MRI Studies of the Fetal Brain and Cranium

Nuno Maria Canto Moreira
Figueira de Almeida
Ultrasound is the primary modality for fetal imaging, but Magnetic Resonance Imaging nowadays has a valuable complementary role as it often reveals findings that alter pregnancy management.

Knowledge on some clinically relevant areas of the normal fetal development is still lacking, and this was the aim of this project. We wanted 1) to obtain reference MRI data of normal brain measurements before 24 gestation weeks (GW), 2) to study the development of the hippocampus, 3) to study the development of the ear and 4) to test the ability of MRI for evaluating the lip and palate.

For this, we retrospectively analysed a database with 464 in vivo and 21 post mortem fetal MRI examinations.

Study I evaluated a series of 70 normal fetuses. A table of normal brain measurements from 17 to 23 GW was built, the first in the literature that includes ages below 20 GW.

Study II focused on the evolution of the hippocampus from 18 to 38 GW by evaluating 3 post mortem and 60 in vivo MRI examinations. Our results suggested this area to develop later and more asymetrically than previously thought.

Study III analysed a series of 122 normal MRI in vivo and 16 MRI post mortem. We described the development of the fetal ear in vivo for the first time in the literature, realizing that the value of MRI is limited by the size of the structures evaluated.

In study IV, 60 brain-targeted MRI examinations of 55 normal fetuses and 5 fetuses with orofacial clefts were blindly reviewed by two readers, focusing on the lips and palates. Our results suggest a high accuracy of MRI in the evaluation of this area, regardless of fetal age or previous ultrasound findings.

This thesis brings new knowledge on the normal development of the fetal brain and cranium.

Keywords: Fetal MRI, Brain, Cranium, Development, Normal, Ear, Lip, Palate, Biometry, Hippocampus, Measurements

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Ao meu Pai

Tillägnad min Far
LIST OF PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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### ABBREVIATIONS

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<thead>
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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADC</td>
<td>Apparent Diffusion Coefficient</td>
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<tr>
<td>BPD</td>
<td>Biparietal Diameter (Cerebral)</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<td>dB</td>
<td>Decibel</td>
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<td>DWI</td>
<td>Diffusion-Weighted Imaging</td>
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<tr>
<td>EAC</td>
<td>External Auditory Canal</td>
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<tr>
<td>FLAIR</td>
<td>Fluid Attenuated Inversion Recovery</td>
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<tr>
<td>FOD</td>
<td>Fronto-Occipital Diameter</td>
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<tr>
<td>FSE</td>
<td>Fast Spin-Echo</td>
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<tr>
<td>GW</td>
<td>Gestation Weeks</td>
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<tr>
<td>LSC</td>
<td>Lateral Semicircular Canal</td>
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<td>MR</td>
<td>Magnetic Resonance</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>RF</td>
<td>Radio Frequency</td>
</tr>
<tr>
<td>SSFP</td>
<td>Steady-State Free Precession</td>
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<tr>
<td>SSFSE</td>
<td>Single-Shot Fast Spin-Echo</td>
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<td>SSH GRE EPI</td>
<td>Single-Shot Half Fourier Gradient-Recalled Echo Planar Imaging</td>
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<tr>
<td>T1W</td>
<td>T1-Weighted</td>
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<tr>
<td>T2W</td>
<td>T2-Weighted</td>
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<tr>
<td>TCD</td>
<td>Transverse Cerebellar Diameter</td>
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<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VAP</td>
<td>Vermian Antero-Posterior Diameter</td>
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<td>VH</td>
<td>Vermian Height</td>
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<td>WK</td>
<td>Weighted Kappa</td>
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INTRODUCTION

How important is fetal MRI?
Ultrasound is the first line modality for fetal imaging. Its accuracy upon detecting anomalies in the low-risk population is nevertheless quite variable, with detection rates that may range from 0 to 100% depending on the type of anomaly to be screened, operator skills, equipment, gestation age, national pregnancy management policy or level of diagnostic centre. In a large European prospective study (Eurofetus) evaluating more than 200,000 fetuses from 14 countries, an average sensitivity of 61% for anomaly detection was reported.

However, some special ultrasound methods - as neurosonography - have high detection rates that have been stated to be equivalent to MRI and there has been a debate concerning the capabilities of the two methods.

