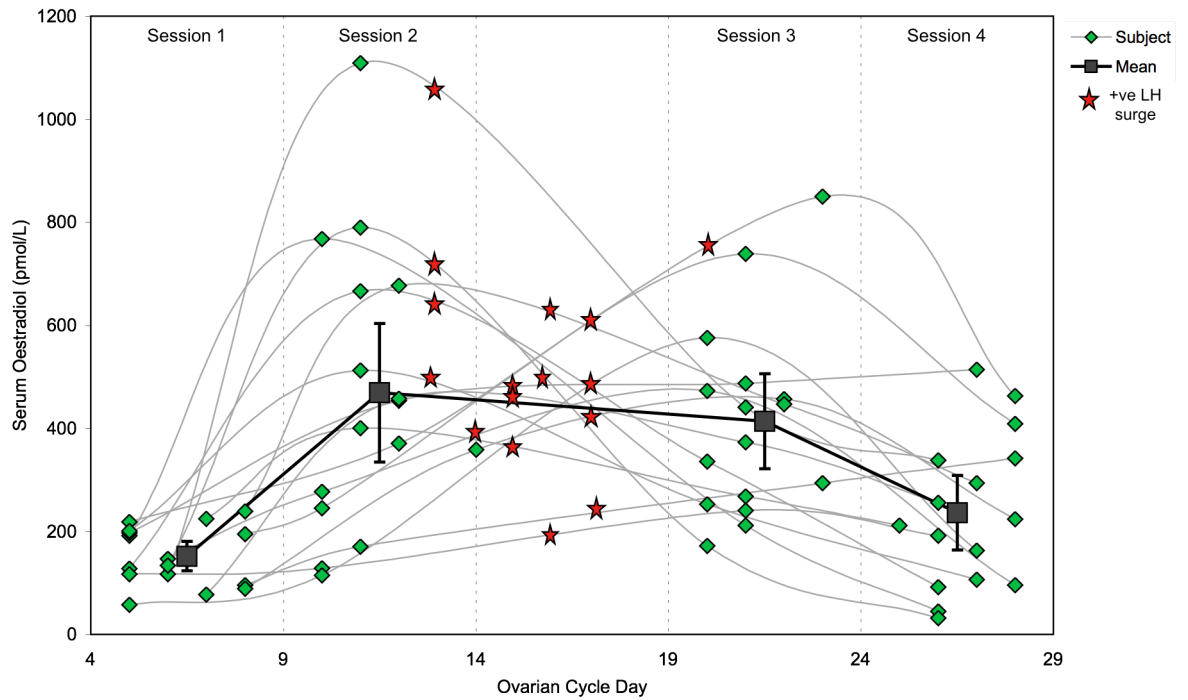
**Figure 7.4.1:** The oestradiol levels plotted against the day of the ovarian cycle, with each line representing one subject's oestradiol levels during the ovarian cycle and the stars indicate the day of the positive LH measured using the ovulatory kit.
The means and 95% confidence intervals of the serum oestradiol levels during the ovarian cycle for all subjects are superimposed.

## 7.4.2 Tympanometry

Tympanometry (method described in section 5.3.1.2) was performed at the start of each test session, before recording otoacoustic emissions to establish normal middle ear function (section 1.2.2).

The middle ear pressure did not change significantly during the repeated testing [LMM, $F_{(3, 47.1)} = 2.01$, $p = 0.13$]. However, the pairwise comparison of the estimated means revealed that the middle ear pressure recorded in session 3 was less than in session 2 ($p = 0.019$), but was still within the normal range as seen in Figure 7.4.2 .
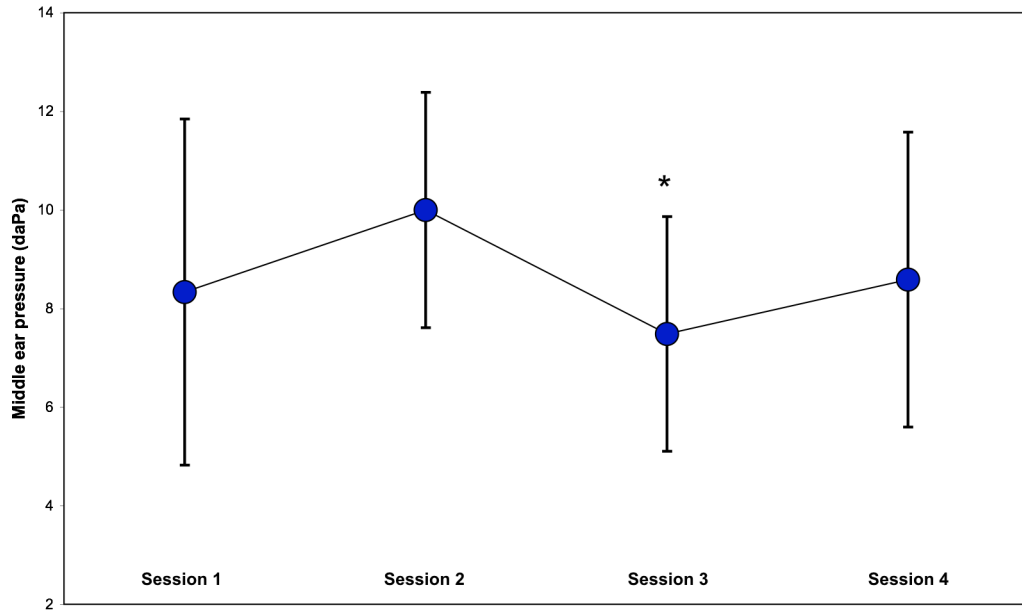
**Figure 7.4.2:** Middle ear pressure (estimated mean and 95 % confidence interval) in the four tested phases of the ovarian cycle. *p < 0.05

The tympanic membrane compliance did not significantly change during the repeated testing [LMM, $F_{(3, 43.7)} = 2.4$, p = 0.083]. However, the pairwise comparison of the estimated means showed tympanic membrane compliance was less in session 4 compared to session 1 (p=0.034) and session 3 (0.049), but still within the normal range as seen in Figure 7.4.3.
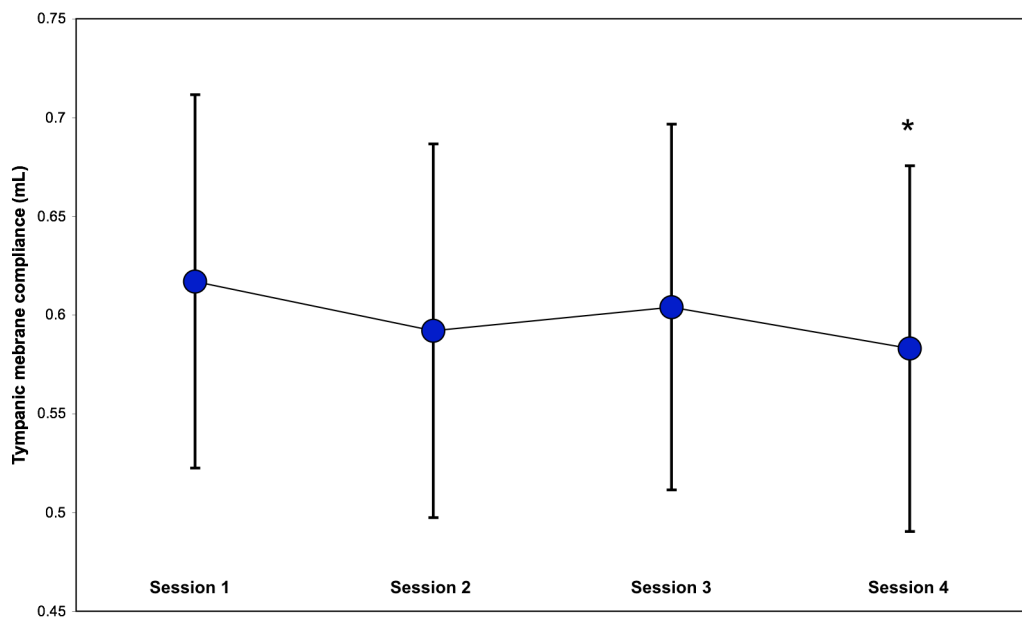


**Figure 7.4.3:** Tympanic membrane compliance (estimated mean and 95 % confidence interval) in the four tested phases of the ovarian cycle. *p < 0.05

The linear regression analysis showed that the middle ear pressure was not significantly correlated with the corresponding oestradiol levels across all four sessions [$r^2 = 0.001$, $F(1,138) = 0.17$, $p = 0.7$]. The middle ear pressure was not significantly correlated with the corresponding oestradiol levels in sessions 1 and 2 (follicular phase) [$r^2 = 0.006$, $F(1, 70) = 0.4$, $p = 0.5$], but was significantly correlated with the oestradiol and progesterone level in session 3 and 4 (luteal phase) [$r^2 = 0.10$, $F(2, 65) = 3.6$, $p = 0.03$]. The correlation with oestradiol was positive [$r = 0.45$, $p = 0.009$], and negative with progesterone [$r = -0.35$, $p = 0.04$].

The tympanic membrane compliance was not significantly correlated with oestradiol levels across all four sessions [$r^2 = 0.003$, $F(1,138) = 0.41$, $p = 0.5$], or with the corresponding oestradiol levels in sessions 1 and 2 (follicular phase) [$r^2 = 0.018$, $F(1, 70) = 1.3$, $p = 0.3$]. There was a significant correlation between the tympanic membrane compliance and oestradiol and progesterone levels in session 3 and 4 (luteal phase) [$r^2 = 0.14$, $F(2, 65) = 5.19$, $p = 0.008$]. The correlation was positive with oestradiol [$r = 0.49$, $p = 0.004$], and negative with progesterone [$r = -0.2$, $p = 0.2$].

### 7.4.3   Otoacoustic emissions

#### 7.4.3.1   *Spontaneous otoacoustic emissions*

SOAE were recorded in 23 of the 36 tested ears (63.9%). Out of the 18 subjects, 13 subjects had recordable SOAE, from both ears in ten subjects, from the right ear only in two subjects, and one had recordable SOAE from the left ear only. The number of SOAE spectral peaks recorded in the session 3 (early luteal phase) was lower than in the other sessions during the ovarian cycle (Table 7.4-B). Most of the SOAE spectral peaks were between 1-3 kHz (65.7%).

**Table 7.4-B:** The number and frequency composition of SOAE spectral peaks during the ovarian cycle.

| SOAE Spectral Peaks | Phase of ovarian cycle | | | |
|---|---|---|---|---|
| | **Session 1** (early follicular) | **Session 2** (late follicular) | **Session 3** (early luteal) | **Session 4** (late luteal) |
| **Number** | 82 | 80 | 65 | 68 |
| | | | | |
| **SOAE frequency** | **< 1kHz** | **1-3kHz** | **3-4kHz** | **>4kHz** |
| proportion | 17.1% | 65.7% | 8.6% | 8.6% |

Of all spectral peaks, 52 SOAE peaks were recorded in all testing sessions.

The SOAE amplitudes increased during the session 2 then gradually decreased during the sessions 3 and 4 as seen in Figure 7.4.4. However, the changes did not reach statistical significance [LMM, $F_{(3, 58.9)} = 1.28$, $p = 0.29$].
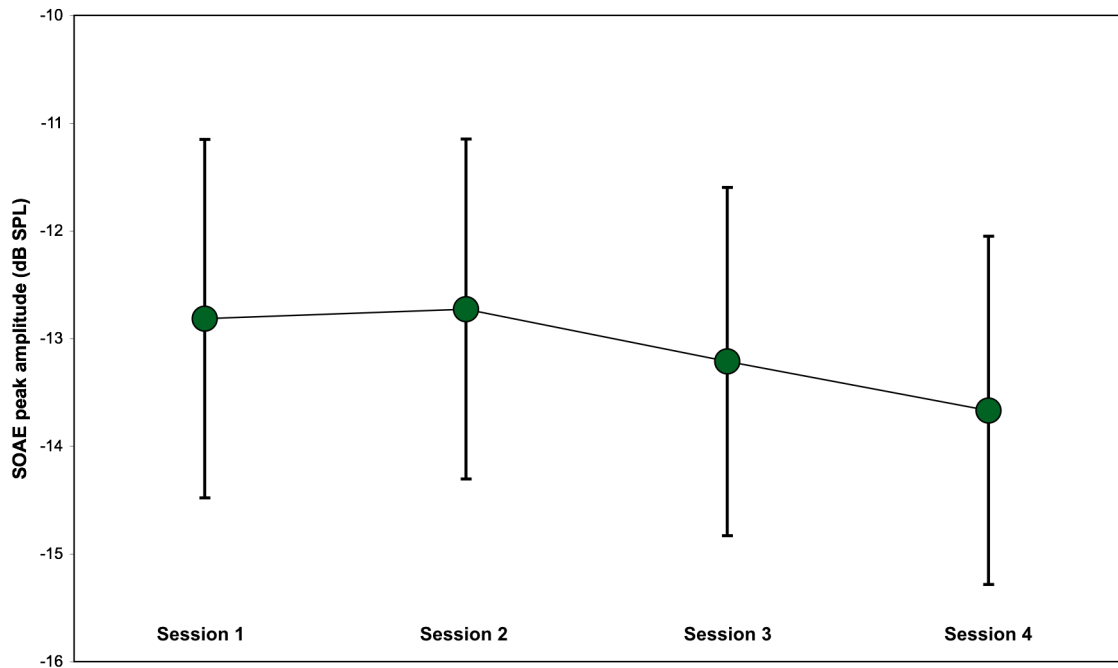
**Figure 7.4.4:** SOAE peak amplitude (estimated mean and 95 % confidence interval) in the four tested phases of the ovarian cycle.

There was a highly significant change in the SOAE frequency shift in relation to the ovarian cycle [LMM, $F_{(3,89.2)} = 14.39$, $p < 0.001$]. SOAE shifted to a higher frequency in session 1 and 2 (the follicular phase) and to a lower frequency in sessions 3 and 4 (the luteal phase). The pairwise comparison of the estimated marginal means demonstrated that SOAE frequency shift in the session 1 was significantly greater than in session 3 ($p = 0.002$), and session 4 ($p < 0.001$), and the SOAE frequency shift in the session 2 was significantly greater than in the session 3 ($p < 0.001$) and session 4 ($p < 0.001$) (Figure 7.4.5).

Serum oestradiol was added to the LMM as a covariate, and was found to have a significant negative effect on SOAE frequency shift during the ovarian cycle [LMM, oestradiol estimate = -0.0002 (SE = 0.0001), df = 133.7, $p = 0.03$], but no effect on the SOAE amplitude [LMM, oestradiol estimate = -0.002 (SE = 0.002), df = 70.5, $p = 0.15$]. The serum oestradiol in session 1 and 2 (follicular phase) had a significant negative effect on SOAE frequency shift [LMM, oestradiol estimate = -0.0005 (SE = 0.0002), df = 97.7, $p = 0.005$], but no effect on SOAE amplitude [LMM, oestradiol estimate = -0.0002 (SE = 0.002), df = 68.2, $p = 0.92$].
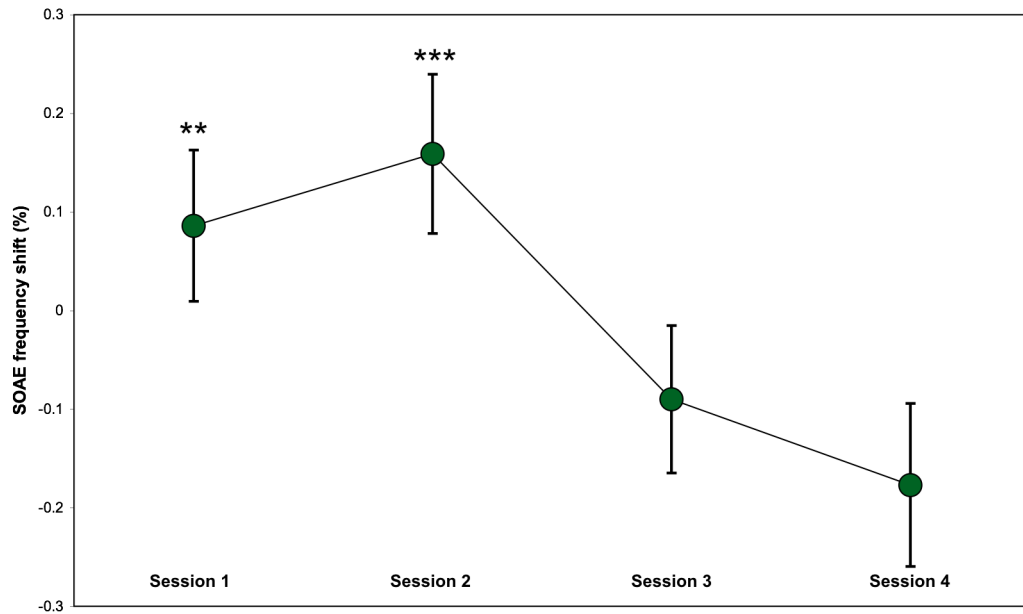
**Figure 7.4.5:** The SOAE frequency shift (mean and 95% confidence interval) in the four tested phases of the ovarian cycle.

The SOAE frequency shift in session 1 significantly greater than in session 3 and session 4; The SOAE frequency shift in session 2 significantly greater than in session 3 and session 4. ** $p < 0.01$, *** $p < 0.001$

In sessions 3 and 4 (the luteal phase), serum progesterone had a small significant negative effect on SOAE frequency shift [LMM, progesterone estimate = -0.004 (SE = 0.002), df = 71.1, p = 0.05], while oestradiol had no significant effect [LMM, oestradiol estimate = 0.0002 (SE = 0.0002), df = 72.9, p = 0.3]. Serum oestradiol in session 3 and 4 had a significant negative effect on the SOAE amplitude [LMM, oestradiol estimate = -0.01 (SE = 0.004), df = 82.9, p = 0.012], while progesterone had no effect [LMM, progesterone estimate = 0.026 (SE = 0.035), df = 82.8, p = 0.5].

The linear regression analysis showed that the SOAE frequency shift was not significantly correlated with the corresponding oestradiol levels, across all four sessions [$r^2$ = 0.001, F(1,196) = 0.23, p = 0.6]. The SOAE frequency shift was not significantly correlated with the corresponding oestradiol levels in sessions one and two (follicular phase) [$r^2$ = 0.017, F(1, 102) = 1.78, p = 0.2] or with the oestradiol and progesterone level in session three and four (luteal phase) [$r^2$ = 0.027, F(2, 91) = 1.28, p = 0.3].

The SOAE amplitude was not significantly correlated with the corresponding oestradiol levels across all four sessions [$r^2 = 0.004$, $F(1, 196) = 0.73$, $p = 0.4$], or with the oestradiol in session 1 and 2 (follicular phase) [$r^2 = 0.014$, $F(1, 102) = 1.46$, $p = 0.2$]. However, the multiple linear regression analysis between the SOAE amplitude and oestradiol and progesterone levels in session 3 and 4 (luteal phase) was significant [$r^2 = 0.13$, $F(2, 91) = 6.6$, $p = 0.002$], with a negative significant correlation with oestradiol level [$r = -0.48$, $p = 0.001$] and a weak positive correlation with progesterone level [$r = 0.26$, $p = 0.057$].

### 7.4.3.2  *Transient evoked otoacoustic emissions*

All 18 subjects (36 ears) had recordable TEOAE from both ears during the repeated testing sessions.

The total TEOAE response increased from session 1 to session 2 and session 3, then decreased in session 4, as seen in Figure 7.4.6. However the changes did not reach statistical significance as demonstrated in Table 7-C
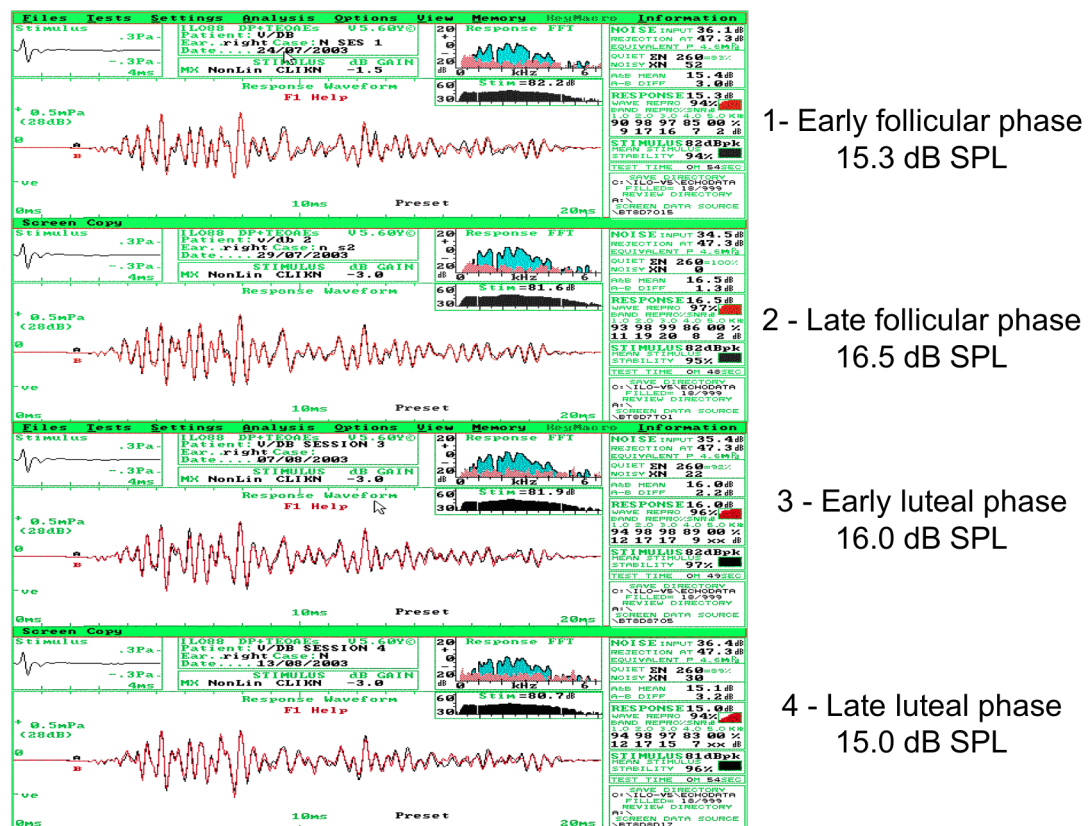


**Figure 7.4.6:** An example of the TEOAE responses in one of the volunteers during the four phases of the ovarian cycle.