It is clear nowadays that MRI and ultrasound do not compete with each other, as they do not occupy a similar place in fetal management. MRI is a tertiary method that is most of the times performed to clarify ultrasound findings, often revealing additional information that may alter patient counselling and case management.

In fact, just by being performed by distinct medical groups (radiologists vs obstetricians), MRI and US provide complementary standpoints that, when part of a team work, contribute to a wider diagnostic capability.

Historical overview
MRI in pregnancy was first described in 1983. By not using ionizing radiation, the potential value of this method as a tool for evaluating the fetus was immediately recognized. However, the long acquisition times of the MRI sequences available at that time rendered the observation of the fetal anatomy very difficult in practice due to motion artefacts. Studies in that decade were mostly T1-weighted and tended to be limited to older, insinuated fetuses, or to be targeted for volumetric measurements that used faster echo-planar sequences. Even if fetal motion control could be achieved in some instances by invasive procedures, such as curarization of the umbilical cord, all these limitations contributed then to restrain the technique to a small number of university centres.

The availability of single-shot MR sequences in the 90’s made it possible to obtain T2-weighted images in 1 second or even less. These sequences allow each image to be acquired separately, thus highly benefiting a movement-prone method such as fetal MRI that became increasingly more popular. The number of available sequences that

Figure 1.
Examples of sequences commonly used in fetal MRI
a) T2-weighted SSFSE, b) T1-weighted FLAIR, c) DWI and d) SSFP thick-slab dynamic sequence.
MRI Studies of the Fetal Brain and Cranium

can efficiently cope with fetal motion is nevertheless quite limited even today (fig. 1) and we cherish the recent development of a very promising single-shot T1-weighted sequence 21, that was still lacking in daily practice.

**Indications for fetal MRI**

Indications for performing a fetal MRI may be related to the mother, the fetus, or both. Maternal causes include obesity or other aspects that might otherwise compromise an US examination, whereas fetal causes most of the times involve a) the clarification of previous US findings, b) the screening for genetic pathology or c) to rule out diseases that might not be detectable by US 22.

Traditionally fetal MRI has been mostly targeted for the central nervous system, as this field is particularly challenging for US and the fast T2-weighted sequences were ideal for providing contrast within the unmyelinated fetal brain and towards the surrounding cerebrospinal fluid. MRI is now currently acknowledged to be also important in body pathology 22, namely of the lungs, kidney and liver, but this is beyond the scope of our work.

Major fetus-related indications for performing a fetal MRI in neuroradiology include all neuronal migration disorders, corpus callosum dysgenesis and posterior fossa malformations 8, 10, 23. However, in order to obtain the best diagnostic result, a precise knowledge of the diagnostic capabilities of US is also needed, or when to combine both methods 6, as summarized in table 1.

Attemptive guidelines for the use of MRI in fetal medicine have been published 6, 24, but the precise indications and timing for this procedure still vary from country to country, in relationship to factors as local legislation for pregnancy management 25, differences in the number of fetal MRI centres, or the existence of specialised ultrasound techniques like neurosonography 3, 4, 26, 27.

<table>
<thead>
<tr>
<th>Pathology</th>
<th>US</th>
<th>MRI</th>
<th>both</th>
<th>either</th>
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<tr>
<td>Screening</td>
<td>X</td>
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<td>MRI contra-indicated or failed</td>
<td>X</td>
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<td>Early diagnosis (until 19 GW)</td>
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<td>Assessment of fetal movements (up to 3rd trimester)</td>
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<tr>
<td>Assessment of cerebral blood flow</td>
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<td>Evaluation of associated abnormalities</td>
<td>X</td>
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<td>Oligohydramnios</td>
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<td>Engaged fetal head and ruptured membranes</td>
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<tr>
<td>Assessment of fetal movements (late 3rd trimester)</td>
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<td>Posterior fossa abnormalities</td>
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<td>Detecting and determining age of intracranial bleeding</td>
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<td>Detecting intracranial tuberous sclerosis</td>
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<td>Corpus callosum and pericallosal abnormalities</td>
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<tr>
<td>Fetal brain death</td>
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<td>Germinial matrix and intraventricular bleeding</td>
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<td>Septo-optic dysplasia</td>
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<td>Holoprosencephaly (after 20 weeks)</td>
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<td>Corpus callosum abnormalities (after 20 weeks)</td>
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<td>Craniosynostosis</td>
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Table 1.

Major indications for fetal MRI versus ultrasound. Modified from Pistorius et al. 6.

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Introduction

MR technique

In modern MRI units, there are no special hardware requisites for performing a fetal examination. Mothers lie supine or in right lateral decubitus (if late in pregnancy), care being taken to maximize comfort and reduce noise. Fetal motion and peristalsis may be reduced by fasting, or by means of a mild sedative given to the mother, but these methods are not in use in our institutions. Instead, we try to provide a calm environment for the mother and to perform the examinations outside the circadian peaks of fetal movement, although realizing that this is not always possible in daily practice.

Cardiac or flexible abdominal coils are placed around the maternal abdomen, trying to obtain the maximum signal-to-noise ratio for the organ to be studied. This attemptive targeting is mostly meant for older fetuses, since the precise position of younger ones that can move more freely inside the abdominal cavity is harder to predict.

The imaging protocol in our institution in Portugal (table 2) changes somewhat according to differences in fetal size or the precise clinical problem at stake but, routinely, ultrafast single-shot T2-weighted images are obtained sequentially in three orthogonal planes of the fetus, followed by axial T1W, axial DWI and a sagittal thick-slab dynamic sequence. Most CNS-targeted studies take less than 30 minutes to perform but, as a safety precaution, they are usually interrupted when 45 minutes of examination time are reached.

Safety concerns

Fetal MRI at 1.5 Tesla is generally accepted to be a safe procedure per se, also with no evidence of long-term harmful consequences. There are, however, three potential areas of concern regarding fetal safety: the static field, the field gradients and the radiofrequency pulses.

The possibility that the static magnetic field of MRI by itself could have an effect on fetal growth in mice was reported in 1988. Even if

<table>
<thead>
<tr>
<th>Order of acquisition</th>
<th>Sequence</th>
<th>Plane</th>
<th>FOV (mm)</th>
<th>Slice thick. (mm)</th>
<th>Gap (mm)</th>
<th>Matrix</th>
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<tr>
<td>1</td>
<td>SSFP Survey</td>
<td>3 - plane</td>
<td>400</td>
<td>8</td>
<td>0</td>
<td>224*209</td>
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<td>2-4</td>
<td>T2W SSFSE</td>
<td>3 - plane</td>
<td>200-300</td>
<td>3-4</td>
<td>0.3</td>
<td>256*256 (1)</td>
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<td>5</td>
<td>DWI (b=1000+ADC)</td>
<td>axial</td>
<td>230-250</td>
<td>4</td>
<td>1</td>
<td>432*104</td>
</tr>
<tr>
<td>6</td>
<td>FLAIR/T1W</td>
<td>axial</td>
<td>270-330</td>
<td>4</td>
<td>1</td>
<td>512*256</td>
</tr>
<tr>
<td>7</td>
<td>SSFP Dynamic scan</td>
<td>sagittal</td>
<td>350</td>
<td>10-15</td>
<td>0</td>
<td>256*192</td>
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<tr>
<td>Optional</td>
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<tr>
<td>8</td>
<td>T2* GRE EPI</td>
<td>axial</td>
<td>230-250</td>
<td>5</td>
<td>1</td>
<td>512*256</td>
</tr>
<tr>
<td>9</td>
<td>FLAIR /T1W</td>
<td>coronal</td>
<td>200-240</td>
<td>4</td>
<td>1</td>
<td>512*256</td>
</tr>
</tbody>
</table>

Table 2.

Standard brain Fetal MR Imaging protocol at Dr. Campos Costa (Philips Intera 1.5T).
(1) Matrices for the T2W SSFSE sequence have been evolving over time, from 256*256 to 512*512.
there is no evidence to date of such an effect in human fetuses when undergoing MRI studies at 1.5 Tesla units, MR examinations are usually only performed upon the completion of organogenesis, after 16 gestational weeks.

Gradients currently used in MRI may reach 120 dB in noise intensity. As such, hearing damage to the fetus has been considered a potential hazard, but this was not confirmed in practice.

The fast imaging sequences that are used to reduce the impact of fetal motion on image quality usually induce a high radiofrequency specific absorption rate (SAR) and therefore depose heat on the mother and fetus. As heat is a known mutagenic, potential increases in fetal body temperature during MR examinations have been investigated, leading to guidelines on the maximum amount of exposure to radiofrequency radiation that is considered to be safe for the fetus.