**Table 7.4-C:** The estimated mean ± SE for total TEOAE response and TEOAE S/N ratio in all ears in the five frequency bands during the ovarian cycle.

| TEOAE response (dB SPL) | Phase of ovarian cycle | | | | Linear mixed-effect model (fixed effect) |
| --- | --- | --- | --- | --- | --- |
| | Session 1 (early follicular) | Session 2 (late follicular) | Session 3 (early luteal) | Session 4 (late luteal) | |
| Total TEOAE (n=36) | 14.98 ± 0.7 | 15.04 ± 0.7 | 15.22 ± 0.7 | 15.04 ± 0.7 | F(3, 55.02) = 0.62, p = 0.602 |
| 1 kHz (n=36) | 13.39 ± 0.7 | 14.08 ± 0.7 | 14.21 ± 0.7 | 13.94 ± 0.7 | F(3, 40.9) = 1.25, p = 0.306 |
| 2 kHz (n=36) | 14.83 ± 0.9 | 14.97 ± 0.9 | 15.03 ± 0.9 | 14.84 ± 0.9 | F(3, 34.2) = 0.09, p = 0.965 |
| 3 kHz (n=36) | 11.44 ± 1 | 11.64 ± 1 | 11.55 ± 1 | 11.37 ± 1 | F(3, 34.2) = 0.19, p = 0.901 |
| 4 kHz (n=36) | 8.39 ± 1.2 | 8.72 ± 1.2 | 8.77 ± 1.2 | 8.29 ± 1.2 | F(3, 33.7) = 0.61, p = 0.615 |
| 5 kHz (n=35) | 3.64 ± 1.2 | 4.32 ± 1.2 | 4.56 ± 1.2 | 3.68 ± 1.2 | F(3, 29.97) = 0.99, p = 0.41 |

Similarly, the TEOAE S/N in all five frequency bands increased during sessions 2 and 3, then decreased in the session 4, however, the changes were not significant (Table 7.4-C).

The serum oestradiol was added to the LMM as a covariate, and was found to have a small significant, positive effect on the total TEOAE response, during the ovarian cycle [LMM, oestradiol estimate = 0.0006 (SE = 0.003), df = 41.6, p = 0.048]. No significant effect was observed in the TEOAE S/N in any of the five frequency bands, as demonstrated in Table 7.4-D .

**Table 7.4-D:** The linear-mixed effect model of the TEOAE response and TEOAE S/N ratio in the five frequency bands. The test session as a fixed factor and oestradiol as a covariate.

| Linear mixed-effect model | Session (fixed effect test) | Oestradiol estimate (SE) | Degrees of freedom (df) | t, p value |
|---|---|---|---|---|
| **Total TEOAE (n=36)** | $F(3, 57.2) = 0.89$, p=0.45 | 0.000647 (0.0003) | 41.6 | 2.03, p = 0.048 |
| **1 kHz (n=36)** | $F(3, 44.8) = 0.66$, p=0.58 | 0.000618 (0.0009) | 60.3 | 0.66, p = 0.51 |
| **2 kHz (n=36)** | $F(3, 45.3) = 0.03$, p = 0.99 | 0.000609 (0.0008) | 44.2 | 0.74, p = 0.46 |
| **3 kHz (n=36)** | $F(3, 41.9) = 0.05$, p = 0.98 | 0.000562 (0.0009) | 63.4 | 0.6, p = 0.55 |
| **4 kHz (n=36)** | $F(3, 41.9) = 0.16$, p=0.92 | 0.000953 (0.0009) | 49.6 | 1.02, p = 0.31 |
| **5 kHz (n=35)** | $F(3, 34.9) = 1.15$, p=0.34 | -0.0012 (0.0016) | 48.8 | -0.77, p = 0.44 |

The serum oestradiol in sessions 1 and 2 (follicular phase) had no significant effect on TEOAE response or TEOAE S/N in the five frequency bands as demonstrated in Table 7.4-E.

**Table 7.4-E:** The linear mixed-effect model of the TEOAE response and TEOAE S/N ratio in the five frequency bands. The test session (session 1 and 2) as a fixed factor and oestradiol as a covariate.

| Linear mixed-effect model | Session (fixed effect test) | Oestradiol estimate (SE) | Degrees of freedom (df) | t, p value |
|---|---|---|---|---|
| **Total TEOAE (n=36)** | $F(1, 35.3) = 0.009$, $p = 0.92$ | 0.0003 (0.001) | 36.6 | 0.31, $p = 0.76$ |
| **1 kHz (n=36)** | $F(1, 37.8) = 0.52$, $p = 0.47$ | 0.0007 (0.0016) | 41.4 | 0.45, $p = 0.66$ |
| **2 kHz (n=36)** | $F(1, 35.8) = 0.04$, $p = 0.85$ | $-2.76 \times 10^{-6}$ (0.002) | 38.4 | -0.002, $p = 0.999$ |
| **3 kHz (n=36)** | $F(1, 35.97) = 0.03$, $p = 0.86$ | 0.00099 (0.0016) | 37.4 | 0.62, $p = 0.54$ |
| **4 kHz (n=36)** | $F(1, 31.9) = 0.26$, $p = 0.62$ | 0.001 (0.0015) | 32.7 | 1.05, $p = 0.3$ |
| **5 kHz (n=33)** | $F(1, 30.8) = 0.003$, $p = 0.95$ | 0.002 (0.002) | 33.5 | 0.84, $p = 0.41$ |

In sessions 3 and 4 (the luteal phase), the TEOAE S/N at 4 and 5 kHz were significantly higher in session 3 compared to session 4 ($p = 0.048$, and $p = 0.002$ respectively). The serum progesterone had a significant negative effect on the TEOAE S/N in the 4 kHz frequency band in sessions 3 and 4 [LMM, progesterone estimate = -0.07 (SE = 0.03), df = 27.3, $p = 0.018$], while oestradiol in sessions 3 and 4, had a significant negative effect on the TEOAE S/N in the 5 kHz frequency band [LMM, oestradiol estimate = -0.01 (SE = 0.004), df = 30.04, $p = 0.013$] as seen in Table 7.4-F.

**Table 7.4-F:** The linear mixed-effect model of the TEOAE response and TEOAE S/N ratio in the five frequency bands. The test session (session 3 and 4) as a fixed factor and oestradiol and progesterone as covariates.

| LMM (n=36) | Session (fixed effect test) | Oestradiol estimate (SE) | df | t, p value | Progesterone estimate (SE) | df | t, p value |
|---|---|---|---|---|---|---|---|
| Total TEOAE | $F(1, 36) = 0.3$, p = 0.58 | 0.0002 (0.001) | 31.7 | 0.15, 0.88 | -0.0003 (0.012) | 35.4 | -0.02, 0.98 |
| 1 kHz | $F(1, 51) = 0.55$, p = 0.46 | -0.002 (0.002) | 39.4 | -1.05, 0.3 | 0.007 (0.021) | 50.7 | 0.32, 0.75 |
| 2 kHz | $F(1, 43.7) = 3.39$, p = 0.072 | 0.0008 (0.003) | 33.96 | 0.29, 0.77 | -0.052 (0.03) | 42.8 | -1.96, 0.056 |
| 3 kHz | $F(1, 36.8) = 1.63$, p = 0.21 | 0.004 (0.002) | 31.6 | 1.93, 0.06 | 0.0004 (0.02) | 36.1 | 0.023, 0.98 |
| 4 kHz | $F(1, 38.6) = 4.18$, p = 0.048 | 0.002 (0.003) | 32.3 | 0.82, 0.42 | -0.066 (0.03) | 27.3 | -2.5, 0.018 |
| 5 kHz (n=31) | $F(1, 38.1) = 11.5$, p = 0.002 | -0.01 (0.004) | 30.04 | -2.6, 0.01 | -0.043 (0.034) | 31.04 | -1.3, 0.2 |

The paired sample t-test analysis of TEOAE inter-phase differences calculated by the ILO compare analysis showed that the difference in the TEOAE responses within the same cycle phase (i.e. either within follicular or luteal phase) was significantly lower than the difference in TEOAE between the cycle phases (i.e. between the follicular and luteal phases) as shown in Figure 7.4.7.
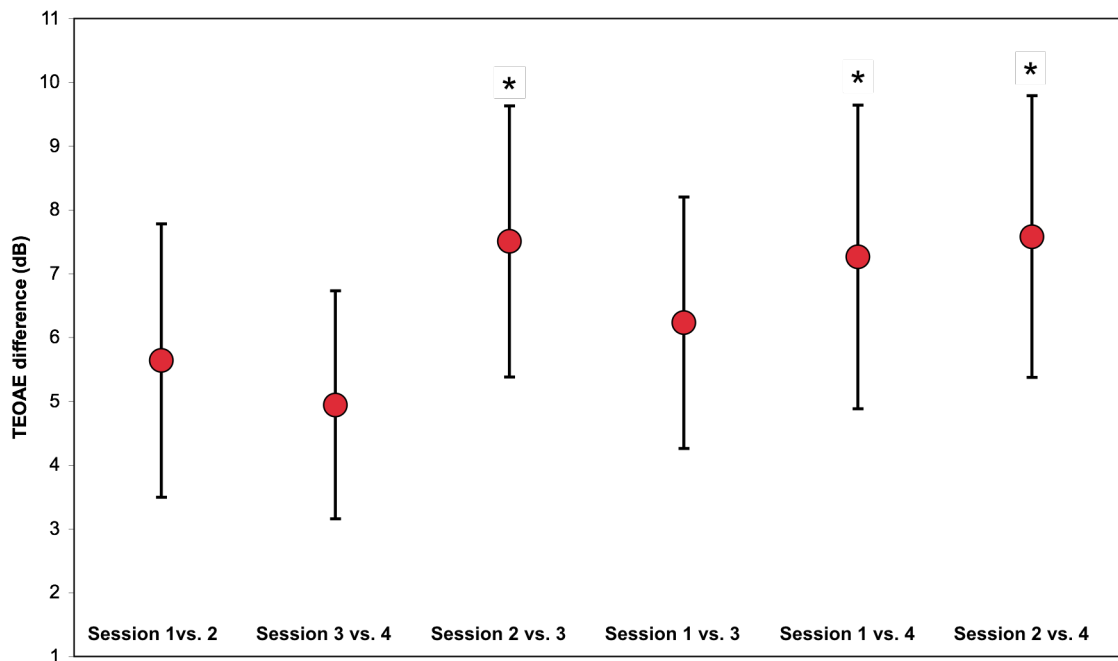
**Figure 7.4.7:** The differences in TEOAE as calculated by the ILO subtraction analysis between the different testing sessions (mean and 95% confidence interval).

The difference in TEOAE responses between sessions 2 and 3 significantly larger than the difference in TEOAE response between sessions 1and 2 and between session 3 and 4; The difference in TEOAE responses between sessions 1 and 4 significantly larger than the difference in TEOAE response between sessions 1 and 2; The difference in TEOAE responses between sessions 2 and 4 significantly larger than the difference in TEOAE response between sessions 1and 2 and between session 3 and 4. *p<0.05

The difference in TEOAE responses between session 2 and session 3 was significantly larger than the difference in the TEOAE responses within the follicular phase (session 1 vs. 2) [t(33) = 2.65, p = 0.012] and within the luteal phase (session 3 vs. 4) [t(31) = 2.23, p = 0.033].

The difference in TEOAE responses between session 2 and session 4 was significantly greater than the difference in the TEOAE responses within the follicular phase [t(33) = 2.46, p = 0.019] and within the luteal phase [t(31) = 2.52, p = 0.017].

The difference in TEOAE responses between session 1 and session 4 was also significantly greater than the difference in the TEOAE responses within the follicular phase [t(33) = 2.36, p = 0.024] and greater, but not statistically significant within the luteal phase [t(31) = 1.96, p = 0.059].

The difference in TEOAE responses between sessions 1 and 3 was larger than the difference in the TEOAE responses between the two sessions within the follicular phase (session 1 and 2) [t(33) = 0.94, p = 0.355] and between the two sessions within the luteal phase (session 3 and 4) [t(31) = 1.31, p = 0.2], but it did not reach statistical significance.

The linear regression analysis showed that the total TEOAE response and the TEOAE S/N in all five frequency bands were not significantly correlated with the corresponding oestradiol levels across all four sessions. However, when regression analysis was applied in the first two sessions only (follicular phase), a significant positive correlation between total TEOAE and oestradiol [$r^2$ = 0.08, p = 0.016, F (1, 70) = 6.1] and TEOAE S/N in the 1kHz [$r^2$ = 0.067, p = 0.028, F (1, 70) = 5.05], 2 kHz [$r^2$ = 0.091, p = 0.01, F (1, 70) = 6.98], and 5 kHz [$r^2$ = 0.073, p = 0.03, F (1, 62) = 4.76] band and oestradiol was found, as demonstrated in Figure 7.4.8.

Regression analysis between total TEOAE response and progesterone and oestrogen levels in sessions 3 and 4 (luteal phase) was significant for the total TEOAE response [$r^2$ = 0.12, p = 0.014, F(2,65) = 4.58], with progesterone having a positive correlation [r = 0.467, p = 0.007] and oestradiol a negative correlation [r = -0.473, p = 0.006]. The regression analysis between the TEOAE S/N in the five frequency bands and progesterone and oestrogen level in sessions 3 and 4 (luteal phase) was significant in the 5 kHz frequency band [$r^2$ = 0.14, p = 0.02, F(1,51) = 4.17], with oestradiol having a significant negative correlation [r = -0.43, p = 0.03] but no significant correlation with progesterone levels [r = 0.08, p = 0.7]. The regression analysis was not significant in any of the other frequency bands.
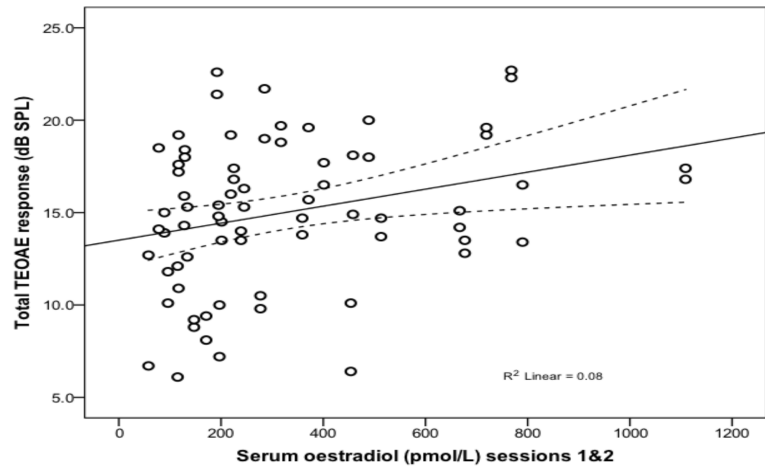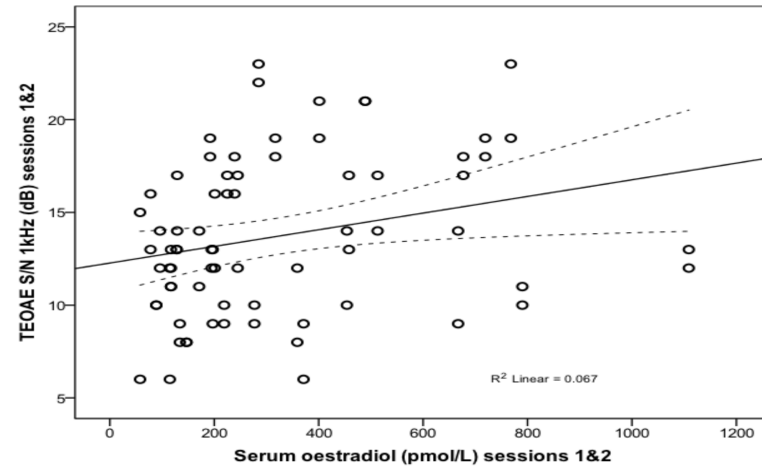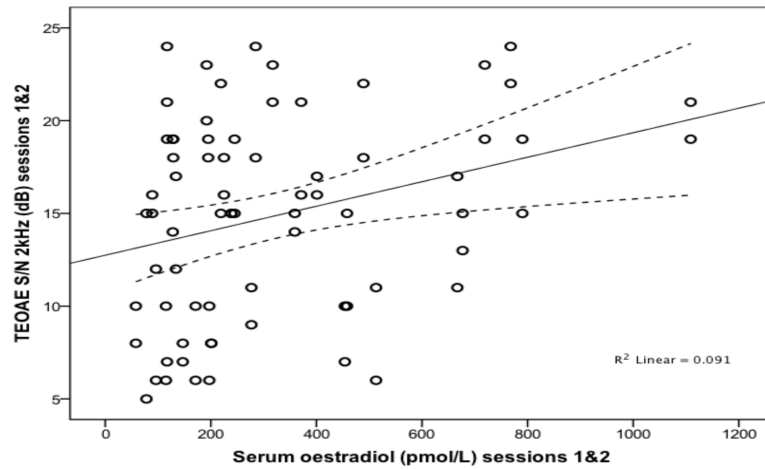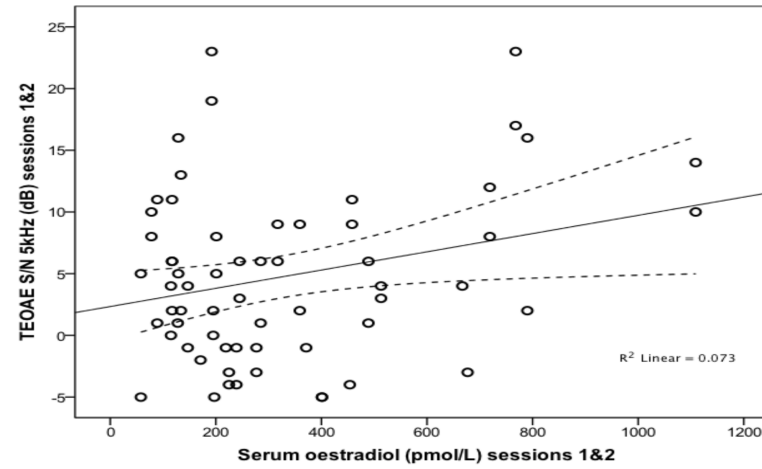
**Figure 7.4.8:** The correlation (R) between the total TEOAE response (a) and serum oestradiol and between the TEOAE S/N in the frequency band centered at 1 kHz (b) 2 kHz (c) and 5 kHz (d) and serum oestradiol in the follicular phase.

### 7.4.4 Medial olivocochlear (MOC) suppression

The MOC suppression significantly changed during the ovarian cycle [LMM, $F(3, 37.2) = 3.99$, $p = 0.015$]. The MOC suppression in sessions 2 and 4 was significantly lower than in session 1 ($p = 0.006$, $0.0004$ respectively), as demonstrated in Figure 7.4.9.
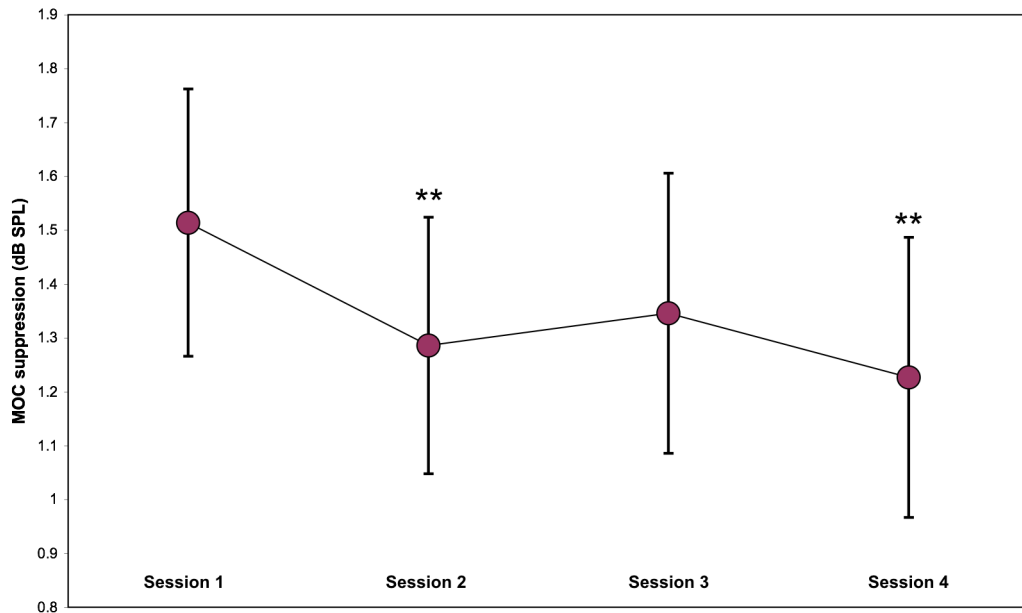


**Figure 7.4.9:** The MOC suppression (mean and 95% confidence interval) in the four phases of the ovarian cycle.

The MOC suppression in session 2 and session 4 was significantly lower than in session 1. **$p < 0.01$

The serum oestradiol level was added to the LMM as a covariate, and was found not to have a significant effect on MOC suppression during the ovarian cycle [LMM, oestradiol estimate = -0.0002 (SE = 0.0002), df = 50.8, p = 0.37], while the session had a significant effect on the MOC suppression [LMM, $F(3,45.7) = 2.85$, p = 0.048]. The serum oestradiol in session 1 and 2 (follicular phase) had no significant effect on MOC suppression [oestradiol estimate = -0.0003 (SE = 0.0003), df = 42.1, p = 0.29]. In sessions 3 and 4 (the luteal phase), the serum progesterone had a significant negative effect on the MOC suppression [progesterone estimate = -0.013 (SE = 0.005), df = 62.3, p = 0.007], while oestradiol in sessions 3 and 4, had a significant positive effect on MOC suppression [oestradiol estimate = 0.0016 (SE = 0.0006), df = 2.7, p = 0.008].

The linear regression analysis showed that MOC suppression was not significantly correlated with the corresponding oestradiol levels across all four sessions [$r^2$ = 0.005, p = 0.4, $F_{(1,138)}$ = 0.7]. However, when the regression analysis was applied in the first two sessions only (follicular phase), a significant negative correlation between MOC suppression and oestradiol [$r^2$ = 0.054, p = 0.049, $F_{(1,70)}$ = 4.03] was found, as demonstrated in Figure 7.4.10.
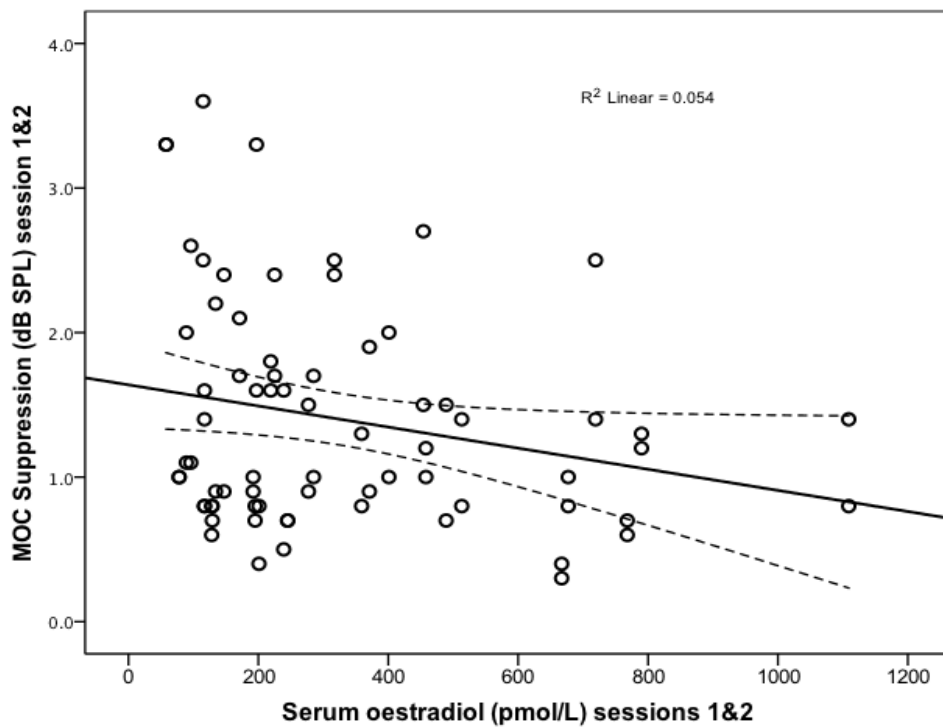


**Figure 7.4.10:** The correlation (R) between the MOC suppression and serum oestradiol in the follicular phase.

The multiple regression analysis between MOC suppression and progesterone and oestrogen level in sessions 3 and 4 (luteal phase) was significant [$r^2$ = 0.09, p = 0.044, $F_{(2,65)}$ = 3.3], with progesterone having a negative correlation [r = -0.36, p = 0.037] and oestradiol a positive correlation [r = 0.43, p = 0.014].

### 7.4.5   Auditory brainstem response

The ABRs were recorded from both the right and left ear in all 18 subjects (36 recordings in total).

The LMM analysis suggests that the ABR latencies were longest in the late follicular phase (session 2), but no significant overall changes were observed Table 7.4-G. The inter-peak intervals tended to shorten during the ovarian cycle and they were shortest during the luteal phase (sessions 3 and 4), but the changes did not reach statistical significance (Table 7.4-G).

**Table 7.4-G:** The estimated mean ± SE ABR wave latencies and inter-peak intervals during the ovarian cycle.

| ABR (n=36) | Phase of ovarian cycle | | | | Linear mixed-effect model (fixed effect) |
| --- | --- | --- | --- | --- | --- |
| | Session 1 (early follicular) | Session 2 (late follicular) | Session 3 (early luteal) | Session 4 (late luteal) | |
| I | 1.6 ± 0.02 | 1.62 ± 0.02 | 1.6 ± 0.02 | 1.6 ± 0.02 | $F_{(3, 38.4)}$ = 2.65, p = 0.06 |
| III | 3.72 ± 0.02 | 3.72 ± 0.02 | 3.72 ± 0.02 | 3.71 ± 0.02 | $F_{(3, 31.1)}$ = 0.68, p = 0.57 |
| V | 5.55 ± 0.03 | 5.56 ± 0.03 | 5.54 ± 0.03 | 5.52 ± 0.03 | $F_{(3, 35.1)}$ = 2.36, p = 0.09 |
| I-III | 2.12 ± 0.03 | 2.1 ± 0.03 | 2.11 ± 0.03 | 2.11 ± 0.03 | $F_{(3, 32.3)}$ = 1.27, p = 0.3 |
| III-V | 1.83 ± 0.02 | 1.83 ± 0.03 | 1.82 ± 0.02 | 1.81 ± 0.03 | $F_{(3, 32.9)}$ = 1.2, p = 0.33 |
| I-V | 3.95 ± 0.03 | 3.94 ± 0.03 | 3.93 ± 0.03 | 3.92 ± 0.03 | $F_{(3, 37.98)}$ = 1.9, p = 0.15 |

The pairwise comparison of the estimated marginal means showed that Wave I latency in session 2 was greater than the latency in session 1 (p = 0.01) and in session 4 (p = 0.03); the Wave V latency in session 4 was significantly shorter compared to session 2 (p = 0.02) and session 1 (p = 0.03); and the wave I-V interval in session 4 was significantly shorter compared to session 1 (p = 0.03).

The serum oestradiol was added to the LMM as a covariate, and was found not to have a significant effect on the ABR latencies during the ovarian cycle as seen in Table 7.4-H. The serum oestradiol in session 1 and 2 (follicular phase) had no significant effect on the ABR latencies as well.

**Table 7.4-H:** The linear mixed-effect model of the ABR wave latencies and interpeak intervals with the test session as a fixed factor and oestradiol as a covariate.

| Linear mixed-effect model (n=36) | Session (fixed effect test) | Oestradiol estimate (SE) | Degrees of freedom (df) | t, p value |
|---|---|---|---|---|
| I | $F(3, 58.5) = 2.28$, p = 0.088 | $-1.22 \times 10^{-5}$ $(1.9 \times 10^{-5})$ | 67.7 | -0.6, p = 0.52 |
| III | $F(3, 43.2) = 0.797$, p = 0.5 | $-1.43 \times 10^{-5}$ $(1.9 \times 10^{-5})$ | 56.01 | -0.8, p = 0.44 |
| V | $F(3, 40.8) = 2.34$, p = 0.087 | $9.88 \times 10^{-6}$ $(3.2 \times 10^{-5})$ | 61.04 | 0.3, p = 0.76 |
| I-III | $F(3, 35.9) = 0.79$, p = 0.51 | $-1.10 \times 10^{-5}$ $(2.3 \times 10^{-5})$ | 57.02 | -0.5, p = 0.64 |
| III-V | $F(3, 39.2) = 1.17$, p = 0.33 | $2.24 \times 10^{-5}$ $(3.3 \times 10^{-5})$ | 58.9 | 0.7, p = 0.5 |
| I-V | $F(3, 42.3) = 2.03$, p = 0.12 | $2.3 \times 10^{-5}$ $(3.4 \times 10^{-5})$ | 58.2 | 0.7, p = 0.5 |

In sessions 3 and 4 (the luteal phase), neither progesterone nor oestradiol had a significant effect on ABR latencies as demonstrated in Table 7.4-I.

**Table 7.4-I:** The linear mixed-effect model ABR wave latencies and interpeak intervals with the test session (session 3 and 4) as a fixed factor and oestradiol and progesterone as covariates.

| LMM (n=36) | Session (fixed effect test) | Oestradiol estimate (SE) | df | t, p value | Progesterone estimate (SE) | df | t, p value |
|---|---|---|---|---|---|---|---|
| I | $F(1, 54.5)$ =1.9, p = 0.17 | $-2.68\times10^{-5}$ $(6.2\times10^{-5})$ | 43.1 | -0.4, 0.67 | -0.0005 (0.0005) | 54.9 | -1.0, 0.32 |
| III | $F(1, 37.8) =$ 1.4, p = 0.24 | $-2.72\times10^{-5}$ $(5.4\times10^{-5})$ | 32.5 | -0.5, 0.62 | -0.0005 (0.0005) | 37.1 | -0.9, 0.35 |
| V | $F(1, 46) =$ 2.15, p = 0.15 | $1.26\times10^{-5}$ $(9.97\times10^{-5})$ | 36.3 | 0.13, 0.9 | -0.0009 (0.0009) | 45.2 | -0.97, 0.34 |
| I-III | $F(1, 37.7) =$ 0.71, p = 0.4 | $-2.65\times10^{-5}$ $(5.8\times10^{-5})$ | 32.4 | -0.4, 0.65 | -0.0004 (0.0006) | 36.97 | -0.7, 0.51 |
| III-V | $F(1, 49.3) =$ 0.47, p = 0.49 | $6.35\times10^{-5}$ $(8.97\times10^{-5})$ | 38.4 | 0.7, 0.48 | -0.0004 (0.0008) | 48.8 | -0.5, 0.64 |
| I-V | $F(1, 48.3) =$ 0.37, p = 0.54 | $2.37\times10^{-5}$ (0.0001) | 37.8 | 0.3, 0.8 | -0.0002 (0.001) | 48.5 | -0.2, 0.82 |

The linear regression analysis indicated a significant positive correlation between the wave III-V interval and the oestradiol level in all four sessions [$r^2 = 0.035$, p = 0.03, F $(1,138) = 5.01$] and when the follicular phase (sessions 1 and 2) was considered [$r^2 = 0.078$, p = 0.02, F $(1,70) = 5.95$]. A weak positive correlation was

found between the Wave V latency and oestradiol level in the follicular phase [$r^2$ = 0.05, p = 0.06, F(1,70) = 3.67]. The regression analysis between the I-V interpeak interval, and progesterone and oestrogen levels in sessions 3 and 4 (luteal phase) just reached significance [$r^2$ = 0.085, p = 0.05, F (1,65) = 3.03] with a positive correlation with progesterone [r = 0.4, p=0.02] and a negative correlation with oestradiol [r = -0.35, p=0.05]. No other significant correlations were observed.

## 7.5   Summary of results

The expected variation in oestradiol and progesterone levels was confirmed and it was consistent with an ovulatory cycle in all women. There was great variability in oestradiol levels between the subjects during the second and third testing sessions (see Figure 7.4.1), which is most likely due to the variability in the length of the ovarian cycle, especially the follicular phase. This is in agreement with previous reports on physiological variability in the length of the follicular phase (Lenton, et al., 1984b; Wilcox, et al., 2000; Fehring, et al., 2006). Due to this variability, a clear demarcation between the late follicular and early luteal phase was difficult to achieve, and some points during the ovarian cycle (e.g. two oestradiol peaks, or progesterone peak) might not have been captured, as only four hormone measurements were obtained.

**Tympanometry**

The middle ear pressure and tympanic membrane compliance did not change significantly during the ovarian cycle. However, during the luteal phase (session 3 and 4), oestradiol was associated with an increase middle ear pressure and tympanic membrane compliance, while progesterone had an opposite effect.

**Cochlear modulation**

The results of otoacoustic emissions in this study showed very subtle changes in cochlear function during the ovarian cycle. The number of SOAE spectral peaks was greater in the follicular phase (sessions 1 and 2) when oestrogen is the dominant hormone, and less in the luteal phase (session 3 and 4) when both progesterone and oestrogen were present. The change in SOAE frequency shift

was significant in relation to the ovarian cycle ($p < 0.0001$), with the greatest SOAE frequency shift occurring during the late follicular phase ($p < 0.001$) associated with highest oestrogen levels. Regression analysis did not demonstrate that these changes were correlated with serum levels of oestrogen or progesterone. However, the results of the LMM analysis revealed that oestradiol decreases the SOAE frequency shift by 0.0002 % across the ovarian cycle and by 0.0005 % during the follicular phase. During the luteal phase, progesterone has a greater effect lowering the SOAE frequency by 0.004 %, while no significant positive effect of oestradiol was recorded.

There was no significant change in the SOAE amplitude in contrast to the change noted in SOAE frequency, which may be due to the SOAE amplitude being highly variable compared to SOAE frequency (reviewed by Ceranic, 2003). However, the amplitude in the follicular phase was higher than in the luteal phase. The regression analysis and LMM analysis demonstrated that oestradiol levels in session 3 and 4 had a significant negative effect in decreasing SOAE amplitude by 0.01 dB SPL.

The overall TEOAE response and the TEOAE S/N in all five frequency bands increased during sessions 2 and 3, then decreased in session 4, but the changes were not significant. The TEOAE inter-session differences calculated by the ILO subtraction analysis revealed that there was a significant difference in TEOAE levels between the two phases of the ovarian cycle (follicular and luteal phase), and this may have been consequent upon a hormonal effect. The LMM analysis revealed that oestradiol increased the total TEOAE response by 0.0006 dB SPL during the ovarian cycle, but had no significant effect on the TEOAE S/N in the five frequency bands. Linear regression analysis revealed a significant positive correlation between total TEOAE and oestradiol ($p = 0.016$) and TEOAE S/N at the 1kHz ($p = 0.03$), 2 kHz ($p = 0.01$) and 5 kHz ($p = 0.03$) frequencies and oestradiol in the follicular phase. However, the positive effect of oestradiol was small, and thus did not reach significance (Table 7.4-E). During the luteal phase, both oestrogen and progesterone were significantly correlated with the total TEOAE response, with progesterone having a positive correlation ($r = 0.5$, $p = 0.007$), and oestradiol a negative correlation ($r = -0.5$, $p = 0.006$) with the TEOAE

response. A significant negative correlation between oestradiol in the luteal phase with the TEOAE S/N in the 5 kHz frequency band was also observed (r = -0.43, p = 0.03). The effect was small for the overall TEOAE response, and thus did not reach significance (Table 7.4-F). However, progesterone was found to decrease the TEOAE S/N in the 2 kHz, 4 kHz and 5 kHz frequencies (Table 7.4-F) and the effect was significant only at the 4 kHz band (p = 0.02). Oestradiol in the luteal phase was found to decrease the TEOAE S/N at the 5 kHz by 0.01 dB. The presence of progesterone in the luteal phase may blunt the effect of oestrogen on the cochlea leading to changes in the frequency composition of the TEOAE that were seen more clearly in the SOAE.

**Olivocochlear suppression**

Olivocochlear suppression decreased in the late follicular phase (p = 0.006), which was characterized by a rising oestradiol level. Suppression then increased in the luteal phase, when both oestradiol and progesterone were secreted, before decreasing again near the end of the cycle, when there were lower levels of both steroids, with a significantly lower suppression in the late luteal compared to the early follicular phase (p = 0.004). A decline in MOC suppression during the late follicular phase was in agreement with the SOAE and TEOAE findings.

There was a significant negative correlation between MOC suppression and oestradiol level in the follicular phase (p = 0.049) suggesting that a decrease in suppression values in the late follicular phase may have been due to an excitatory effect of oestrogen, leading to a reduction of cochlear inhibition. The LMM analysis revealed that oestradiol had a small negative effect [LMM, oestradiol estimate = -0.0003] on the MOC suppression but the effect was not significant (p = 0.3). However, during the luteal phase, progesterone lowered the MOC suppression by 0.013 dB and oestradiol increased it by 0.002 dB. This was further confirmed by the significant negative correlation with progesterone (r = -0.36, p = 0.04) and positive correlation with oestradiol (r = 0.43, p = 0.01) in the luteal phase. These findings suggested that the effects of ovarian steroids on the olivocochlear reflex arc were more complex, with oestradiol possibly having a dual action possibly due to the presence of progesterone.

**Auditory brainstem evoked responses**

The ABR responses showed some significant change during the ovarian cycle, with an increase in the wave I and V latencies in the follicular phase (sessions 1 and 2) and a decrease in the late luteal phase ( session 4). There was also shortening of the wave I-V interval (p = 0.03) in the luteal phase. The longer latencies in the follicular phase (sessions 1 and 2) suggested that oestradiol might have been involved, and the shorter latencies in the luteal phase suggested progesterone might also play a role. The positive correlation between oestradiol and III-V interval and wave V latency suggested that higher oestradiol levels were associated with longer latencies. However, neither oestradiol nor progesterone were found to have a significant effect on ABR latencies by LMM analysis (Table 7.4-H and Table 7.4-I).

# Chapter 8 : Comparison of auditory function between women and men over a similar period of time

## 8.1 Introduction

Gender differences in sensory functions, including the sense of hearing, have been described in both humans and animals (reviewed by Velle, 1987, Nelson, 2000, Becker, 2002). The major sex differences in auditory function have been described above in section 3.1.3, and suggested more sensitive hearing in females. The underlying mechanisms of these observations are not fully elucidated, but may include sexual dimorphism in the central nervous system and auditory structures, exposure to reproductive hormones during development, and adulthood (reviewed in section 3.1.3).

The possible role of ovarian hormones in the gender differences in ABR latencies has been suggested previously (Trune et al., 1988, Dehan and Jerger, 1990, Elkind-Hirsch et al., 1994). However, only a few studies have compared the auditory function between women and men, across a similar period of time to record any fluctuation in auditory function that could be due to physiological cycles in women and men. The majority of studies have examined ABRs (Fagan and Church, 1986, Dehan and Jerger, 1990, Wharton and Church, 1990, Elkind-Hirsch et al., 1994), and only one report studied spontaneous OAE (Haggerty et al., 1993).

The purpose of this study was to compare several aspects of the auditory function between men and women across a similar period of time. This corresponded to one naturally occurring ovarian cycle for women and across a month in the men.

## 8.2 Study protocol

Normal hearing sensitivity (defined in section 1.2.1) was determined by PTA, before participating in the study or during the first testing session.

All volunteers underwent auditory tests (described in section 5.3.1 except for PTA) four times while participating in this study. The women were tested during one ovarian cycle as described in section 7.2, while the men had the auditory tests once a week for four consecutive weeks to correspond with the ovarian cycle measurements, but with no hormonal assessment.

### 8.2.1 Statistical analysis

The statistical tests were performed using SPSS version 17.0 (SPSS Inc., 2008) and included the following procedures:

- *Linear mixed-effect modeling (LMM):* was used for changes in auditory function measures during the repeated testing in men and women (McCulloch & Searle, 2001; Garson, 2009). The auditory function measure was the dependent variable and the test session was both the fixed and repeated factor with pairwise comparison of the estimated marginal means between the different test sessions. Gender was also used as a between subject factor, and the interaction between gender and test session was examined.

- *Paired sample t-test:* was used to analyse the TEOAE inter-session differences calculated by the ILO software (section 5.3.1.3) in each group of subjects.

- *Independent sample t-test:* was used to compare the TEOAE inter-session differences calculated by the ILO software between women and men, and the other auditory function measures at each testing session.

## 8.3 Subjects

The study involved two groups of subjects:

- *Group of women:* 18 women (36 ears) who had taken part in the previous study (section 7.3).

- *Group of men:* 15 men (30 ears) volunteered, of whom one completed only two test sessions and another man completed only three test sessions.

The average age of the women was 32.33 (SD = 8) years, with 30 as the median age (range 22-49). The average age of the men was 31.8 (SD = 7.4) years with 33

as the median age (range 21-48). The t test showed no significant difference in age between men and women [t(31) = 0.2, p = 0.8].

## 8.4 Results

### 8.4.1 Pure tone audiometry

All subjects had normal hearing thresholds. Women tend to have higher thresholds in the low frequencies (250 and 500 Hz) while men tend to have higher thresholds in the mid to high frequencies (1000-4000 Hz). However the differences were not significant (Figure 8.4.1).
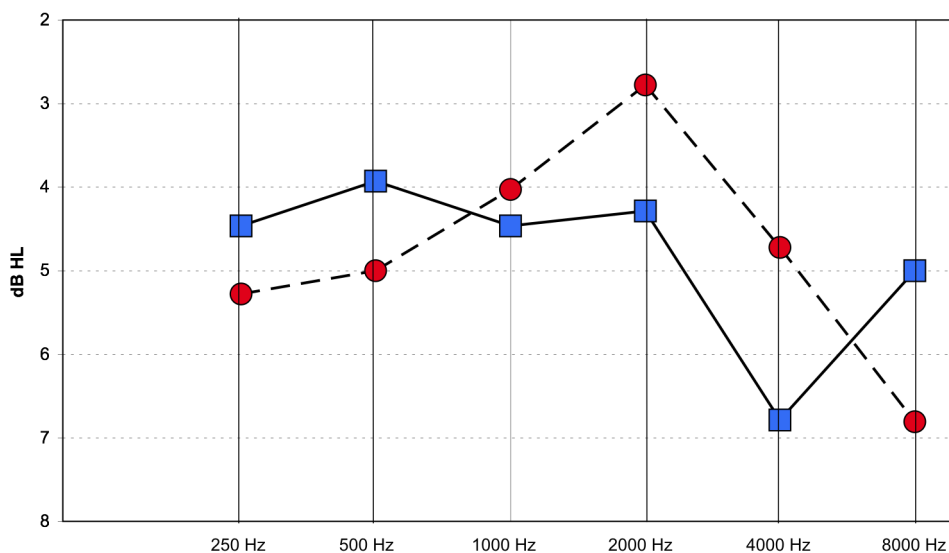


**Figure 8.4.1:** Mean PTA thresholds in men and women (women = ●, men = ■).

### 8.4.2 Tympanometry

All subjects had normal middle ear pressures and tympanic membrane compliance during the repeated testing. There was no significant difference in middle ear pressure between the women and men [LMM, F(1, 64.4) = 0.89, p = 0.35]. However, the tympanic membrane compliance was larger in men [LMM, gender

estimate = 0.33 (SE = 0.15)] compared to the women, which was significant [LMM, $F(1, 63.7) = 4.95$, $p = 0.03$].

### 8.4.3 Otoacoustic emissions

#### 8.4.3.1 *Spontaneous otoacoustic emissions*

SOAE were recorded in 63.9 % of female ears and 36.7% of male ears. The number of SOAE spectral peaks recorded in the four test session are summarised in Table 8.4-A.

**Table 8.4-A:** The number of SOAE spectral peaks during the four testing sessions in women and men.

| | Number of SOAE | | | |
| --- | --- | --- | --- | --- |
| | **Session 1** | **Session 2** | **Session 3** | **Session 4** |
| **Women (n=23 ears)** | 82 | 80 | 65 | 68 |
| **Men (n=11 ears)** | 23 | 19 | 24 | 18 |

There were 66 SOAE spectral peaks recorded consistently in the repeated testing sessions; 52 in the women and 14 in the men.

The SOAE amplitude did not significantly change during the four testing sessions in both the women [LMM, $F(3,58.9) = 1.28$, $p = 0.29$] and men [LMM, $F(3,18.1) = 1.7$; $p = 0.2$]. The SOAE amplitudes in the women were larger than in the men [LMM, gender estimate = 2.45 (SE = 1.55)] but the effect was not significant [LMM, $F(1,64.03) = 2.49$; $p = 0.12$].