It is known however that in fetal MRI the actual RF exposure period does not normally exceed a third of the examination time and a potential harmful heating of the fetus has not been proven in clinical practice, but the issue is at the centre of the present limitation to scan fetuses at magnets no stronger than 1.5 Tesla. Otherwise, the expected better signal-to-noise ratio of higher field strengths could be highly beneficial in this area that deals with very small subjects, even if early reports suggested that increasing artefacts could in fact reduce the quality of fetal MRI at 3T.

**Evaluation of the normal development of the brain and cranium**

To rule out pathology, knowledge about the normal development of the fetal brain and cranium in vivo is needed. MRI is an excellent tool for that purpose, in particular for the evaluation of 1) the gyration pattern, 2) the layering of the cerebral mantle, 3) premyelinating and myelinating processes and 4) posterior fossa. Of interest for our project, we must also refer to it’s role for the study of 5) the hippocampus and 6) the cranium/base of the skull.

1) **Gyration**

The value of recognizing a specific gyration pattern for each gestational age in order to assess maturation has long been recognized in vitro. This has also been attempted by ultrasound, but it was only with MRI that the chartering of the sulcal and gyral development of the human brain in vivo was conclusively obtained.

The evolution of brain sulcation can be used to evaluate fetal age after approximately 24 GW, as the brain is essentially agyric until then (fig. 2).

![Figure 2.](image)

Fetuses of 20, 24 and 34 GW, from left to right. Note the progressive deepening of the Sylvian fissures with age (black arrows). At 24 GW, the calcarine sulci start to be delineable (white arrow). By 34 GW, the primary and secondary sulcation is complete.
75% of fetuses, the parietooccipital sulcus and the callosal sulcus can be detected by 23 GW, whereas the calcarine sulcus is visible by 24–25 GW and the central sulcus by 26 GW \(^{46}\). All primary and some secondary sulci are supposed to be visible on fetal MRI by 34 GW \(^{46}\). To be noticed, however, that the gyration pattern on MRI appears to lag by an average of two GW when compared with autopsy specimens \(^{46}\).

As gyration is not usable as a marker for brain maturation in young fetuses, measurements of brain growth are needed for that purpose. This approach has been the gold-standard for fetal age evaluation with ultrasound \(^{47, 48}\), but data for MRI are also needed, as this method partly evaluates different structures and takes measurements in a different manner than US.

Research has been made with MRI on volumetric assessment of the brain growth by using segmentation methods \(^{49-52}\), but these are time-consuming and difficult to perform in daily practice. More clinically-applicable tables with linear brain measurements are still scarce \(^{29, 46, 53-55}\), in particular data for young ages, as only one published study exists engaging fetuses before 23 GW \(^{54}\), and none before 20 GW.

2) Cerebral layering

A layering pattern of the cerebral mantle can already be seen at histology and in vitro MRI at 10 GW \(^{56}\). This is constituted by the T2-hypointense ventricular zone and cortical plate, both separated by the cell-sparse intermediate zone.

By the time clinical MRI is performed, after the end of embryogenesis, a more complex 5-layer pattern is definable, as seen in figure 3. This transient appearance fades out on MRI by approximately 28-29 GW, even if it is still recognizable on histology later in pregnancy \(^{56, 57}\).

The first group of future cortical neurons and migrating glia is produced at the ventricular zone from 8 to 16 GW \(^{58}\). Later on, the outer subventricular zone becomes the major source of glial cells, and also of the more superficially located neurons of the developing six-layered cortex \(^{59}\).

The intermediate zone - that will become the white matter proper - contains growing axonal pathways, but also an intense proliferation of oligodendrocytes and astrocytes to guide neuronal migration, this cell density being the responsible for the comparatively low signal the layer has on T2W images \(^{60}\).

The subplate is also an important transient structure of the mid-fetal life. This is where most neuron apoptosis occurs \(^{58}\), but it is also a “waiting” area for growing cortical afferents that are surrounded by a very large extracellular space \(^{61}\). This T2-hyperintense layer becomes progressively less evident as synaptogenesis evolves at the third trimester, totally fading on MRI after 28-29 GW.

The outermost layer, the cortical plate, receives the afferents from the subplate and it remains the only laminar compartment that is well-delineated in MRI scans until birth \(^{56}\).

Figure 3.