There was a highly significant change in the SOAE frequency shift in women during the repeated testing [LMM, $F(3,89.2) = 14.39$, $p < 0.001$]. The SOAE frequency gradually shifted during the repeated testing in men and just reached

significance [LMM, F(3,19.01) = 3.14; p = 0.05]. The SOAE frequency shift was greater in women [LMM, gender estimate = 0.004 (SE = 0.04)], but it did not reach significance [LMM, F(1,231.96) = 0.01; p = 0.93], because there was a significant interaction between the test session and gender [LMM, F(1,108.6) = 7.6; p < 0.001]. The SOAE frequency shift was significantly different between men and women at each test session using the independent sample T test as seen in Figure 8.4.2.
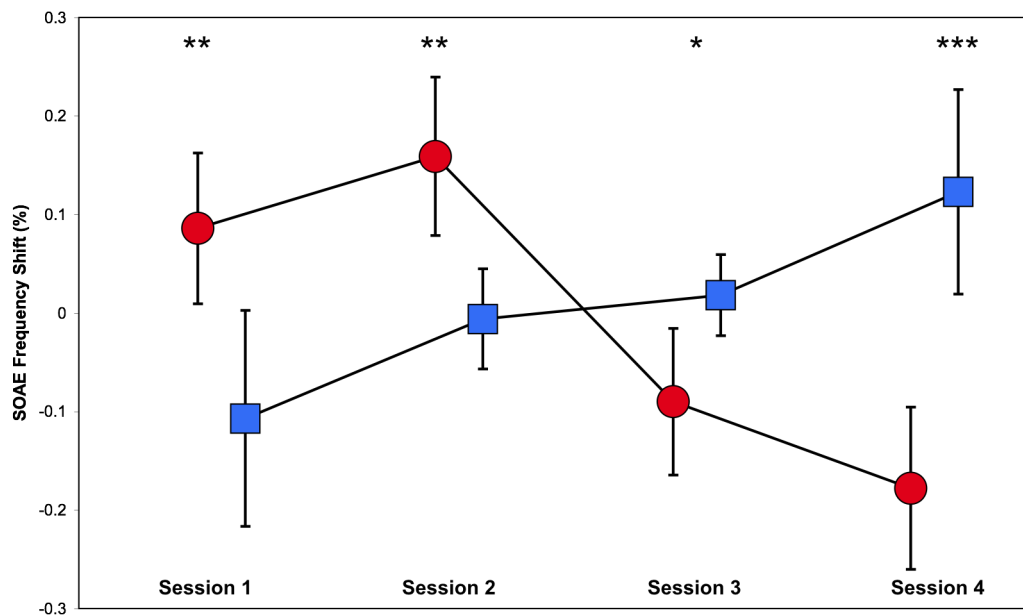


**Figure 8.4.2:** The mean (± 95% confidence interval) of the SOAE peak frequency shift in women and men which were significantly different in all test sessions (women = ●, men = ■) *p < 0.05, **p < 0.01, ***p < 0.001.

### 8.4.3.2 *Transient evoked otoacoustic emissions*

All subjects had recordable TEOAE from both ears, so in total there were TEOAE from the 36 ears of the women and the 30 ears of the men. There was no significant change in the level of the TEOAE response during the four testing session in either women [LMM, F(3, 55.02) = 0.62, p = 0.6 ] or men [LMM, F(3, 27.8) = 1.35, p = 0.3 ]. However, the overall TEOAE responses were larger in the women [LMM, gender estimate = 3.16 (SE = 0.99)] and the effect was significant [LMM, F(1, 63.9) = 13.3, p = 0.001] across the four test sessions.

The TEOAE S/N in the five frequency bands did not change significantly during the testing sessions in either the women or men (Table 8.4-B-8.4-F). The TEOAE S/N in the 1 kHz frequency band was larger in the women [LMM, gender estimate = 1.7 (SE = 1.1)], but the effect did not reach significance [LMM, $F(1, 63.1)$ = 2.3; p = 0.13] as seen in Table 8.4-B.

**Table 8.4-B:** TEOAE S/N in 1 kHz frequency band (estimated marginal means ± SE) in women and men.

| TEOAE S/N 1 kHz | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 13.4 ± 0.73 | 14.1 ± 0.71 | 14.2 ± 0.69 | 13.9 ± 0.68 | $F(3, 40.9)$ = 1.2, p = 0.31 |
| **Men (n=30)** | 12.4 ± 1.02 | 12.1 ± 0.98 | 12 ± 1.01 | 12.4 ± 0.98 | $F(3, 32.2)$ = 0.3, p = 0.84 |
| **Gender t-test*** | $t(64)$ = 0.9 p = 0.38 | $t(64)$ = 1.5 p = 0.13 | $t(64)$ = 1.7 p = 0.09 | $t(64)$ = 1.6 p = 0.06 | $F(1, 63.1)$ = 2.3, p = 0.13 |

*equal variances assumed

The TEOAE S/N in the 2 kHz [LMM, gender estimate = 4.9 (1.3)], 3 kHz [LMM, gender estimate = 6.05 (1.4)], 4 kHz [LMM, gender estimate = 6.2 (SE = 1.4)], and 5 kHz [LMM, gender estimate = 5.06 (SE = 1.4)] frequency bands were significantly higher in the women compared to the men in all test sessions (Table 8.4-C-8.4-F).

**Table 8.4-C:** TEOAE S/N in 2 kHz frequency band (estimated marginal means ± SE) in women and men.

| TEOAE S/N 2 kHz | Test session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| Women (n=36) | 14.8 ± 0.9 | 15 ± 0.9 | 15 ± 0.9 | 14.8 ± 0.9 | F(3, 34.2) = 0.09, p = 0.96 |
| Men (n=30) | 9.8 ± 1 | 10.5 ± 0.91 | 9.1 ± 1 | 9.7 ± 0.95 | F(3, 32.4 ) = 2.1, p = 0.12 |
| Gender t-test* | t(64) = 3.7 p < 0.001 | t(64) = 3.5 p = 0.001 | t(64) = 4 p < 0.001 | t(64) = 4.1 p < 0.001 | F(3, 63.9) = 15.3, p < 0.001 |

*equal variances assumed

**Table 8.4-D:** TEOAE S/N in 3 kHz frequency band (estimated marginal means± SE) in women and men.

| TEOAE S/N 3 kHz | Test session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| Women (n=36) | 11.4 ± 1.03 | 11.6 ± 1.03 | 11.5 ± 1.01 | 11.4 ± 1.01 | F(3, 34.2) = 0.19, p = 0.9 |
| Men (n=30) | 5.8 ± 0.99 | 5.7 ± 0.97 | 5.4 ± 0.96 | 5.1 ± 0.96 | F(3, 32.7) = 0.88; p = 0.46 |
| Gender t-test* | t(64) = 4.1 p < 0.001 | t(64) = 4 p < 0.001 | t(60) = 4.3 p < 0.001 | t(58) = 4.1 p < 0.001 | F(1, 63.9) = 19.9, p < 0.001 |

*equal variances assumed

**Table 8.4-E:** TEOAE S/N in 4 kHz frequency band (estimated marginal means ± SE) in women and men.

| TEOAE S/N 4 kHz | Test session | | | | Linear mixed-effect model (fixed effect) |
| --- | --- | --- | --- | --- | --- |
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 8.4 ± 1.19 | 8.7 ± 1.18 | 8.8 ± 1.2 | 8.3 ± 1.19 | $F_{(3, 33.7)} = 0.6$, p=0.6 |
| **Men (n=30)** | 2.6 ± 0.82 | 2.2 ± 0.79 | 2.1 ± 0.87 | 2.6 ± 0.81 | $F_{(3, 26.2)} = 0.5$, p = 0.68 |
| **Gender t-test\*** | $t_{(58.7)} = 3.8$, p < 0.001 | $t_{(54.5)} = 4.7$, p < 0.001 | $t_{(56.5)} = 3.6$, p < 0.001 | $t_{(51.4)} = 3.9$, p < 0.001 | $F_{(3, 63.8)} = 18.1$, p < 0.001 |

\*equal variances not assumed

**Table 8.4-F:** TEOAE S/N in 5 kHz frequency band (estimated marginal means ± SE) in women and men.

| TEOAE S/N 5 kHz | Test session | | | | Linear mixed-effect model (fixed effect) |
| --- | --- | --- | --- | --- | --- |
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=35)** | 3.6 ± 1.23 | 4.3 ± 1.23 | 4.6 ± 1.25 | 3.7 ± 1.18 | $F_{(3, 30)} = 0.99$, p = 0.41 |
| **Men (n=26)** | -1.2 ± 0.69 | -0.9 ± 0.54 | -0.9 ± 0.79 | -1 ± 0.62 | $F_{(3, 18.2)} = 0.15$, p=0.93 |
| **Gender t-test\*** | $t_{(45.8)} = 3.9$, p < 0.001 | $t_{(42.2)} = 3.8$, p < 0.001 | $t_{(39)} = 3.7$, p = 0.001 | $t_{(35)} = 2.6$, p = 0.014 | $F_{(1, 60.7)} = 12.5$, p = 0.001 |

\*equal variances not assumed

The overall difference in the level of TEOAE between the sessions calculated by the ILO software was larger and more variable in the female group (Figure 8.4.3).
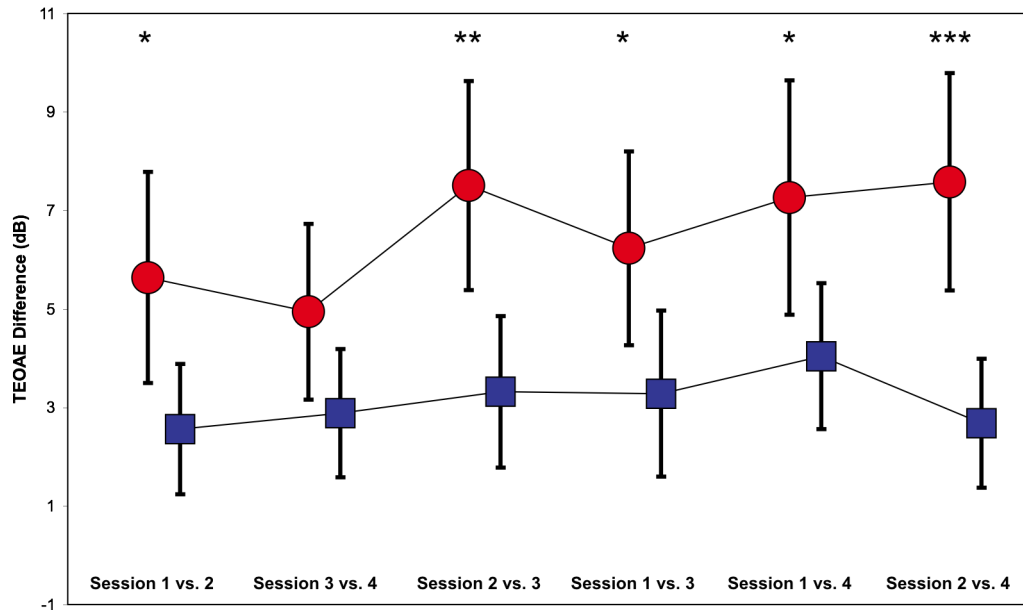
**Figure 8.4.3:** The mean (± 95% confidence interval) difference in TOAE responses between the different testing sessions in women and men calculated by the ILO software (women = ●, men = ■). * p < 0.05, ** p < 0.01, *** p < 0.001.

The inter session differences in TEOAE levels were significantly larger in women compared to men except for the difference in TEOAE levels between session 3 and 4 as see in Table 8.4-G.

**Table 8.4-G:** The mean (±SD) difference in TEOAE levels calculated by the ILO software in women and men.

| | The inter-session TEOAE Difference | | | | | |
|---|---|---|---|---|---|---|
| | **Session 1 vs. 2** | **Session 3 vs. 4** | **Session 2 vs. 3** | **Session 1 vs. 3** | **Session 1 vs. 4** | **Session 2 vs. 4** |
| **Women (n=36)** | 5.6 ± 6.5 | 4.9 ± 5.2 | 7.5 ± 6.3 | 6.2 ± 5.8 | 7.3 ± 7 | 7.6 ± 6.5 |
| **Men (n=30)** | 2.6 ± 3.7 | 2.9 ± 3.4 | 3.3 ± 4.1 | 3.3 ± 4.6 | 4 ± 3.9 | 2.7 ± 3.4 |
| **Gender t-test*** | $t(56.9) = 2.4$, $p = 0.02$ | $t(53.9) = 1.8$, $p = 0.07$ | $t(57.4) = 3.1$, $p = 0.003$ | $t(56.9) = 2.2$, $p = 0.03$ | $t(53.2) = 2.3$, $p = 0.03$ | $t(51.99) = 3.7$, $p < 0.001$ |

*equal variances not assumed

The inter session difference was significantly larger between the luteal and follicular phase of the ovarian cycle in the group of women (section 7.4.3.2). However, the inter session difference in the group of men was similar during the repeated testing (Figure 8.4.3), except for the difference in TEOAE responses between session 1 and session 4 which was significantly larger than the difference in the TEOAE responses between sessions 1 and 2 [t(25) = 2.8, p = 0.01].

### 8.4.4  Medial olivocochlear (MOC) suppression

The MOC suppression in the women significantly changed during the repeated testing [LMM, F(3, 37.2) = 3.99, p = 0.015]. The MOC suppression in men decreased during the repeated testing but did not change significantly [LMM, F(3, 25.03) = 1.11, p = 0.36]. The suppression was less marked in the women compared to the men in all the test sessions [LMM, gender estimate = -0.3 (SE = 0.2)], but the difference was not statistically significant [LMM, F(1, 61.4) = 2.1, p = 0.15] as seen in Table 8.4-H.

**Table 8.4-H:** The MOC suppression (estimated marginal means± SE) in women and men.

| MOC Suppression | Test session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 1.51 ± 0.13 | 1.29 ± 0.12 | 1.35 ± 0.13 | 1.23 ± 0.13 | F(3, 37.2) = 4, p = 0.01 |
| **Men (n=30)** | 1.88 ± 0.23 | 1.58 ± 0.2 | 1.57 ± 0.2 | 1.58 ± 0.22 | F(3, 25) = 1.1, p=0.36 |
| **Gender t-test\*** | t(64) = -1.5, p = 0.14 | t(64) = -1.2, p = 0.22 | t(60) = -1.04, p = 0.3 | t(58) = -1.7, p = 0.1 | F(1, 61.4) = 2.1, p=0.15 |

*equal variances assumed

### 8.4.5   Auditory brainstem response

The absolute wave latencies were significantly shorter in women compared with men in all test sessions (Figure 8.4.4).
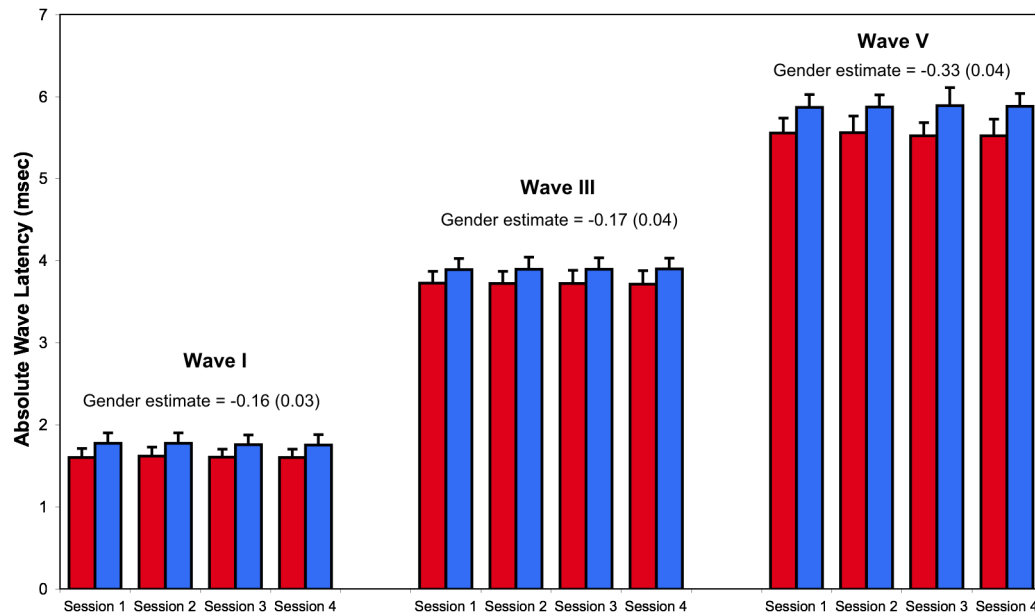


**Figure 8.4.4:** The mean (+SD) of the ABR latencies in women (■) and men (■), with the LMM gender estimates and SE.

The ABR latencies did not change significantly during the repeated testing in either men or women, as seen in Tables 8.4-I -8.4-K. However, there was a significant interaction between the test session and gender for the Wave V latency [LMM, $F(3, 62.2) = 3.6$, $p = 0.018$].

**Table 8.4-I:** The absolute Wave I latency (estimated mean ± SE) in women and men.

| Wave I absolute latency | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 1.6 ± 0.02 | 1.62 ± 0.02 | 1.6 ± 0.02 | 1.6 ± 0.02 | $F(3, 38.4) = 2.65$ $p = 0.06$ |
| **Men (n=30)** | 1.77 ± 0.02 | 1.77 ± 0.02 | 1.76 ± 0.02 | 1.78 ± 0.02 | $F(1, 26.1) = 0.84$ p $= 0.48$ |
| **Gender t-test\*** | t(64) = -5.8 p < 0.001 | t(64) = -5.2 p < 0.001 | t(60) = -5.5 p < 0.001 | t(58) = -5.2 p < 0.001 | $F(1, 63.5) = 35.4$, p<0.001 |

\*equal variances assumed

**Table 8.4-J:** The absolute Wave III latency (estimated mean ± SE) in women and men.

| Wave III absolute latency | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 3.72 ± 0.02 | 3.72 ± 0.02 | 3.72 ± 0.02 | 3.71 ± 0.02 | $F(3, 31.1) = 0.68$ $p = 0.57$ |
| **Men (n=30)** | 3.89 ± 0.02 | 3.89 ± 0.03 | 3.89 ± 0.02 | 3.9 ± 0.02 | $F(1, 31.9) = 1.81$, $p = 0.16$ |
| **Gender t-test\*** | t(64) = -4.8 p < 0.001 | t(64) = -4.7 p < 0.001 | t(60) = -4.5 p < 0.001 | t(58) = -4.6 p < 0.001 | $F(1, 63.97) = 24.8$, $p < 0.001$ |

\*equal variances assumed

**Table 8.4-K:** The absolute Wave V latency (estimated mean ± SE) in women and men.

| Wave V absolute latency | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | **Session 1** | **Session 2** | **Session 3** | **Session 4** | |
| **Women (n=36)** | 5.55 ± 0.03 | 5.56 ± 0.03 | 5.54± 0.03 | 5.52 ± 0.03 | $F(3, 35.1) = 2.36$, $p = 0.09$ |
| **Men (n=30)** | 5.87 ± 0.03 | 5.87 ± 0.03 | 5.88 ± 0.03 | 5.88 ± 0.03 | $F(1, 28.5) = 1.06$, $p = 0.38$ |
| **Gender t-test\*** | $t(64) =$ -7.5 $p<0.001$ | $t(64) =$ -7.1 $p<0.001$ | $t(60) =$ -7.4 $p < 0.001$ | $t(58) =$ -7.4 $p < 0.001$ | $F(1,63.9) = 59.3$, $p < 0.001$ |

*equal variances assumed

The interpeak intervals did not change significantly during the repeated testing in both women and men (Table 8.4-L-8.4-N). The I-III interpeak intervals were shorter in women [LMM, gender estimate = -0.012 (SE = 0.03)], but the difference was not significant [LMM, $F(1, 64) = 0.13$, $p = 0.7$].

**Table 8.4-L:** The I-III interpeak interval (estimated mean ± SE) in women and men.

| I-III interpeak interval | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | **Session 1** | **Session 2** | **Session 3** | **Session 4** | |
| **Women (n=36)** | 2.12 ± 0.03 | 2.1 ± 0.03 | 2.11 ± 0.03 | 2.11 ± 0.03 | $F(3, 32.3) = 1.27$, $p = 0.3$ |
| **Men (n=30)** | 2.12 ± 0.02 | 2.12 ± 0.02 | 2.14 ± 0.02 | 2.14 ± 0.02 | $F(1, 31.6) = 0.9$, $p=0.45$ |
| **Gender t-test*** | $t(62.6) = 0.2$ $p = 0.87$ | $t(63.1) = -0.49$ $p = 0.6$ | $t(55) = -0.86$ $p = 0.4$ | $t(52.4) = -0.86$ $p = 0.39$ | $F(1, 63.96) = 0.13$, $p = 0.72$ |

*equal variances assumed

The III-V [LMM, gender estimate = -0.15 (SE = 0.03)] and the I-V [LMM, gender estimate = -0.17 (SE = 0.04)] interpeak intervals were significantly shorter in the women in all test sessions (Table 8.4-M and 8.4-N).

**Table 8.4-M:** The III-V interpeak interval (estimated mean ± SE) in women and men.

| III-V interpeak interval | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 1.83 ± 0.02 | 1.83 ± 0.03 | 1.82 ± 0.02 | 1.81 ± 0.03 | F(3, 32.9) = 1.2 p = 0.33 |
| **Men (n=30)** | 1.97 ± 0.03 | 1.98 ± 0.03 | 2.06 ± 0.07 | 1.97 ± 0.03 | F(1, 28.3) = 0.51, p = 0.68 |
| **Gender t-test\*** | t(64) = -4.1 p < 0.001 | t(64) = -3.9 p = 0.001 | t(60) = -3.05 p = 0.003 | t(58) = -4.2 p < 0.001 | F(1, 64.1) = 18.7, p < 0.001 |

\*equal variances assumed

**Table 8.4-N:** The I-V interpeak interval (estimated mean ± SE) in women and men.

| I-V interpeak interval | Test Session | | | | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|---|
| | Session 1 | Session 2 | Session 3 | Session 4 | |
| **Women (n=36)** | 3.95 ± 0.03 | 3.94 ± 0.03 | 3.93 ± 0.03 | 3.92 ± 0.03 | F(3, 37.98) = 1.9 p = 0.15 |
| **Men (n=30)** | 4.1 ± 0.03 | 4.1 ± 0.03 | 4.12 ± 0.03 | 4.11 ± 0.03 | F(1, 29.6) = 0.86 p = 0.47 |
| **Gender t-test\*** | t(64) = -3.2 p = 0.002 | t(64) = -3.5 p = 0.001 | t(60) = -4.3 p < 0.001 | t(58) = -4.4 p < 0.001 | F(1, 63.9) = 14.9, p < 0.001 |

\*equal variances assumed

## 8.5   Summary of results

This study examined several aspects of auditory function, including cochlear modulation (changes in SOAE and TEOAE), olivocochlear suppression and auditory brainstem evoked responses, between men and women. The results suggest gender differences in auditory function.


**Tympanometry**

The middle ear pressure was similar in both men and women, while the tympanic membrane compliance was significantly larger in men compared to the women ($p = 0.03$).


**Cochlear modulation**

The SOAE were recorded in 72.2 % of the women (in 13 out of 18) and in 46.7% of the men  (in 7 out of the 15), which was slightly higher than previously reported in the literature (Penner and Zhang, 1997). The SOAE amplitudes in women were higher than in the men, but the differences did not reach significance, and this is possibly due to the high variability in  SOAE amplitudes compared to SOAE frequency (van Dijk & Wit, 1990; Wit, 1993).

The SOAE frequency shift significantly changed during the repeated testing sessions in women, but not in, men.

The overall TEOAE responses were three times larger in women compared to men (gender estimate= 3.16). The larger overall TEOAE response recorded in the women was in agreement with earlier findings reported in the literature (Probst et al., 1991, McFadden, 1998, Hall, 2000). The TEOAE S/N ratios in the 2, 3, 4 and 5 kHz frequency bands were between five to six time larger in women compared to the men, which was significant, but the TEOAE S/N in the 1 kHz frequency band was only two times larger in women compared to the men and was not statistically significant (section 8.4.3.2).

The inter-session difference in the level of TEOAE calculated by the ILO software was significantly larger in the female group and more variable compared to the men.

**Olivocochlear suppression**

The MOC suppression was lower in the women compared to the men (gender estimate = -0.3), but the difference was not statistically significant.

The level of MOC suppression significantly changed during the ovarian cycle in the women but not in the men. The lower MOC suppression in women might suggest that the MOC fibers were less inhibitory to the OHC leading to lower efferent suppression and greater TEOAE response amplitudes being seen in the women.

**Auditory brainstem evoked responses**

The absolute wave latencies of the ABR were significantly shorter in the women (by 0.2-0.3 msec) compared to the men, in all testing sessions. The III-V and I-V interpeak intervals were also significantly shorter in the women by 0.2 msec compared to the men. The shorter ABR latencies in women may be attributed to the excitatory effect of oestrogen and sexual dimorphism in the CNS (Cahill, 2006).

# Chapter 9 : Auditory function in women undergoing assisted conception treatment

## 9.1 Introduction

Subfertility is defined as failure to achieve pregnancy after twelve months or more of regular unprotected sexual intercourse (Zegers-Hochschild, et al., 2009). The choice of treatment for subfertile couples depends on the underlying cause of subfertility and the results of their investigations (Lass, 1999).

There are several treatment techniques, including in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), intrauterine insemination (IUI)  and ovulation induction (OI).

The first baby born through IVF was in July, 1978. The oocyte used was obtained from a natural ovarian cycle (Steptoe & Edwards, 1978). Further research revealed that the pregnancy rate with IVF was greatly improved if more than one embryo was replaced in the uterus (Edwards & Steptoe, 1983; Fishel, et al., 1985; Wood, et al., 1985). This is the rationale behind ovarian stimulation in current IVF treatment protocols.  The aim of ovarian stimulation or superovulation is to stimulate the woman's ovaries to produce a larger number of follicles, to collect greater number of oocytes for fertilization, which enables a greater chance for a number of high-quality embryos for transfer to the uterus and cryopreservation (Macnamee & Brinsden, 1999). There are several protocols for ovarian stimulation (Hugues, 2002; Cohen, 2003), but there is always a risk of ovarian hyperstimulation syndrome (Rizk & Aboulghar, 1999). Patients undergoing ovarian stimulation are usually monitored by serum oestradiol levels and ultrasonography to anticipate ovarian hyperstimulation syndrome (reviewed by Rizk & Smitz, 1992; Kwan, et al., 2008).

The main ovarian stimulation protocol involves the use of gonadotrophin releasing hormone (GnRH) analogues with gonadotrophins. The standard long protocol is widely used clinically, and involves the following:

- A GnRH analogue is administered subcutaneously or as a nasal spray for a minimum of 14 days (Porter, et al., 1984), leading to suppression of the pituitary and thus suppression of ovarian function with ovarian steroid down-regulation to the levels similar to those in postmenopausal women (Akagbosu, 1999). The rationale behind ovarian steroid down-regulation is to prevent premature LH surge that may lead to cancellation of treatment.

- Ovarian stimulation is started by the administration of gonadotrophins after establishing that ovarian down-regulation has been achieved by ultrasound to measure endometrial thickness and serum oestradiol levels (Macnamee & Brinsden, 1999). The patient continues taking the GnRH analogue along with the gonadotrophins to prevent premature LH surge and increase the chance of more oocytes available for collection (Loumaye, 1990). The ovaries are stimulated to produce several follicles and oestradiol levels rise significantly, compared to the natural ovarian cycle. The length of treatment is usually eight to ten days (Macnamee & Brinsden, 1999). Once the size of at least three dominant follicles reach more than or equal to 16-17 mm, human chorionic gonadotrophin (HCG) is given to complete oocyte maturation in preparation for oocyte collection, which is scheduled 35-36 hours later.

- Progesterone supplement is started after oocyte collection and is continued after embryo transfer. This supplement is given as a support because the hormone production in luteal phase of a stimulated cycle is different from a natural cycle (Loumaye, 1990; Hugues, 2002). A pregnancy test is usually performed two weeks after embryo transfer.

The typical timeline of the long protocol IVF treatment is described in Figure 9.1.1. The dosage and length of the medications is tailored to each subject by the treating gynecologist.
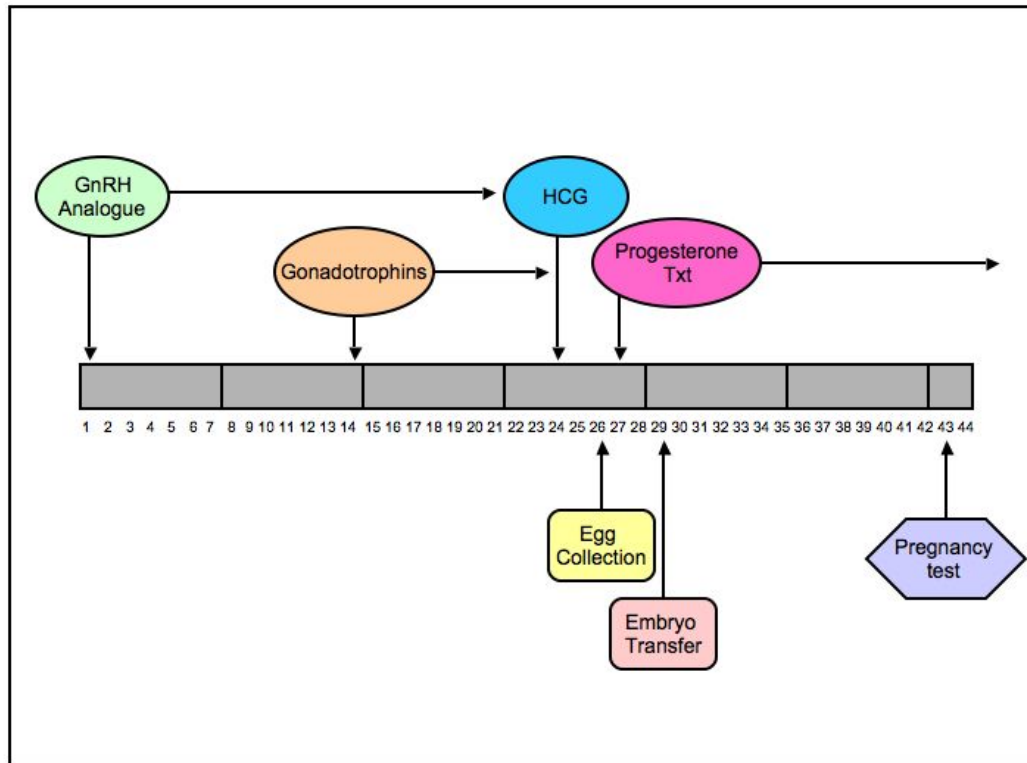
**Figure 9.1.1:** Timeline of a standard long protocol IVF treatment cycle. (HCG= Human chorionic gonadotrophin).

The assisted conception treatment alters the women's hormonal profile and may affect the auditory system, as seen during the normal ovarian cycle. There has been one case report of a patient, who was undergoing IVF treatment, presenting with sudden sensorineural hearing loss, tinnitus and vertigo (Hajioff, et al., 2003). The underlying cause was attributed to a throboembolic vascular event and was not considered a direct hormonal cause due to her normal physiological oestrogen level. A thrombotic stroke has been described previously in a patient undergoing a similar treatment (Rizk, et al., 1990).

The supraphysiological oestrogen levels in women undergoing assisted conception treatment provide a unique cohort for researchers, to evaluate the possible effects of ovarian steroids, especially oestrogen on auditory or other physiological system. The patient's hormones are closely monitored making the timing for testing more accurate than during the natural ovarian cycle. The possible effect of oestradiol on Eustachian tube function (Nir, et al., 1991) and ABR (Ben David, et al., 1995) has been studied in women undergoing ovulation

induction. Nir and co-workers (1991) correlated their patients (n = 25) oestrogen levels with the shift in tympanometric peak pressure, which reflected the Eustachian tube function. They found that the Eustachian tube function did not change significantly with rising oestrogen levels in the majority of their subjects. Ben David and his colleagues (1995) reported longer ABR latencies in the group of women with higher oestrogen levels compared to women with lower oestrogen levels and women with both higher oestrogen and progesterone, but the differences were not significant.

GnRH may also have an effect on the auditory system. Auditory stimuli associated with reproductive behaviour in birds and amphibians leads to stimulation of GnRH production (Cheng, et al., 1998; Maney, et al., 2007). GnRH receptors have been identified in several areas of CNS (reviewed by Wang, et al., 2010) including some sensory and motor areas in some species (Forlano, et al., 2000; Kawai, et al., 2009), some of which may be relevant to auditory processing (Ubuka & Bentley, 2009). Recently GnRH was found to elevate the auditory threshold in fish (Maruska & Tricas, 2011). Another possible action of GnRH on auditory system is through its effect on the GABA-ergic system, and GnRH analogues have been used to treat some forms of catamenial epilepsy (Bauer, et al., 1992; Herzog, 2009; Reddy, 2009).

The aim of this study was to assess the auditory function in a group of women undergoing a standard assisted conception treatment with simultaneous measurements of their hormone levels at three points during their treatment.


## 9.2   Study protocol

Normal hearing sensitivity (defined in section 1.2.1) was determined by PTA before participating in the study or during the first testing session.

Auditory tests (described in section 5.3.1 except for PTA) were performed at three points during the assisted conception treatment as follows:

**Session 1**: minimum 14 days following GnRH stimulation (ovarian steroid down-regulation).

**Session 2:** about 8-10 days following GnRH plus gonadotrophin stimulation (ovarian stimulation) before egg collection.

**Session 3:** about 10-14 days following egg collection when the subject is taking progesterone supplement which raises the level of progesterone (post embryo transfer).

Blood samples were taken at each test session to measure serum oestradiol and progesterone levels. The patients were also monitored by ultrasound by their treating gynecologists as part of their treatment.

### 9.2.1   Statistical analysis

Statistical analysis was as described above in section 7.2.1.
Due to the small sample size, the inter-session difference in TEOAE calculated by the ILO software was analysed using the non-parametric related samples test (Wilcoxon Signed-rank test), instead of the paired sample t-test.

## 9.3   Subjects

Eighty seven potential subjects initially expressed an interest to participate, but when they were asked to take part in the study, 62 declined, as they were offered a different fertility protocol, failed to attend the first testing session and could not be contacted, became pregnant naturally, or they were still waiting to have their fertility treatment.

Twenty five women, who were scheduled to receive assisted conception treatment, volunteered for the study. Two women were excluded from the study after the first test due to concomitant medical conditions (seronegative arthritis and insulin dependent diabetes mellitus) which may have affected their hearing

tests. Six women had only the initial first test but did not continue due to their treatment being terminated, because they did not respond to either the pituitary suppression or the ovarian stimulation. A further three had only one hearing test then were unable to continue engagement in the study due to other family or work commitments.

The remaining fourteen completed at least two hearing test sessions, with seven having completed all three hearing test sessions (subject 1-4 and subject 6-7), but some of the serum hormones were not measured as detailed in Table 9.3-A. One subject from the 14 was unable to come for the first session (subject 9), one did not attend her second session (subject 5), and five could not come for the third session, because their treatment failed and they started to bleed (subject 10-14). A summary of the auditory test sessions and blood samples performed on the 14 volunteers is presented in Table 9.3-A.

The average age of the 14 subjects who completed at least two test sessions was 33.5 (± 3.5) years old (median 34, range 28-40 years). The average age of the seven women who completed the three test sessions was 33.6 (± 3.4) (median 34, range 29-40 years).

**Table 9.3-A:** Summary of the age, auditory tests and serum hormone levels performed in the three test sessions in the 14 volunteers.

| Subject | Age | Session 1 Auditory tests Serum E2/P | Session 2 Auditory tests Serum E2/P | Session 3 Auditory tests Serum E2/P |
|---|---|---|---|---|
| **1** | 40 | All except ABR<br>E2 only | All except ABR<br>E2 only | All except ABR<br>E2 and P |
| **2** | 29 | All<br>E2 and P | All<br>E2 and P | All<br>E2 and P |
| **3** | 34 | All<br>E2 and P | All<br>E2 and P | All<br>E2 and P |
| **4** | 34 | All<br>E2 only | All<br>E2 only | All<br>E2 and P |
| **5** | 33 | All<br>E2 and P | DNA | All except ABR<br>E2 and P |
| **6** | 33 | All<br>E2 and P | All<br>E2 and P | All<br>E2 and P |
| **7** | 31 | All<br>E2 and P | All<br>NM | All<br>E2 and P |
| **8** | 34 | All<br>E2 and P | All<br>E2 and P | All<br>E2 and P |
| **9** | 29 | DNA | All<br>E2 and P | All<br>E2 and P |
| **10** | 35 | All<br>E2 only | All<br>E2 only | DNA |
| **11** | 28 | All<br>E2 and P | All<br>E2 and P | DNA |
| **12** | 34 | All<br>E2 and P | All<br>NM | DNA |
| **13** | 36 | All<br>E2 and P | All<br>E2 and P | DNA |
| **14** | 39 | All<br>NM | All<br>E2 only | DNA |

(DNA: did not attend test session, E2: oestradiol, P: progesterone, NM: E2 and P not measured)

## 9.4 Results

### 9.4.1 Serum hormone levels

The serum oestradiol levels in 14 subjects was not measured in some test sessions (Table 9.3-A) as summarized in Table 9.4-A.

The serum oestradiol levels changed significantly during the treatment [LMM, $F_{(2, 29)} = 9.14$, $p = 0.001$]. The serum oestradiol levels were lowest in session 1 and reached their highest levels in session 2.

**Table 9.4-A:** Serum oestradiol levels (pmol/L) in the 14 volunteers.

| Subject | Session 1 (ovarian steroid down-regulation) | Session 2 (ovarian stimulation) | Session 3 (post embryo transfer) |
|---|---|---|---|
| 1 | 74 | 1229 | 488 |
| 2 | 192 | 4343 | 6571 |
| 3 | 110 | 5558 | 164 |
| 4 | 49 | 948 | 208 |
| 5 | 77 | DNA | 6094 |
| 6 | 76 | 2232 | 2390 |
| 7 | 74 | NM | 124 |
| 8 | 116 | 4442 | 313 |
| 9 | DNA | 11241 | 180 |
| 10 | 75 | 3077 | DNA |
| 11 | 167 | 11674 | DNA |
| 12 | 99 | NM | DNA |
| 13 | 209 | 5705 | DNA |
| 14 | NM | 1393 | DNA |

(NM: not measured; DNA: did not attend test session)

The serum progesterone levels in 14 subjects was not measured in some test sessions (Table 9.3-A) as summarized in Table 9.4-B

**Table 9.4-B:** Serum progesterone levels (nmol/L) in the 14 volunteers.

| Subject | Session 1 (ovarian steroid down-regulation) | Session 2 (ovarian stimulation) | Session 3 (post embryo transfer) |
|---|---|---|---|
| 1 | NM | NM | 50 |
| 2 | 4 | 4 | 190.8 |
| 3 | 1 | 4 | 40 |
| 4 | NM | NM | 49 |
| 5 | 2 | DNA | 190.8 |
| 6 | 2 | 2 | 79 |
| 7 | 2 | NM | 42 |
| 8 | 2 | 3 | 48 |
| 9 | DNA | 7 | 48 |
| 10 | NM | NM | DNA |
| 11 | 3 | 10 | DNA |
| 12 | 2 | NM | DNA |
| 13 | 1 | 3 | DNA |
| 14 | NM | NM | DNA |

(NM: not measured; DNA: did not attend test session)

The serum progesterone changed significantly during the treatment [LMM, $F_{(2, 16.6)} = 12.5$, $p < 0.001$]. The serum progesterone level was highest in session 3.

Two of the subjects (subject 2 and 5) who were tested in session 3 were probably pregnant (Table 9.4-B). They both had a high level of progesterone (190.8 nmol/L), which was significantly greater [$t_{(7)} = -14.53$, $p < 0.0001$] than the progesterone measured in the other seven women tested in session 3.

### 9.4.2 Tympanometry

Tympanometry (method described in section 5.3.1.2) was performed in each test session before recoding otoacoustic emissions to establish normal middle ear function (section 1.2.2).

The middle ear pressure significantly changed during the repeated testing [LMM, $F_{(2, 19.8)} = 4.4$, $p = 0.03$]. The pairwise comparison of the estimated means revealed that the middle ear pressure recorded during session 2 was lower than during session 1 ($p = 0.013$) and session 3 ($p = 0.04$), but was still within the normal middle ear pressure range (-50 to +50 daPa, Section 1.2.2) as seen in Figure 9.4.1.
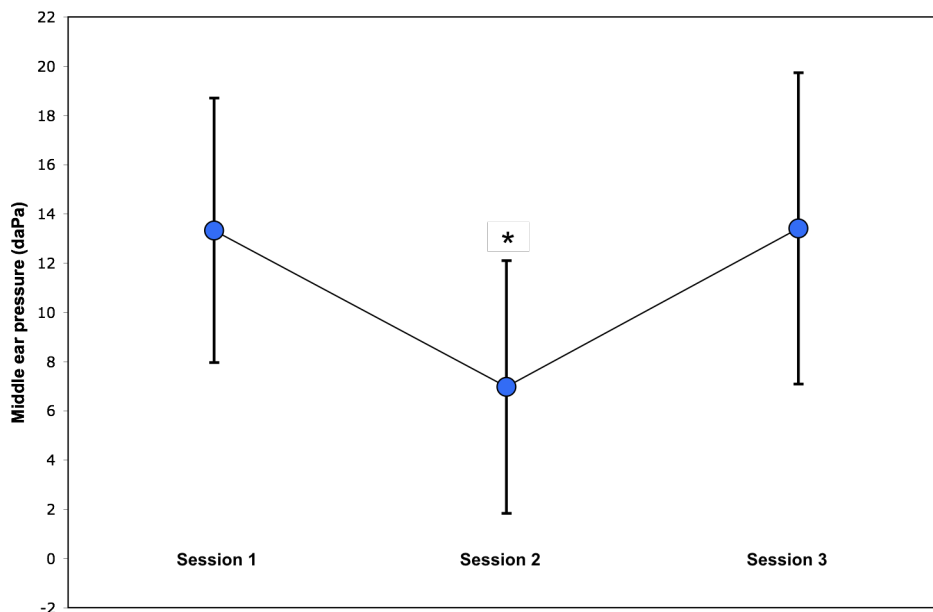


**Figure 9.4.1:** Middle ear pressure (estimated mean and 95 % confidence interval) in the three test sessions. The middle ear pressure in session 2 significantly less compared to session 1. *p < 0.05.

The tympanic membrane compliance was highest in the third test session as seen in Figure 9.4.2, but the change was not significant [LMM, $F_{(2, 41.03)} = 1.7$, $p = 0.2$].

The linear regression analysis demonstrated no significant correlation between the middle ear pressure [$r2 = 0.045$, $F_{(2,49)} = 1.1$, $p = 0.34$] or the tympanic membrane compliance [$r2 = 0.035$, $F_{(2,49)} = 1.3$, $p = 0.43$] and corresponding oestradiol and progesterone across all three sessions.
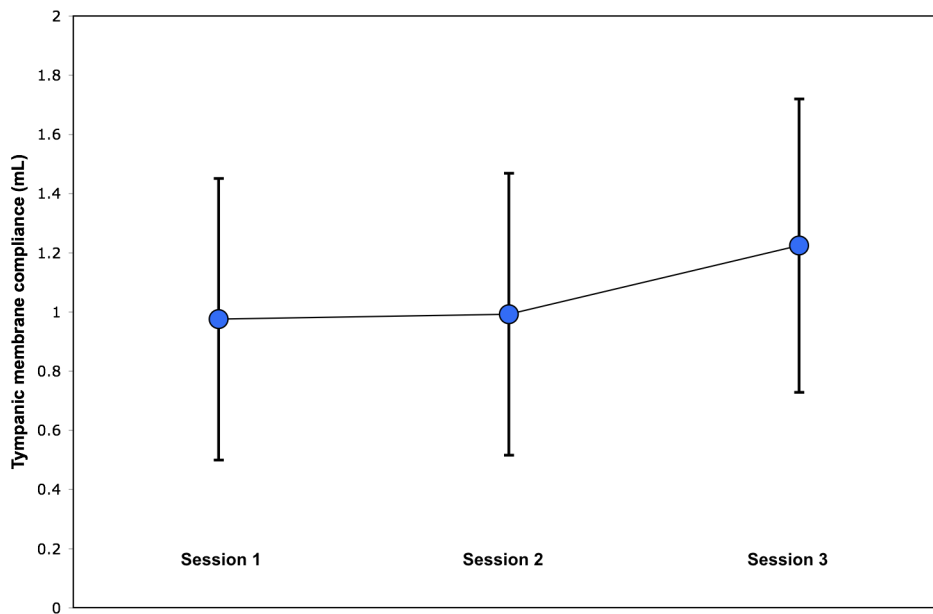
**Figure 9.4.2:** Tympanic membrane compliance (estimated mean and 95 % confidence interval) in the three test sessions.