Fetuses of 25 (left) and 29 GW (right).
The ventricular zone (VZ), the subventricular zone (SVZ), the intermediate zone (IZ), subplate (SP) and cortex (CP) can be identified at the younger fetus, but the layering is much less distinct at the older one.
Other transient brain structures of importance are the ganglionic eminences - that will later form the basal ganglia and thalamus - and the recently described periventricular crossroads \(^\text{62}\). The latter are major areas of intersection of fiber systems, situated at predilection sites for hypoxic-ischemic injuries \(^\text{62}\), thus suggesting a selective local vulnerability by means that are not yet fully understood.

### 3) Myelination

Ultrasound, on the contrary to MRI, is unable to assess myelination. This progressive process that spans from mid-gestation to adulthood, has known milestones on MRI that can be trustfully used to evaluate the maturation of the brain \(^\text{63}\). To know these milestones is very important clinically, since disturbances in myelination are a fairly common component of hundreds of diseases, from hypoxic-ischemic to metabolic processes.

In fetal MRI \textit{in vivo} (fig. 4), a T2-hyposignal in relationship to myelination can already be seen at 28 GW in the posterior fossa, namely at the gracile and cuneate nuclei, vestibular nuclei, cerebellar vermis, inferior and superior cerebellar peduncles, dentate nucleus, medial longitudinal fasciculus, inferior olivary nuclei, medial lemnisci, lateral lemnisci and inferior colliculi, but also at parts of the diencephalon as the medial geniculate bodies, subthalamic nuclei and ventrolateral nuclei of the thalamus \(^\text{64}\). From this gestational age on, myelination is not visualized at any new site until 36 GW, when myelin can be seen in the corona radiata, posterior limb of the internal capsule, corticospinal tracts and lateral geniculate bodies \(^\text{64}\).

### 4) Posterior fossa

MRI is excellent for the evaluation of the posterior fossa, as it allows a direct visualization of the cerebellar hemispheres, vermis and brainstem in three orthogonal planes. In a recent study \(^\text{65}\) fetal MRI was able to rule out pathology in 28% of 90 fetuses referred for suspected posterior fossa anomalies on ultrasound. However, in the same study, only a 60% agreement existed between the pre- and postnatal MRI findings and an early gestational age at the time of examination was considered an important factor for such a disparity. Therefore, opinion has built \(^\text{23, 66}\) that MRI findings should be taken with caution when evaluating the posterior fossa early in gestation, especially in the case of the vermis or brainstem.

### 5) Hippocampus

Morphological studies of the fetal hippocampus have been focusing more on the cellular types and organization than on its general shape. Three studies dealt with MRI evaluations of formalin-fixed specimens \(^\text{56, 67, 68}\), but with this type of approach the anatomical shape and proportions may change when the brain is fixed in formalin or prepared for the histological examination. Therefore, studying

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**Figure 4.**

Fetuses of 28 GW (left side) and 37 GW (right side). The T2-hyposignal of myelination (arrows) can be seen at the colliculi, dorsal pons and gracile nucleus in the younger fetus, and at the posterior limb of the internal capsule (arrow) in the older one.
the hippocampal development in vivo is pertinent.

The hippocampal development begins at 8 GW (fig. 5) and the hippocampal sulcus becomes visible at 10 GW 69. This sulcus progressively deepens as a part of the hippocampal inversion process, that has been reported to be completed at approximately 21 GW 67-71, even if some data suggest it might take longer 72. The fully developed hippocampus is oval in coronal slices, and Baker and Barkovich have suggested that this oval shape is preceded by a round or pyramidal shape 73. Indeed, this non-oval shape has been shown to be more common in younger than older premature neonates in an ultrasound study 72 but it may persist throughout life in 19% of the general population, most often on the left side 74, 75.

On MRI in vivo, the hippocampal sulcus can be seen at 22-23 GW and the collateral sulcus at 23-26 GW 76. There is one in vivo fetal MR study focusing on the hippocampal infolding process, from 20 GW until 37 GW 77, but developmental changes in its shape have not been assessed before with this technique.

5) Cranium

Detailed in vitro MRI studies regarding the development of the fetal cranium have been published 78, 79, but there is paucity in fetal MRI literature in vivo in what concerns this area, probably due to the fact that the bone and muscle prevailing here do not provide the same high T2 contrast that is available for the endocranium. The exceptions are reports on some structures with a high water content, as the eye globes 80, 81 or the lacrimal ducts 82.

In particular, prenatal data in vivo for the evaluation by MRI of the ear and the normal lip and palate are scarce, but they would be of great clinical relevance:

a) Ear

A vast amount of congenital syndromes are accompanied by ear involvement 83, 84. 3D ultrasound is useful to delineate the pinna, it helps to rule out local malformations and can give evidence of aneuploidy 85-87 but the capacity of the method to rule out temporal bone pathology is very limited 88.

Figure 5.
Schematic representation of the fetal hippocampal development.
The ear is also very difficult to evaluate at brain-targeted fetal MRI studies, as these are prone to suffer from movement, slice tilting and also from partial-volume averaging. Some isolated reports do exist on inner ear malformations depicted by fetal MRI and there is even a published series addressing the development of the middle ear, but a comprehensive evaluation of which structures of the ear can be seen on fetal MRI at different stages of pregnancy is still lacking in the literature.

b) Lip and palate

Ultrasound has been the gold standard for the prenatal screening of the lip and palate, but its accuracy is very heterogeneous, depending heavily on the user and examination protocol. However, its ability to identify facial anomalies has been improving significantly in recent years, even in non-selected screening examinations.

The most common facial malformations are cleft lips, with or without an associated cleft palate, attaining approximately 1/700 live births. The importance of detecting these entities in the prenatal period is several-fold as it modifies patient management and parental expectations, but also due to the fact that a cleft lip is associated to a genetic syndrome in more than 10% of patients.

At least five prospective studies and one retrospective study have dealt with the role of MRI in the evaluation of orofacial clefts. All agree that MRI provides a better evaluation of the secondary palate than US and only one study did not find the method to be more helpful when identifying primary palate involvement. MRI has also been shown to be important in the evaluation of the intracranial anomalies that may exist in association to clefts, these ranging from 6.3% to 23% in previous reports.

In only one of these MRI series, regarding fetuses in the third trimester, the examinations were read in a blind fashion. In all other studies the reader, the MRI protocol or both were not blinded to previous ultrasound findings. Therefore, the question of how well does MRI independently detect orofacial anomalies remains unanswered, even knowing that in one series with 34 fetuses the method disclosed 5 (14.7%) clefts that were not previously seen by ultrasound.
AIMS OF THE STUDY

General aim
To evaluate with MRI the normal development of some clinically relevant structures of the fetal brain and cranium that previously are insufficiently described. We were particularly focused on the period before 25 GW, as this is a time limit for pregnancy management in a large number of countries 25.

Specific aims
1) To obtain brain measurements for MRI of normal fetuses before the end of the second trimester of gestation.

2) To evaluate with MRI in vivo the development of the fetal hippocampus.

3) To evaluate with MRI in vivo the development of the fetal ear.

4) To test the reliability of brain-targeted MRI in vivo for the evaluation of the lip and palate across gestation.
Table 3, (study IV).
Post-natally confirmed facial clefts

<table>
<thead>
<tr>
<th>Fetus no</th>
<th>GW</th>
<th>Cleft lip</th>
<th>Cleft primary palate</th>
<th>Cleft secondary palate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>Bilateral</td>
<td>Bilateral</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>Unilateral</td>
<td>Unilateral</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>Unilateral</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>Unilateral</td>
<td>Unilateral</td>
<td>No</td>
</tr>
</tbody>
</table>

GW = gestational week
MATERIAL

The material for this project came from three sources:

A) A database of 71 in vivo fetal MRI examinations performed between 2004 and 2006 at Uppsala University Hospital (Sweden). This source contributed with fetuses for studies I and II.

B) A database of 393 in vivo fetal MRI examinations performed between 2002 and 2010 at the Consultório Dr. Campos Costa, Porto (Portugal). This source contributed with fetuses for all studies.

C) A database of 21 post mortem fetal MRI performed between 2006 and 2010 at Uppsala University Hospital of non-fixed, aborted fetuses that were examined before autopsy. This source contributed with fetuses for studies II and III.

Study I

Out of all MRI examinations of singleton fetuses aged from 17 to 23 GW from databases A and B, we selected the studies that fulfilled the following criteria: 1) to have been evaluated as normal in what concerned the brain examination, and 2) to have T2-weighted images in orthogonal sagittal, coronal and axial planes, with no obliquities or movement artefacts. A total of 70 examinations was obtained.