### 9.4.3 Otoacoustic emissions

#### 9.4.3.1 Spontaneous otoacoustic emissions

SOAE were recorded in 22 of the 28 tested ears (78.6%). Out of the 14 subjects, 11 subjects had recordable SOAE, from both ears.

The number of SOAE spectral peaks recorded during session 2 was greater than in the other two test sessions (Table 9.4-C). Most of the SOAE spectral peaks were between 1-3 kHz (58.6%).

Of all spectral peaks, 118 SOAE peaks were recorded in at least two consecutive test sessions.

**Table 9.4-C:** The number and frequency composition of SOAE spectral peaks during the IVF treatment.

| SOAE Spectral Peaks | Session 1 (ovarian steroid down-regulation) | Session 2 (ovarian stimulation) | Session 3 (post embryo transfer) |
|---|---|---|---|
| **Number** | 115 | 125 | 94 |
| **SOAE frequency** | < 1kHz | 1-3kHz | 3-4kHz | >4kHz |
| proportion | 17.2% | 58.6% | 14.4% | 9.8% |

The SOAE amplitudes were highest during session 2 and lowest during session 3 as seen in Figure 9.4.3. However, the changes did not reach statistical significance [LMM, $F_{(2, 105.2)} = 0.67$, $p = 0.5$].
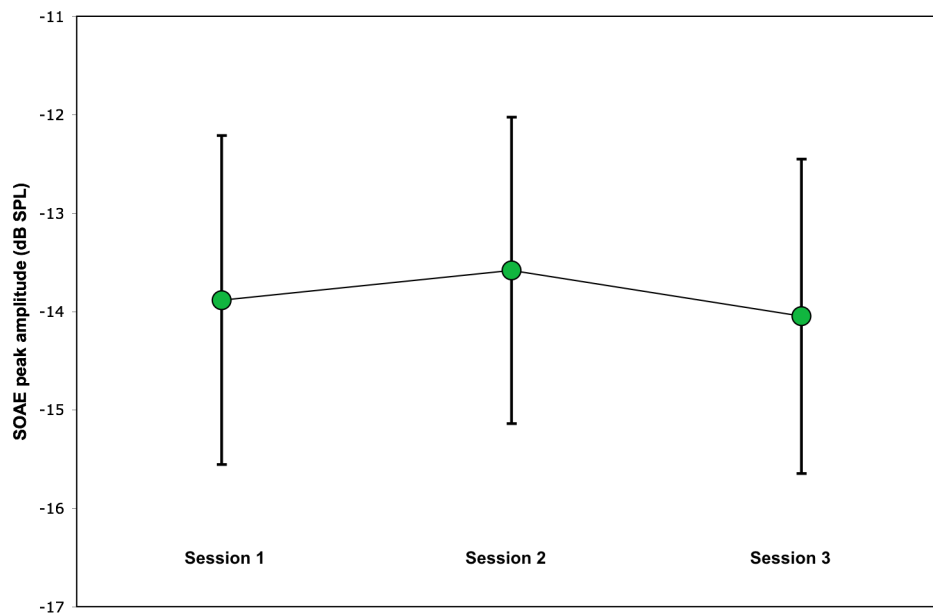


**Figure 9.4.3:** SOAE peak amplitude (estimated mean and 95 % confidence interval) in the three test sessions.

There was a highly significant change in the SOAE frequency shift during the three test sessions [LMM, $F_{(2, 205.9)} = 18.9$, $p < 0.001$]. The SOAE shifted to a higher frequency during session 2 and to a lower frequency during session 3 as seen in Figure 9.4.4. The pairwise comparison of the estimated marginal means revealed that SOAE frequency shift in the session 1 and 2 was significantly greater than in session 3 ($p < 0.001$).
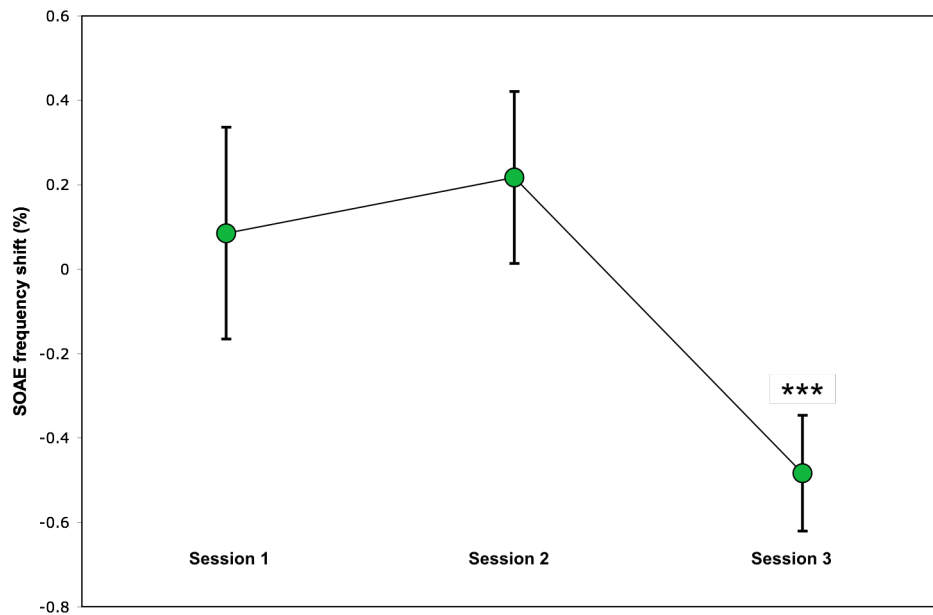


**Figure 9.4.4:** The SOAE frequency shift (mean and 95% confidence interval) in the three test sessions: The SOAE frequency shift in session 3 significantly lower than in session 1 and session 2. *** $p < 0.001$.

The serum oestradiol was added to the LMM as a covariate and was found to have a small positive effect on SOAE amplitude [LMM, oestradiol estimate = $3.8 \times 10^{-5}$ (SE = $7.5 \times 10^{-5}$), df = 68.6, $p = 0.6$], and small negative effect on SOAE frequency shift [LMM, oestradiol estimate = $-2.5 \times 10^{-5}$ (SE = $2.03 \times 10^{-5}$), df = 149.7, $p = 0.2$], during the three test sessions, but did not reach significance. Serum progesterone was added to the LMM as a covariate along with oestradiol and was found to have a small negative effect on SOAE frequency shift [LMM, progesterone estimate = $-0.001$ (SE = $0.002$), df = 118.4, $p = 0.6$] and SOAE amplitude [LMM, progesterone estimate = $-0.018$ (SE = $0.01$), df = 108.7, $p = 0.07$] without reaching significance.

The linear regression analysis revealed that the SOAE frequency shift was significantly correlated with the corresponding oestradiol and progesterone levels across all three sessions [$r^2 = 0.07$, $F(2,218) = 8.2$, $p < 0.001$]. There was a positive correlation with oestradiol level [$r = 0.115$, $p = 0.08$] and a significant negative correlation with progesterone level [$r = -0.263$, $p < 0.001$]. There was a significant correlation between the SOAE amplitude and serum oestradiol and progesterone during the three test sessions [$r^2 = 0.04$, $F(1,218) = 4.5$, $p = 0.01$]. There was a significant positive correlation with oestradiol level [$r = 0.201$, $p = 0.003$] and a negative correlation with progesterone level [$r = -0.06$, $p = 0.34$].

### 9.4.3.2  *Transient evoked otoacoustic emissions*

All 14 subjects had recordable TEOAE from both ears. In total TEOAE were recorded from 28 ears.

The total TEOAE responses increased in session 2 and decreased in session 3, but the change was not statistically significant [LMM, $F(2, 20.7) = 0.86$, $p = 0.44$], as seen in Figure 9.4.5.
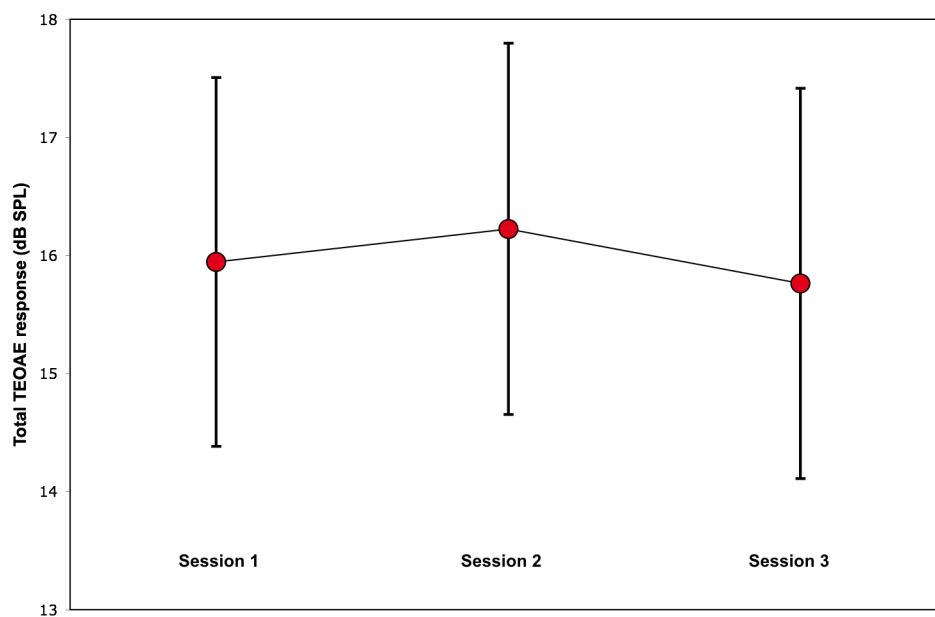


**Figure 9.4.5:** The total TEOAE response (estimated mean and 95% confidence interval) during the three test session

The majority of the tested ears (54.2%) had higher total TEOAE responses in the session 2 compared to session 1.

Similarly, the TEOAE S/N in all five frequency bands were lower in session 3 compared to session 1 or session 2, but the differences were not significant (Table 9.4-D).

**Table 9.4-D:** The estimated mean ± SE for the TEOAE S/N ratio in all ears in the five frequency bands during IVF treatment.

| TEOAE S/N (dB SPL) (n = 28) | Session 1 (Ovarian steroid down-regulation) | Session 2 (Ovarian stimulation) | Session 3 (Post embryo transfer) | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|
| **1 kHz** | 16.12 ± 1.19 | 15.62 ± 1.22 | 15.14 ± 1.16 | $F_{(2, 22.9)} = 2.3$, $p = 0.12$ |
| **2 kHz** | 13.51 ± 0.86 | 13.41 ± 0.96 | 12.9 ± 1.37 | $F_{(2, 18.96)} = 0.14$, $p = 0.87$ |
| **3 kHz** | 12.09 ± 0.98 | 12.12 ± 0.92 | 12.1 ± 0.91 | $F_{(2, 25.6)} = 0.002$, $p = 0.998$ |
| **4 kHz** | 11.09 ± 1.18 | 10.31 ± 1.25 | 10.46 ± 1.32 | $F_{(2, 21.2)} = 1.65$, $p = 0.22$ |
| **5 kHz** | 3.86 ± 1.1 | 4.78 ± 1.1 | 3.67 ± 1.2 | $F_{(2, 20.2)} = 1.45$, $p = 0.26$ |

The serum oestradiol was added to the LMM as a covariate, and was found to have a very small negative effect on the total TEOAE response during the IVF treatment [LMM, oestradiol estimate = -0.0001 (SE = $6.22 \times 10^{-5}$), df = 24.7, p = 0.08] that was not significant. No significant effect was found in TEOAE frequency bands as demonstrated in Table 9.4-E.

**Table 9.4-E:** The linear mixed effect model of the TEOAE response and TEOAE S/N ratio in the five frequency bands. The test session as a fixed factor and oestradiol as a covariate.

| Linear mixed-effect model (n = 28) | Session (fixed effect test) | Oestradiol estimate (SE) | Degrees of freedom (df) | t, p value |
|---|---|---|---|---|
| **Total TEOAE** | $F(2, 19.1) = 2.3$, p=0.13 | -0.0001 $(6.22 \times 10^{-5})$ | 24.7 | -1.8, p = 0.08 |
| **1 kHz** | $F(2, 21.2) = 2.04$, p=0.15 | -0.0001 $(9.82 \times 10^{-5})$ | 29.2 | -1.37, p = 0.18 |
| **2 kHz** | $F(2, 18.7) = 1.4$, p = 0.17 | 0.0003 (0.0002) | 27.1 | 1.9, p = 0.07 |
| **3 kHz** | $F(2, 18.2) = 0.84$, p = 0.45 | $7.78 \times 10^{-5}$ $(7.72 \times 10^{-5})$ | 18.8 | 1.01, p = 0.33 |
| **4 kHz** | $F(2, 19.7) = 3.44$, p=0.05 | 0.0002 (0.0001) | 25.7 | 1.37, p = 0.18 |
| **5 kHz** | $F(2, 20.4) = 0.5$, p=0.62 | $3.66 \times 10^{-5}$ (0.0001) | 29.4 | 0.25, p = 0.81 |

The serum progesterone was added to the LMM as a covariate along with oestradiol, and was found to have a small negative effect on the total TEOAE response during the IVF treatment [LMM, progesterone estimate = -0.006 (SE = 0.007), df = 25.1, p = 0.38] that was not significant. No significant effect was observed in the TEOAE S/N in the five frequency bands as demonstrated in Table 9.4-F.

**Table 9.4-F:** The linear mixed effect model of the TEOAE response and TEOAE S/N ratio in 24 ears in the five frequency bands: the test session as a fixed factor and oestradiol and progesterone as covariates.

| LMM (n = 24) | Session (fixed effect test) | Oestradiol estimate (SE) | df | t, p value | Progesterone estimate (SE) | df | t, p value |
|---|---|---|---|---|---|---|---|
| Total TEOAE | $F_{(1, 23.1)}$ = 0.68, p = 0.52 | -0.0001 (0.0001) | 23.02 | -0.89, 0.38 | -0.004 (0.008) | 23.5 | -0.44, 0.66 |
| 1 kHz | $F_{(1, 22.8)}$ = 1.63, p = 0.22 | -0.0001 (0.0001) | 22.7 | -0.99, 0.33 | 0.0038 (0.01) | 23.1 | 0.36, 0.72 |
| 2 kHz | $F_{(1, 29.3)}$ = 0.06, p = 0.94 | 0.0002 (0.0003) | 29.6 | 0.73, 0.47 | -0.015 (0.021) | 32.7 | -0.68, 0.5 |
| 3 kHz | $F_{(1, 24.5)}$ = 0.04, p = 0.96 | $3.59 \times 10^{-5}$ (0.0002) | 24.3 | 0.17, 0.87 | -0.0002 (0.015) | 25.7 | -0.01, 0.99 |
| 4 kHz | $F_{(1, 23.3)}$ = 0.58, p = 0.57 | $1.48 \times 10^{-5}$ (0.0002) | 23.2 | 0.08, 0.93 | 0.006 (0.013) | 23.6 | 0.49, 0.63 |
| 5 kHz (n=23) | $F_{(1, 25.1)}$ = 0.42, p = 0.66 | 0.0001 (0.0002) | 25.1 | 0.56, 0.58 | -0.016 (0.017) | 26.3 | -0.91, 0.37 |

The Wilcoxon Signed-rank test of TEOAE inter-session differences calculated by the ILO compare analysis showed that the inter-session difference in the TEOAE responses between session 2 and session 3 was greater that the inter-session difference in TEOAE responses between session 1 and session 2 as shown in Figure 9.4.6, but the difference did not reach statistical significance (p = 0.07).
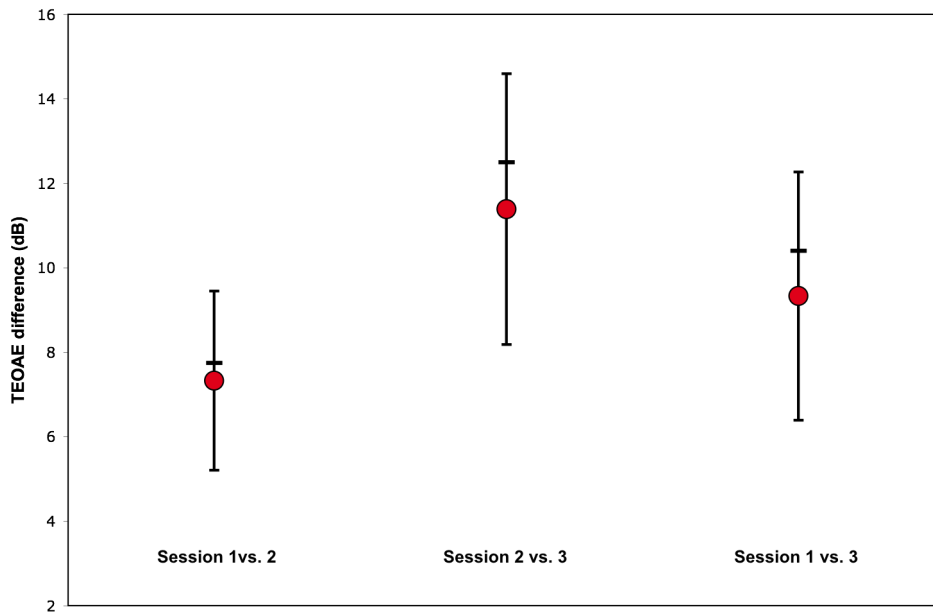
**Figure 9.4.6:** The differences in TEOAE as calculated by the ILO subtraction analysis between the different testing sessions (mean and 95% confidence interval). The bar (**-**) indicates the median value.

The linear regression analysis showed that the total TEOAE responses were not significantly correlated with the corresponding oestradiol and progesterone levels across the three sessions [$r^2 = 0.08$, $p = 0.14$, $F(2, 47) = 2.05$]. The correlation with oestradiol was significantly positive [$r = 0.3$, $p = 0.049$], while the correlation with progesterone was negative [$r = -0.07$, $p = 0.6$] but not significant. The TEOAE S/N in all five frequency bands were not significantly correlated with the corresponding oestradiol and progesterone levels across the three sessions.

### 9.4.4 Medial olivocochlear (MOC) suppression

The MOC suppression recorded from the 28 ears, decreased slightly during session 2 and session 3, but the change was not significant [LMM, $F(2, 15.7) = 0.98$, $p = 0.4$], as seen in Figure 9.4.7.
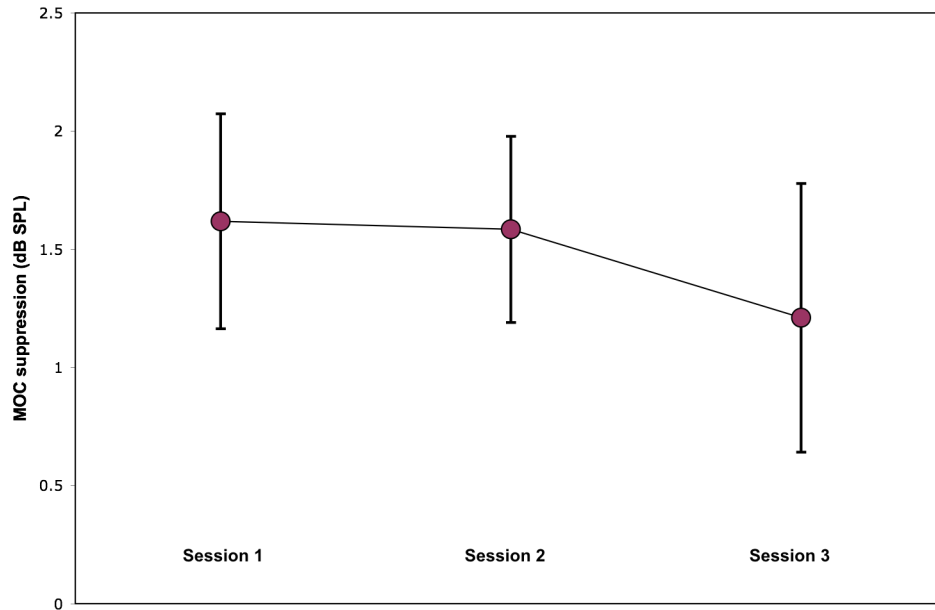
**Figure 9.4.7:** The MOC suppression (mean and 95% confidence interval) in the three test sessions.

The serum oestradiol was added to the LMM as a covariate, and was found to have a small positive effect on MOC suppression [LMM, oestradiol estimate = $7.13 \times 10^{-5}$ (SE = $4.94 \times 10^{-5}$), df = 31.96, p = 0.16]. The serum progesterone was added to the LMM as a covariate along with oestradiol, and was found to have a small negative effect on the MOC suppression during the three test sessions. [LMM, progesterone estimate = -0.0036 (SE = 0.006), df = 27.9, p = 0.54] that was not significant.

The linear regression analysis showed that the MOC suppression was not significantly correlated with the corresponding oestradiol levels and progesterone across the three test sessions [$r^2 = 0.05$, p = 0.3, F(1,47) = 1.19]. The correlation with oestradiol was positive [r = 0.2, p = 0.15], while the correlation with progesterone was negative [r = -0.12, p = 0.41] but not significant.

### 9.4.5   Auditory brainstem response

The ABRs were recorded from both the right and left ear in 13 subjects (26 ears), one subject from the 13 did not have ABR recoded in her last test only (Table 9.3-A).

**Table 9.4-G:** The estimated mean ± SE of ABR wave latencies and interpeak intervals during the three test sessions.

| ABR (n = 26) | Session 1 (ovarian steroid down-regulation) | Session 2 (ovarian stimulation) | Session 3 (post embryo transfer) | Linear mixed-effect model (fixed effect) |
|---|---|---|---|---|
| I | 1.64 ± 0.02 | 1.64 ± 0.02 | 1.59 ± 0.02 | $F(2, 15.4) = 3.18$, $p = 0.07$ |
| III | 3.76 ± 0.03 | 3.76 ± 0.03 | 3.74 ± 0.03 | $F(2, 19.9) = 0.33$ $p = 0.72$ |
| V | 5.6 ± 0.04 | 5.65 ± 0.04 | 5.58 ± 0.04 | $F(2, 16.2) = 6.6$, $p = 0.008$ |
| I-III | 2.12 ± 0.03 | 2.14 ± 0.03 | 2.15 ± 0.03 | $F(2, 16.3) = 1.05$, $p = 0.37$ |
| III-V | 1.84 ± 0.03 | 1.88 ± 0.03 | 1.83 ± 0.03 | $F(2, 19.2) = 2.97$, $p = 0.07$ |
| I-V | 3.96 ± 0.04 | 4.02 ± 0.04 | 3.98 ± 0.04 | $F(2, 16.3) = 4.7$ $p = 0.02$ |

The LMM analysis suggests that the absolute wave latencies were shorter in session 3 and longer during session 2 (Table 9.4-G), with the change being significant for the Wave V latency [LMM, $F(2, 16.2) = 6.6$, $p = 0.008$] and the I-V interpeak interval [LMM, $F(2, 16.3) = 4.7$, $p = 0.02$] . The pairwise comparison revealed that the Wave I latency was significantly shorter in session 3 compared to session 1 ($p = 0.03$) and session 2 ($p = 0.047$). The Wave V latency was significantly longer in the second session compared to the first ($p = 0.005$) and third test session ($p = 0.01$). The pairwise comparison on the interpeak intervals demonstrated that the I-V interpeak interval in the second session was significantly longer compared to the first session ($p = 0.006$). The III-V interpeak

interval in the second session was longer than in third session, but the difference did not reach significance (p = 0.054).

The serum oestradiol was added to the LMM as a covariate, and was not found to have any significant effect on the ABR as demonstrated in Table 9.4-H.

**Table 9.4-H:** The linear mixed effect model of the ABR wave latencies and interpeak intervals with the test session as a fixed factor and oestradiol as a covariate.

| Linear mixed-effect model (n=26) | Session (fixed effect test) | Oestradiol estimate (SE) | Degrees of freedom (df) | t, p value |
|---|---|---|---|---|
| I | $F(2, 16.7) = 2.99$, $p = 0.08$ | $-3.45 \times 10^{-6}$ $(3.4 \times 10^{-6})$ | 24.3 | $-1.01$, $p = 0.32$ |
| III | $F(2, 13.9) = 1.07$, $p = 0.37$ | $3.22 \times 10^{-6}$ $(3.5 \times 10^{-6})$ | 17.8 | $0.93$, $p = 0.37$ |
| V | $F(2, 14.7) = 2.62$, $p = 0.11$ | $-1.62 \times 10^{-6}$ $(5.2 \times 10^{-6})$ | 24.3 | $-0.31$, $p = 0.76$ |
| I-III | $F(2, 18.4) = 0.84$, $p = 0.45$ | $3.45 \times 10^{-6}$ $(4.4 \times 10^{-6})$ | 22.1 | $0.77$, $p = 0.45$ |
| III-V | $F(2, 18.05) = 1.9$, $p = 0.17$ | $-4.75 \times 10^{-7}$ $(4.98 \times 10^{-6})$ | 19.4 | $-0.09$, $p = 0.92$ |
| I-V | $F(2, 15.7) = 1.96$, $p = 0.17$ | $1.3 \times 10^{-7}$ $(5.1 \times 10^{-6})$ | 25.1 | $0.03$, $p = 0.98$ |

The serum progesterone was added to the LMM as a covariate along with oestradiol, and was found to have a small positive effect on ABR wave latencies and interpeak intervals as seen in Table 9.4-I. The effect was significant only on the Wave III and Wave V latencies (p = 0.003 and p = 0.004 respectively).

**Table 9.4-I** The linear mixed effect model ABR wave latencies and interpeak intervals with the test session as a fixed factor and oestradiol and progesterone as a covariates.

| LMM (n=22) | Session (fixed effect test) | Oestradiol estimate (SE) | df | t, p value | Progesterone estimate (SE) | df | t, p value |
|---|---|---|---|---|---|---|---|
| I | $F_{(2, 16.4)}$ = 2.3, p = 0.1 | $-4.58\times10^{-7}$ $(3.7\times10^{-6})$ | 12.7 | -1.2, 0.24 | 0.0005 (0.0005) | 16.4 | 1.06, 0.31 |
| III | $F_{(2, 13.4)}$ = 10.3, p = 0.002 | $-2.65\times10^{-6}$ $(3.5\times10^{-6})$ | 8.4 | -0.7, 0.47 | 0.001 (0.0003) | 12.4 | 3.7, 0.003 |
| V | $F_{(2, 11.2)}$ = 9.3, p = 0.004 | $-3.5\times10^{-6}$ $(4.2\times10^{-6})$ | 8.5 | -0.8, 0.43 | 0.0015 (0.0004) | 7.2 | 4.2, 0.004 |
| I-III | $F_{(2, 19.6)}$ = 0.35, p = 0.7 | $-1.5\times10^{-6}$ $(5.6\times10^{-6})$ | 14.5 | -0.3, 0.79 | 0.0006 (0.0005) | 17.1 | 1.3, 0.21 |
| III-V | $F_{(2, 15.5)}$ = 0.96, p = 0.4 | $-6.68\times10^{-7}$ $(5.6\times10^{-6})$ | 12.8 | -0.1, 0.91 | 0.0004 (0.0005) | 12.8 | 0.86, 0.4 |
| I-V | $F_{(2, 17.1)}$ = 1.6, p = 0.23 | $-1.98\times10^{-6}$ $(6.6\times10^{-6})$ | 15.4 | -0.3, 0.77 | 0.0009 (0.0007) | 14.8 | 1.23, 0.24 |

The regression analyses between ABR latencies or interpeak intervals with oestradiol and progesterone levels across the three sessions were not significant.

## 9.5 Summary of results

The study had examined several aspects of auditory function with simultaneous measurements of serum oestrogen and progesterone levels in a group of women undergoing assisted conception treatment, IVF.

The serum oestradiol and progesterone levels were not measured in some subjects as mentioned in Table 9.4-A and Table 9.4-B and, however their auditory tests were undertaken following an ultrasound assessment, which had confirmed, although less accurately than hormone analysis, that they were in the corresponding stage of their treatment as described in the protocol (section 9.2).

The results of the serum oestradiol in the three test sessions were as expected with oestradiol levels being highest during the second test session following ovarian stimulation. The progesterone levels were highest during the third test session after embryo transfer as a result of progesterone administration and possible pregnancy in two subjects who had the highest progesterone levels (Howles & Macnamee, 1990).

The progesterone levels in two of the volunteers (subject 9 and 11 as seen in Table 9.4-B) following ovarian stimulation (session 2) was higher than expected ( serum progesterone was 7 and 10 nmol/L respectively). Serum progesterone levels following ovarian down-regulation and ovarian stimulation are not expected to rise above 5 nmol/L, before ovarian rupture (Djahanbakhch, et al., 1981). However, the rise was small and was not expected to have a major effect on the results, as the corresponding oestradiol levels were high (11241 and 11674 pmol/L respectively as seen in Table 9.4-A).

**Tympanometry**

The middle ear pressure significantly changed during the three test sessions [LMM, $F_{(2, 19.8)} = 4.4$, $p = 0.03$], and was significantly lower following ovarian stimulation (session 2). However, the middle ear pressure was still within the normal middle ear pressure range (-50 to +50 daPa, Section 1.2.2), and not expected to have an effect on OAE results (discussed in Section 10.1.1) .

The tympanic membrane compliance did not change significantly during the repeated testing.


**Cochlear modulation**

The results of the otoacoustic emissions showed very subtle changes in cochlear function during assisted conception treatment. Greater number of SOAE peaks was recorded during ovarian steroid down regulation and ovarian stimulation (sessions 1 and 2) when oestrogen was the main hormone, and progesterone levels were low. The number of SOAE peaks was reduced post embryo transfer (session 3) where progesterone levels are higher.

The SOAE frequencies significantly shifted during the three test sessions ($p < 0.001$). The SOAE frequency shifted to a higher frequency during ovarian stimulation (session 2), when oestrogen levels were highest, and to a lower frequency post embryo transfer (session 3) when progesterone levels were greater. The results suggested that oestrogen led to a positive shift in SOAE frequency, while progesterone shifted the SOAE frequency in the opposite direction. There was no significant change in the SOAE amplitude in contrast to the change noted in SOAE frequency as was observed during the ovarian cycle. However, the amplitude following ovarian stimulation (session 2) was higher than the during post embryo transfer (session 3).

The TEOAE response and the TEOAE S/N in all five frequency bands tend to be lower post embryo transfer (session 3) compared to the other two sessions and slightly higher after ovarian stimulation (session 2), but the changes were not significant. The TEOAE inter-session differences calculated by the ILO subtraction analysis revealed that there was a greater difference in TEOAE levels between the sessions when oestrogen levels is highest (session 2) and the session when progesterone levels is high (session 3) but the change was not statistically significant.

There was a significant positive correlation between oestrogen levels and the overall TEOAE responses during the three test sessions. The regression analysis suggested that oestrogen increased, while progesterone decreased the TEOAE

responses. However, from the LMM analysis, the effect of oestradiol was mainly positive (i.e. increases TEOAE S/N) in the higher frequency bands, and negative (i.e. decreases TEOAE S/N) in the lower frequency bands (Table 9.4-F and Table 9.4-G). This dual effect may explain absence of a clear change during the repeated testing of the overall TEOAE response.

**Olivocochlear suppression**

The results showed that olivocochlear suppression was lowest during post embryo transfer (session 3) but the change was not significant. Oestrogen effect on the MOC suppression was found to be a positive one, where the MOC suppression tended to increase with an increase in oestradiol levels, but the effect was small [oestradiol estimate = $7.13 \times 10^{-5}$]. Progesterone, on the other hand, tended to be associated with a decrease the MOC suppression [progesterone estimate = -0.0036]. However the effect was not significant, but it may be sufficient to blunt the positive effect of oestrogen.

**Auditory brainstem evoked responses**

Significant changes were observed in the Wave I and V absolute latencies, with the longer latencies during ovarian stimulation (session 2) when the oestradiol levels were highest. The III-V and I-V interpeak intervals were also longer during ovarian stimulation.

The regression analysis revealed that the increase in ABR latencies was not associated with oestradiol levels. The effect of oestradiol was mainly negative but very small, while progesterone effect was positive (Table 9.4-I), which seems to be contradictory to the observed ABR latencies.

# Chapter 10 : Discussion

The results of the studies demonstrated that subtle changes in auditory function are associated with the fluctuation of the ovarian steroids during the natural ovarian cycle and in pharmacologically controlled cycles in women undergoing assisted conception treatment. These changes were not observed in men over a similar period of time.

## 10.1 The changes in auditory function

### 10.1.1 Tympanometry

The middle ear pressure and tympanic membrane compliance did not change during the repeated testing in women throughout the natural ovarian cycle (section 7.4.2). However, the middle ear pressure was lower during ovarian stimulation in women undergoing assisted conception treatment, but still within the normal range (section 9.4.2).

The supraphysiological levels of oestradiol seen in ovarian stimulation may be associated with greater capillary permeability (Rizk & Aboulghar, 1999) and thus lead to congestion and oedema in the middle ear or effect the Eustachian tube function leading to lower middle ear pressure. However, the lower middle ear pressure recorded was still within the normal range (section 1.2.2) . The change was small and can be compared to the slight non-significant decrease in Eustachian tube function with higher oestrogen levels observed by Nir et al (1991).

Therefore it can be assumed that the small changes in tympanometry observed in the study had no mechanical interference on the middle ear transducer function and thus did not have an effect on the properties of OAE transduction (Trine, et al., 1993; Johansson & Arlinger, 2003).

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There was no gender difference in middle ear pressure, but the tympanic membrane compliance was significantly larger in men by about 0.3 ml.

### 10.1.2 Cochlear modulation

The cochlear function was evaluated using OAE, including TEOAE and SOAE. In general, the higher levels of both classes of OAE reflect higher cochlear gain and they are associated with better hearing sensitivity (Kemp, et al., 1990; Probst, et al., 1991; Hurley & Musiek, 1994). The presence of SOAE, reflect exquisite hearing sensitivity that correspond to the best thresholds at homologous frequencies (Probst et al., 1987; Bonfils, 1989).

In agreement with the literature, the prevalence of SOAE were greater in the female ears (70.3%) than in the male ears (36.7%), and the TEOAE responses were significantly larger in female ears (section 8.4.3.2).

In women, the number of SOAE peaks were more prevalent in women in the first two test sessions of the natural ovarian cycle (Table 7.4-B) and during assisted conception treatment (Table 9.4-C) when oestrogen was the dominate hormone, and were less prevalent during the third session in both groups, when the levels of progesterone were highest. These changes were similar to Yellin and Stillman's (1999) findings of a greater number of SOAE early in the cycle and less near the end. The other studies in the literature that examined SOAE in relation to the menstrual cycle (Bell, 1992; Haggerty, et al., 1993; Penner, et al., 1994; Penner, 1995), did not report any changes in the number of SOAE spectral peaks, but concentrated on frequency changes, which seemed to change in relation to the cycle.

Significant changes in the frequency shift of SOAE, were noted in women but not in the men during repeated testing. These results were similar to those found by Bell (1992) and Haggerty and colleagues (1993) in their subjects, in whom no variations in SOAE frequency were reported in male subjects but a change was found in the female subjects.

The SOAE shifted to a higher frequency when oestradiol levels were highest (session 2 in both the natural ovarian cycle and after ovarian stimulation) and then shifted to a lower frequency when progesterone was present (luteal phase of the ovarian cycle and post embryo transfer). These findings are in a similar direction to those found by Bell (1992), Haggerty et al (1993), Penner et al (1994) and Penner (1995). The negative effect of progesterone on the SOAE frequency shift was greater than the positive effect of oestrogen during the luteal phase of the ovarian cycle (the approximate progesterone effect was -0.004 %, while the oestradiol effect was 0.0002 %). The same effect was identified during assisted conception treatment (the approximate progesterone effect was -0.001 % while the oestradiol effect was $-2.5 \times 10^{-5}$ %). These findings suggest that the SOAE frequency shift to a lower frequency during the luteal phase of the natural ovarian cycle and following progesterone supplementation in assisted conception treatment is probably due to the effect of progesterone.

The SOAE amplitudes were higher in the follicular phase compared to the luteal phase of the ovarian cycle and following ovarian stimulation compared to post embryo transfer during assisted conception treatment. However, the change in SOAE amplitudes were not statistically significant. A possible explanation is that SOAE amplitudes were more variable than SOAE frequency (reviewed by Ceranic, 2003). Previous studies on SOAEs during the menstrual cycle (section 3.1.4.1) did not report SOAE amplitudes.

The TEOAE responses were larger in women compared to men. It may be hypothesised that the gender difference in the overall TEOAE amplitude is mainly due to the difference in the responses seen in the 2-5 kHz frequency bands and not in the 1 kHz frequency band. This finding may also explain the reported observation that women have more sensitive hearing in the higher frequencies compared to men (reviewed by Velle, 1987; McFadden, 1993; Davis, 1995).

The results of OAE suggest more sensitive hearing in women than in men. This could be, in part, due to sexual dimorphism in the cochlea and CNS (section 2.1.3). Another contributing factor for the lower OAE in men is the higher middle

ear compliance that has been associated with lower the levels of TEOAE (Johansson & Arlinger, 2003).

The overall TEOAE response and the TEOAE S/N ratio in the five frequency bands were slightly greater in the test sessions, in which oestrogen levels were highest, and were lower when progesterone was present. However the changes were not significant. As mentioned above (section 3.1.4.1), only a few studies evaluated TEOAE during the ovarian cycle, and two of them did not report any changes (Yellin & Stillman, 1999; Arruda & Silva, 2008). Amit and Animesh's (2004) finding of lower TEOAE responses during the mid and luteal phase of the ovarian cycle compared to the menses phase, may have been confounded by possible cochlear dysfunction in their subjects (section 3.1.4.1 and Table 3.1-A).

The TEOAE inter-session differences calculated by the ILO subtraction analysis demonstrated a greater difference in TEOAE levels between the sessions in which oestrogen was highest (late follicular phase in the natural ovarian cycle and following ovarian stimulation in assisted conception treatment) and the sessions where progesterone levels were highest (luteal phase of the ovarian cycle and post embryo transfer in assisted conception treatment). These changes were not observed in the male subjects, suggesting a possible hormonal influence on TEOAE.

The changes seen in the SOAE and the greater inter-session differences in TEOAE suggest a hormonal effect and that oestrogen and/or progesterone may play a role in cochlear function, because these changes were not seen in men.

The interpretation of SOAE shift in terms of auditory processing is speculative, as SOAE generation is poorly understood and physiological significance still unclear. Nevertheless, SOAE are very sensitive indicators of active cochlear processes and their shift may reflect a change in cochlear transducer operating point. In addition, it may reflect changes in frequency sensitivity that may be similar to those which were found by the change in auditory sensitivity in the midshipman fish when treated with oestrogen (Sisneros & Bass, 2003).

Women undergoing assisted conception treatment have displayed changes in auditory function tests similar to those observed during the natural ovarian cycle. A greater variation in OAE was hypothesised in women undergoing assisted conception treatment (section 4.3), but was not observed. One possible explanation is that the supraphysiological levels of oestradiol may lead to greater capillary permeability (Rizk & Aboulghar, 1999) and thus in theory may also have an effect on the fluid and electrolyte balance of the cochlear fluids. Changes in the cochlear homeostasis may affect the cochlear hair cell function, and thus diminish the TEOAE responses. There may also be an effect of the sample size in these two studies.

### 10.1.3  Olivocochlear suppression

Cochlear function is modulated by the complex efferent feedback system, a part of which is the medial olivocochlear (MOC) system. Although the MOC system is considered to be mainly inhibitory (Wiederhold, 1986), the presence of the receptors for excitatory (cholinergic) and suppressive (GABA-ergic) neurotransmitters at the MOC terminals to the OHCs (Altschuler & Fex, 1986; Plinkert, et al., 1993; Puel, 1995), reflects the complex function of this system, which may be modulated by both oestrogen and progesterone (section 1.3.2). In the quiet background, the MOC system displays a predominately suppressive effect, but when there is efferent activity in a noisy background, they exhibit an enhancement of the transient response (Kawase, et al., 1993) . This is in agreement with the limited knowledge on the roles of the olivocochlear system, which include signal detection and discrimination (Guinan, 2006).

MOC suppression was lower in women compared to men, but without reaching significance. Durante and Carvalho (2002) noted that the contralateral suppression of TEOAE was significantly greater in male neonates compared to female neonates. However, Ferguson and co-workers (2001) did not find any gender difference in TEOAE suppression in a group of adults, while other studies have not reported a gender difference in MOC suppression (Williams, et al., 1994; Hood, et al., 1996; Abdala, et al., 1999; De Ceulaer, et al., 2001). The MOC suppression test is still not widely used in the clinical setting and there are

different methodologies in research laboratories, which may explain the conflicting findings.

MOC suppression significantly changed during the natural ovarian cycle, but not during the assisted conception treatment or in men. During the ovarian cycle, the MOC suppression decreased in the late follicular phase, but did not change after ovarian stimulation. Suppression was negatively correlated with oestradiol levels during the follicular phase of the ovarian cycle, and was positively correlated with oestradiol during the assisted conception treatment.

Oestrogen is known as a modulator of the acetylcholine system, which is one of the major neurotransmitters of the MOC bundle that leads to inhibition of the cochlear amplifier. GABA is also involved and is known to be modulated in other parts of the CNS by both oestrogen and progesterone.

The lower suppression during the ovarian cycle could relate to the negative effect of oestrogen on GABA that can occur at higher levels of oestrogen. The supraphysiological levels of oestrogen in the assisted conception treatment may have a greater effect on the acetylcholine system and, thus, increase suppression. Oestrogen has a dual effect on the GABA system in the CNS (section 2.2.1.1), and higher levels of oestrogen are associated with attenuation of GABA production leading to the LH surge (Wagner, et al., 2001). However, during ovarian stimulation, GnRH analogues were administered to counteract the effect of oestrogen and to suppress the LH production, and this may involve the GABA-ergic system. GnRH analogues have been used in treatment of epilepsy triggered by menstruation (catamenial epilepsy), which involves the GABAergic system (Bauer, et al., 1992; Herzog, 2009; Reddy, 2009).

### 10.1.4 Auditory brainstem evoked responses

The ABR latencies and interpeak intervals were significantly shorter in women compared to men except for the I-III interpeak interval. Previous studies comparing ABR parameters in women and men during the ovarian cycle have reported significantly shorter wave III and V absolute latencies in women (Fagan

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& Church, 1986; Dehan & Jerger, 1990), while wave I latency was shorter in women, but the difference did not reach significance. Elkind -Hirsch et al (1994) only described the wave V latency, which was shorter in normal reproductive women. Fagan and Church (1986) reported that only the I-III interpeak interval was significantly shorter in women, while no difference was noted in the III-V and I-V interpeak interval. On the other hand, Wharton and Church (1990), found that all interpeak intervals including the III-V and I-V were shorter in young females compared to young men. Dehan and Jerger (1990) and Elkind-Hirsch et al (1994) did not report the interpeak intervals in their studies. These conflicting findings may be the result of different methodologies.

Dehan and Jerger (1990) and Elkind-Hirsch et al (1994) measured the serum hormone levels in women to confirm the hormonal changes that occur in the ovarian cycle and noted that wave V latency was significantly longer near the time of ovulation, which was also noted in our study, while no changes were seen in the men. The shorter ABR latencies in women may be attributed to the excitatory effect of oestrogen and sexual dimorphism in the CNS (Cahill, 2006).

The Wave I and Wave V latencies significantly changed during the ovarian cycle and during assisted conception treatment. Longer latencies were observed when oestradiol levels were the highest (late follicular phase in the natural ovarian cycle, and following ovarian stimulation in the assisted conception treatment) and were shorter when progesterone was present and when both ovarian steroids were lowest at the end of the ovarian cycle. A significant lengthening of the wave V peak latency during the mid ovarian cycle and the shorter wave V latency during the luteal phase was also reported by Elkind-Hirsch et al (1992a), in a study in which the ovarian cycle was also defined by measurements of oestradiol.

There was no strong association between oestradiol or progesterone levels with ABR latencies (see Table 7.4-H, Table 7.4-I, Table 9.4-H and Table 9.4-I). While oestrogen, generally, has an excitatory effect and it is expected to facilitate transmission of the auditory signals, on the other hand, the higher levels of oestrogen in the late follicular phase and ovarian stimulation are possibly associated with higher levels of neurosteroids, especially allopregnalone (Stomati,

et al., 2002; Bernardi, et al., 2003), which is a potent GABA-A receptor agonist (Majewska, et al., 1986; Baulieu, 1998) and may have an inhibitory effect on the auditory brainstem (Disney & Calford, 2001). The neurosteriods may have a greater inhibitory effect on the auditory brainstem compared to the small excitatory effect of oestrogen.

## 10.2 Potential effects of ovarian steroids on auditory function

The gender differences in auditory function and the correlation between some of the auditory function tests with oestradiol and progesterone observed in this thesis, suggest that ovarian steroids have an effect on the auditory system as was suggested from the previous literature. The following paragraphs consolidate the study observations with the previous physiological literature.

At the cochlear level, the effect of oestradiol may be more direct, due to the presence of the oestrogen receptors in the sensory and non sensory areas of the cochlea (see section 2.2.1.1). The changes observed in otoacoustic emissions, which reflect cochlear function, were associated with higher levels of oestradiol and may be evidence of this effect. However, the effect of oestrogen may be two fold: excitatory at one level, such as the cochlea but inhibitory at another, as seen in the auditory brainstem. Other confounding effects may dampen the excitatory effect of oestrogen. The supraphysiological levels of oestrogen in ovulation induction may have a congestive effect by increasing capillary permeability (Rizk & Aboulghar, 1999), and thus the total TEOAE response may not be as high as expected.

The effect of oestrogen in the proximal and central parts of the auditory system is possibly more complex. The results of the ABR show, that oestrogen has an inhibitory effect, as demonstrated by longer ABR latencies during the late follicular phase and ovulation stimulation. A similar finding was observed during the breeding season in sparrows were oestradiol levels were highest (Caras, et al., 2010). The mechanism behind these longer latencies is still speculative but may involve other neurosteroids and not be directly due to oestrogen (see section 10.1.4), or alternatively GnRH (section 9.1), which has been reported to increase

auditory thresholds in fish (Maruska & Tricas, 2011) and may in theory, influence the auditory system in other vertebrates as well.

The progesterone effect on the auditory system seems to be more inhibitory as demonstrated by the mainly negative correlation with auditory function tests reported in the above studies, and supported in the literature (section 3.1.4.2, 3.1.5, and 3.1.6). The presence of a higher level of progesterone seems to counteract the effect of oestrogen, both in the cochlea and brainstem as demonstrated in the results of these studies.

Ovarian hormones may play a role in maintaining homeostasis in the auditory system, and the rapid decline in the levels of both hormones at the end of the ovarian cycle (late luteal phase) may alter GABA function, leading to lower inhibition (Maguire & Mody, 2009; Gangisetty & Reddy, 2010), and, thus, may explain the lower MOC suppression and shorter ABR latencies observed during the ovarian cycle (section 7.4.4 and 7.4.5). This may also partly explain some of the auditory symptoms or pathologies observed in some women associated with the ovarian cycle (section 3.1.4.2 and 3.2) or following the menopause (section 3.1.6).

The changes observed in auditory function associated with oestrogen and/or progesterone may also be associated with more central auditory processes in the auditory cortex, which may, in turn influence the peripheral auditory system. Oestrogen has been found to play an important role in modulating auditory processing in birds (reviewed by Maney & Pinaud, 2010) and improved the ability to discriminate communication signals (Tremere & Pinaud, 2011). These findings may in theory also be relevant in other vertebrates, including humans.

## 10.3 Conclusion

This thesis presents for the first time a detailed correlation of auditory function tests and precise levels of female reproductive hormones.

The findings suggest that ovarian steroids influence the auditory system. The difference in the auditory function tests between men and women suggest better hearing sensitivity in women. The inter-session differences seen in women, but not in the men, suggest that the auditory system is sensitive to the fluctuations in ovarian steroids during the ovarian cycle and may alter the frequency sensitivity of the cochlea, and thus contribute to the observed gender differences in auditory function.

The effect of oestrogen is mainly excitatory, while progesterone is mainly inhibitory and balances the effect of oestrogen. The decline in the levels of both hormones may be associated with greater excitability due to loss of tonic inhibition by the ovarian steroids.

## 10.4 Study limitations:

A number of limitations were recognized, the major ones are listed below:

- The difficulty in precisely monitoring the natural ovarian cycle confounded the results. As seen in Figure 7.4.1, even though all subjects reported regular menstrual cycles, the results of the hormone tests suggest that the ideal day that was planned to test to coincide with the peak of oestradiol or progesterone may have been missed.

- Progesterone levels were not measured in the first two test sessions of the ovarian cycle and in a number subjects during the assisted conception treatment. These levels might have allowed evaluation of the possible effect of progesterone during the whole ovarian cycle and assisted conception treatment, and might have revealed that some of the subjects ovulated before the expected time.

- The number of patients who took part in the last study was relatively small, due to difficulty in recruiting and retraining women in the study and time restraints.

- The power of the study has been affected by the missing data from some of the subjects. The use of LMM analysis was helpful in minimizing the effect, due to its ability to handle all available data so not to lose the subjects with missing results.

However the results obtained show a trend, which requires confirmation.

## 10.5 Suggested further studies:

- Auditory function in pregnant women during the three trimesters and post partum. The higher levels of both hormones during pregnancy may have a different effect on auditory function and also the levels stay stable over a longer period. The levels of both hormones drop dramatically postpartum and thus may effect auditory function, similar to that which is seen at the end of the ovarian cycle.

- Auditory function in post menopausal women pre and post hormone replacement therapy with monitoring of the hormone levels.

- Monitoring of auditory function in women with auditory pathology, such as Menière disease or tinnitus during their ovarian cycle with measurement of the ovarian steroids levels.

- Central auditory function in women during their ovarian cycle. This study may provide more information about the effect of ovarian steroids on the auditory system.

- Evaluating auditory function in women with pre-menstrual syndrome with measurement of the ovarian steroids levels.

- Measuring the sex hormone binding globulin (SHBG) along with the oestradiol serum levels (section 2.2.1.1), to calculate the bioactive oestradiol level (Sodergard, et al., 1982; Rinaldi, et al., 2002). This may

help in providing a more accurate reflection of the possible correlation between oestradiol and auditory function tests.

These studies along with the recent animal studies may provide potential pharmacological treatments for women suffering from auditory pathology and a greater understanding of the auditory system.

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# Appendix I:    Subject Questionnaire

## *A Study of the Effect of Ovarian Hormones on Auditory Function*

**Name:**                                      **Code:**
**Date of Birth:**
**Profession:**

## *Menstrual History:*

*Age of menarche:*

*Regular cycle:*          Yes     No

*Length of cycle*

*Did you have menstrual irregularities in the past?*   Yes     No

*If yes, was it due to a hormonal problem?*          Yes     No

*Did you receive hormonal treatment?*               Yes     No

*Do you experience symptoms during your cycle?*   Yes     No

*Describe (esp. hearing)*

*Are you using the Pill?*          Yes     No

*Did you take it in the last 3 months?*  Yes     No

## *Medical History:*

Drug history:

Allergic history:

Do you have migraines?        Yes     No

If Yes, are they related to your cycle?

Other medical conditions:

### *Family History:*

*Hormonal irregularities in the family:*     Yes     No

*Family history of HL:*          Yes     No

*Family history of migraine:*   Yes     No

### *Auditory History\*:*

*Fullness in the ear:*   Yes     No

*Tinnitus:*       Yes     No

*Hyperacusis:*   Yes     No

*Distortion:*     Yes     No

*Change in hearing sensitivity:*          Yes     No

*Related to cycle?*     Yes     No

\*ask at each session

# Appendix II:    Subject Information Sheets

The following pages are copies of the information sheets for the study volunteers.

# University College London Hospitals NHS

NHS Trust
**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
Box 127,
Queen Square, London
WC1N 3BG

Telephone: 020 7837 3611
Fax: 020 7829 8775

CONFIDENTIAL
INFORMATION SHEET FOR HEALTHY VOLUNTEER

## *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

*Version 2, 6th November 2002*

We would like to ask you to participate in this research project, the purpose of which is to investigate the effect of female hormones on hearing.

Many healthy women experience changes in hearing related to the menstrual cycle, pregnancy or the menopause. Similarly, some patients with inner ear/hearing problems have also reported changes in their symptoms, as a result of hormonal changes. This study may improve our knowledge of how hormonal variations during the menstrual cycles influence hearing and, in the long term, help to identify the contribution of hormonal changes in some hearing disorders.

If you consent to participate, you will be asked to undergo several hearing tests and, on the same day, an intravenous blood sample, about two teaspoonfuls (10 ml), will be taken for hormone tests. Blood taking would cause a slight discomfort due to the vein puncture and sometimes may cause some bruising. The tests (both hearing tests and blood samples) will be performed four times during your menstrual cycle: first, between 5th-7th day, second, between 9th-11th day, third, between 18th-24th day and fourth, between 24th-26th day. If you take the oral contraceptive pills, we would be unable to include you in the study. The dates will be arranged during your first visit.

To assess the time of ovulation, we will also ask you to use the ovulatory kit during your cycle. Ovulation is predicted by testing your urine daily using the provided dipstick from day 10 of your cycle until you have a positive result (further information will be provided with the kit).

Details about your age, menstrual history and hearing will be obtained by an interview.

We are planning to perform the following hearing tests:

1) *Standard hearing test*: quiet tones at different frequencies are presented through earphones, and you will be asked to respond by pressing the button when you hear the sound.

1) *A test to assess middle ear function:* a small probe is inserted into your ear and you will hear a tone, while the pressure in your ear canal is changed gently. This gives the sensation you may experience passing through a tunnel in a train.
2) *A test to assess inner ear function:* Clicking sounds are presented through foam probes inserted into you ears and the inner ear responses are recorded and analysed by computer.
3) *A test of hearing by recording electrical responses from the brain:* Clicking sounds are presented through earphones and the auditory responses in the brain will be recorded from the electrodes attached to your scalp with a special gel.

These tests are harmless and do not cause any discomfort. Except for the first test in which you are asked to signal when you hear a sound, the others do not require your active participation. They will take about 45 min to perform. We will be pleased to reimburse your travel expenses.

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights. Details about you will be stored on a computer during this research project and will be in coded form with the code known only to the research team. The data from the study will be kept for ten years and the researchers may use them for further research. The security will be the responsibility of Prof. Linda M. Luxon.

We would be pleased to inform you of the results of your investigations and, if we identify any abnormality of your hearing or hormone tests, we would inform your General Practitioner.

This research project has been reviewed by the National Hospital for Neurology & Neurosurgery and the Institute of Neurology Joint Research Ethics Committee.

Thank you for your time and attention

## Investigators:

*Dr. Deena Al-Mana*    020 7837 3611 ext 3386/ 07947 041954 (out of working hours)
*Dr Borka Ceranic*      020 7837 3611 ext 3386
*Professor Linda M. Luxon*    Department of Neuro-otology, The National Hospital for Neurology & Neurosurgery, Queen Square, London WC1N 3BG    Tel. 020 7837 3611 ext 3385
*Professor Ovrang Djahanbakhch*      Department of Obstetrics & Gynaecology, Barts and the London School of Medicine and Dentistry, IV Floor, Holland Wing, The Royal London Hospital, Whitechapel, London E1 1BB   Tel: 020 7363 8096

# University College London Hospitals NHS

**NHS Trust**

**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
Box 127,
Queen Square, London
WC1N 3BG

Telephone: 020 7837 3611
Fax: 020 7829 8775

CONFIDENTIAL
INFORMATION SHEET FOR HEALTHY VOLUNTEER

## *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

*Version 2a, 13<sup>th</sup> February 2004*

We would like to ask you to participate in this research project, the purpose of which is to investigate the effect of female hormones on hearing. The study also includes a group of men, to allow assessment of hearing in the absence of hormonal changes, which occur in women.

This study may improve our knowledge of how hormonal variations in women influences hearing and, in the long term, help to identify the contribution of hormonal changes in some hearing disorders.

If you consent to participate, you will be asked to attend our clinic to undergo several hearing tests once a week for four consecutive weeks. In total, you will be attending our clinic four times during one month. The dates will be arranged during your first visit.

Details about your age and hearing will be obtained by an interview.

We are planning to perform the following hearing tests:

1) *Standard hearing test*: quiet tones at different frequencies are presented through earphones, and you will be asked to respond by pressing the button when you hear the sound.
2) *A test to assess middle ear function:* a small probe is inserted into your ear and you will hear a tone, while the pressure in your ear canal is changed gently with no or minimal discomfort.
3) *A test to assess inner ear function:* Clicking sounds are presented through foam probes inserted into you ears and the inner ear responses are recorded and analysed by computer.
4) *A test of hearing by recording electrical responses from the brain:* Clicking sounds are presented through earphones and the auditory responses in the brain will be recorded from the electrodes attached to your scalp with a special gel.

These tests are harmless and do not cause any discomfort. Except for the first test in which you are asked to signal when you hear a sound, the others do not require your active participation. They will take about 45 min to perform. We will be pleased to reimburse your travel expenses.

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights. Details about you will be stored on a computer during this research project and will be in coded form with the code known only to the research team. The data from the study will be kept for ten years and the researchers may use them for further research. The security will be the responsibility of Prof. Linda M. Luxon.

We would be pleased to inform you of the results of your investigations and, if we identify any abnormality of your hearing, we would inform your General Practitioner.

This research project has been reviewed by the National Hospital for Neurology & Neurosurgery and the Institute of Neurology Joint Research Ethics Committee.

Thank you for your time and attention

## Investigators:

*Dr. Deena Al-Mana*    020 7837 3611 ext 3386/ 07947 041954 (out of working hours)
*Dr Borka Ceranic*      020 7837 3611 ext 3386
*Professor Linda M. Luxon*    Department of Neuro-otology, The National Hospital for Neurology & Neurosurgery, Queen Square, London WC1N 3BG    Tel. 020 7837 3611 ext 3385
*Professor Ovrang Djahanbakhch*    Department of Obstetrics & Gynaecology, Barts and the London School of Medicine and Dentistry, IV Floor, Holland Wing, The Royal London Hospital, Whitechapel, London E1 1BB   Tel: 020 7363 8096

# University College London Hospitals NHS

**NHS Trust**
**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
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Telephone: 020 7837 3611
Fax: 020 7829 8775

CONFIDENTIAL

INFORMATION SHEET FOR PATIENTS

## *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

*Version 2, 6th November 2002*

We would like to ask you to participate in this research project, the purpose of which is to investigate the effect of female hormones on hearing. As your treatment involves changes in your hormone levels, which are carefully measured, we are asking women undergoing conception-assisted treatment to take part

Many healthy women experience changes in hearing related to the menstrual cycle, pregnancy or the menopause. Similarly, some patients with an inner ear/hearing problem have also reported changes in their symptoms, as a result of hormonal changes. This study may improve our knowledge of how hormonal variations during the menstrual cycles influence hearing and, in the long term, help to identify the relationship of hormonal changes to some hearing disorders.

If you consent to participate, you will be asked to undergo several hearing tests on the same day as your scan appointments. The hearing and blood tests will be performed on the same day, three times during your treatment under the Gynaecologist.

Details about your age, menstrual history and hearing will be obtained by a verbal interview.

We are planning to perform the following hearing tests:

1) *Standard hearing test*: quiet tones at different frequencies are presented through earphones, and you will be asked to respond by pressing the button when you hear the sound.
2) *A test to assess middle ear function*: a small probe is inserted into your ear and you will hear a tone, while the pressure in your ear canal is changed gently. This gives the sensation you may experience passing through a tunnel in a train.
3) *A test to assess inner ear function*: Clicking sounds are presented through foam probes inserted into you ears and the inner ear responses are recorded and analysed by computer.

4) ***A test of hearing by recording electrical responses from the brain***: Clicking sounds are presented through earphones and the auditory responses in the brain will be recorded from the electrodes attached to your scalp with a special gel.

These tests are harmless and do not cause any discomfort. Except for the first test in which you are asked to signal when you hear a sound, the others do not require your active participation. They will take about 45 min to perform. We will be pleased to reimburse your travel expenses.

Your participation in the trial is entirely voluntary. You are free to decline to enter or to withdraw from the study at any time without having to give a reason. If you choose not to enter the trial, or to withdraw once entered, this will in no way affect your future medical care. All information regarding your medical records will be treated as strictly confidential and will only be used for medical purposes. Your medical records may be inspected by competent authorities and properly authorised persons, but if any information is released this will be done so in coded form so that confidentiality is strictly maintained. Participation in this study will in no way affect your legal rights. Details about you will be stored on a computer during this research project in a coded form known only to the researchers involved in the study. The data from the study will be kept for ten years and the researches may use them for further research. The security will be the responsibility of Prof. Linda M. Luxon.

We would be pleased to inform you of the results of your investigations and, if we identify any abnormality of your hearing, we would inform your General Practitioner.

This research project has been reviewed by the National Hospital for Neurology & Neurosurgery and the Institute of Neurology Joint Research Ethics Committee.

Thank you for your time and attention

## Investigators:
*Dr. Deena Al-Mana*   08451555000 ext 723385 / 07947 041954/
                       D.Al-Mana@ich.ucl.ac.uk
*Dr Borka Ceranic*     08451555000 ext 723385
*Professor Linda M. Luxon*   Department of Neuro-otology, The National Hospital for Neurology & Neurosurgery, Queen Square, London WC1N 3BG
Tel. 08451555000 ext 723385
*Professor Ovrang Djahanbakhch*   Department of Obstetrics & Gynaecology, Barts and the London School of Medicine and Dentistry, IV Floor, Holland Wing, The Royal London Hospital, Whitechapel, London E1 1BB   Tel: 020 7363 8096

## Appendix III:      Subject consent forms

The following pages are copies of the consent forms for the study volunteers.

# University College London Hospitals NHS

**NHS Trust**

**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
Box 127,
Queen Square, London
WC1N 3BG
Fax: 020 7829 8775

Centre Number:

Study Number: 02/N112

Patient Identification Number for this trial:

## CONSENT FORM FOR HEALTHY VOLUNTEERS

Title of project: *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

Researchers:
Dr. Deena Al-Mana        020 7837 3611 ext 3386/ 07947 041954 (out of working hours)
Dr Borka Ceranic         020 7837 3611 ext 3386
Professor Linda M. Luxon     020 7837 3611 ext 3385
Professor Ovrang Djahanbakhch   020 7363 8096

*PLEASE INITIAL BOX*

1. I confirm that I have read and understood the information sheet dated 6th November 2002 (version 2) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree that information from this study may be used in further research

5. I agree to take part in the above study.

_____     _____     _____
Name of patient                          Date                   Signature

_____     _____     _____
Name of Person taking consent            Date                   Signature
(if different from researcher)

_____     _____     _____
Researcher                               Date                   Signature

**Comments or concerns during the study**
**If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.**

1 copy for Patient        1 copy for researcher        1 copy to be kept with hospital note

The National Hospital for Neurology and Neurosurgery is part of UCL Hospitals NHS Trust which also includes the Eastman Dental Hospital, Elizabeth Garrett Anderson and Obstetric Hospital, The Heart Hospital, Hospital for Tropical Diseases, The Middlesex Hospital and University College Hospital.

# University College London Hospitals **NHS**

**NHS Trust**

**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
Box 127,
Queen Square, London
WC1N 3BG
Fax: 020 7829 8775

Centre Number:

Study Number: 02/N112

Patient Identification Number for this trial:

## CONSENT FORM FOR HEALTHY VOLUNTEERS

Title of project: *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

Researchers:
Dr. Deena Al-Mana        020 7837 3611 ext 3386/ 07947 041954 (out of working hours)
Dr Borka Ceranic          020 7837 3611 ext 3386
Professor Linda M. Luxon        020 7837 3611 ext 3385
Professor Ovrang Djahanbakhch          020 7363 8096

*PLEASE INITIAL BOX*

1. I confirm that I have read and understood the information sheet dated 13th February 2004 (version 2a) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree that information from this study may be used in further research

5. I agree to take part in the above study.

_____        _____        _____
Name of patient                                    Date                              Signature

_____        _____        _____
Name of Person taking consent                Date                              Signature
(if different from researcher)

_____        _____        _____
Researcher                                          Date                              Signature

**Comments or concerns during the study**
**If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.**

1 copy for Patient        1 copy for researcher        1 copy to be kept with hospital note

**UCL HOSPITALS**        The National Hospital for Neurology and Neurosurgery is part of UCL Hospitals NHS Trust which also includes the Eastman Dental Hospital, Elizabeth Garrett Anderson and Obstetric Hospital, The Heart Hospital, Hospital for Tropical Diseases, The Middlesex Hospital and University College Hospital.

# University College London Hospitals **NHS**

Centre Number:

Study Number: 02/N112

Patient Identification Number for this trial:

**The National Hospital for Neurology and Neurosurgery**
**Department of Neuro-otology**
Box 127,
Queen Square, London
WC1N 3BG
Fax: 020 7829 8775

## CONSENT FORM FOR PATIENTS

Title of project: *A Study of the Effect of Ovarian Steroid Hormones on Auditory Function*

Researchers:
Dr. Deena Al-Mana          020 7837 3611 ext 3386/ 07947 041954 (out of working hours)
Dr Borka Ceranic          020 7837 3611 ext 3386
Professor Linda M. Luxon          020 7837 3611 ext 3385
Professor Ovrang Djahanbakhch          020 7363 8096

*PLEASE INITIAL BOX*

1. I confirm that I have read and understood the information sheet dated 6th November 2002 (version 2) for the above study and have had the opportunity to ask questions.

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

3. I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records.

4. I agree that information from this study may be used in further research

5. I agree to take part in the above study.

_____          _____          _____
Name of patient          Date          Signature

_____          _____          _____
Name of Person taking consent          Date          Signature
(if different from researcher)

_____          _____          _____
Researcher          Date          Signature

**Comments or concerns during the study**
**If you have any comments or concerns you may discuss these with the investigator. If you wish to go further and complain about any aspect of the way you have been approached or treated during the course of the study, you should write or get in touch with the Complaints Manager, UCL hospitals. Please quote the UCLH project number at the top this consent form.**

1 copy for Patient          1 copy for researcher          1 copy to be kept with hospital note

The National Hospital for Neurology and Neurosurgery is part of UCL Hospitals NHS Trust which also includes the Eastman Dental Hospital, Elizabeth Garrett Anderson and Obstetric Hospital, The Heart Hospital, Hospital for Tropical Diseases, The Middlesex Hospital and University College Hospital.

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# Appendix IV:     Published papers

The following are copies of the two published papers.

Removed due to copyright reasons