The main indications for the MRI studies were suspicion of mild ventriculomegaly (n=35), brain screening in case of non-CNS abnormalities (n=13), abnormal biochemical screening tests (n=9) and US suspicion of structural brain anomaly (n=9).

In nine cases, the pregnancy was terminated due to non-CNS disorders. All these fetuses had normal CNS findings at autopsy. The remaining 61 fetuses were delivered, and all neonates had a normal neurological status and clinical biometric parameters between the 10th and 90th percentiles at birth.

Study II

Twelve fetuses from database C and 306 from databases A and B were assessed. All the fetuses that underwent post mortem examinations in which central nervous system pathology was revealed in autopsy or MRI were excluded. For the in vivo group, all the subjects with brain pathology, including ventriculomegaly (even mild), and the examinations in which the hippocampal regions were not well assessable bilaterally or the coronal slices were not symmetrically positioned were also excluded. Three post mortem examinations and 60 in vivo examinations were accepted for final analysis.

Study III

Post mortem group: 16 fetuses from database C, with ages ranging from 16 to 22 GW.

In vivo group: From database B, we retrospectively reviewed the brain investigations that fulfilled the following criteria: 1) to have been reported as normal or with mild ventriculomegaly, 2) to have T2-weighted images in orthogonal sagittal, coronal and axial planes, with no movement artefact and 3) to have a normal clinical evaluation at the perinatal period, including normal audimetry values at the newborn hearing-screening test (UNHS). 122 examinations were obtained in total, from 116 fetuses.

Study IV

From database B, we selected all the examinations in which a cleft lip or cleft palate had been diagnosed and confirmed post-natally. A total of five fetuses were found, presenting with seven cleft lips, six cleft primary palates and two cleft secondary palates (table 3, see left). All of them had a previous suspicion of orofacial pathology at ultrasound that lead to the MRI examination.

A group of 55 gestational age-matched normal brain MRI studies was also selected from the same database, covering fetuses from 20 to 38 GW. All of them had a post-natal confirmation of a normal clinical status.
MRI Studies of the Fetal Brain and Cranium

METHODS

The in vivo MR studies were performed in Portugal and Sweden using 1.5 Tesla MRI units (Philips Intera, Eindhoven, Netherlands). Abdominal phased-array flexible coils were used. No sedation was given to the mothers-to-be.

The imaging protocols in vivo can be seen in table 2.

The post mortem examinations were performed using a birdcage knee-foot coil. T2-weighted 2D FSE sequences were obtained in the sagittal, coronal and transverse planes, with a slice thickness of 2 mm and in-plane resolution of 0.50x0.53 mm (transverse plane) and 0.59x0.62 mm (coronal and sagittal planes).

The gestational age at the moment of the MRI studies was based on ultrasound data and defined as the number of complete gestational weeks.

One reviewer of study II (DB) was a pediatric radiologist with a large experience in MRI. The other reviewers of the studies - NCM, JT and VR - were senior neuroradiologists with 12, 10 and 6 six years experience in fetal MRI, respectively.

The statistical treatment of the data included Mann-Whitney non-parametric tests to evaluate scoring levels, and kappa evaluation of interobserver disagreement.

The ethics committees of the two centres approved the study protocol.

Study I

Five measurements of the brain were taken, according to the criteria published by Garel et al (see fig. 6).

The examinations were reviewed and measurements made by two neuroradiologists (NCM and JT). In case of disparity, a measurement consensus was obtained. For each GW and parameter, the median, maximum and minimum values were recorded and rounded to the nearest millimetre.

![Figure 6.](image)

Fetus of 20 GW. T2-W-SSFSE.

a) Fronto-occipital diameter (FOD). Maximal occipital to frontal distance of the brain. Sagittal plane.

b) Cerebral biparietal diameter (CBD). Maximal transversal diameter of the brain above the Sylvian fissures. Coronal plane.

c) Transverse cerebellar diameter (TCD). Largest cerebellar diameter. Coronal plane.

d) Vermian height (VH) (dashed line) and antero-posterior diameter (VAP) (full line). Sagittal plane.
Materials and Methods

Study II

The hippocampal regions were assessed in one or more coronal slices (fig. 7). A comparison between the right and left side was made in every case. The side of the heart was regarded as the left side of the fetus, but the liver and the gastric chamber were also used as additional visual references.

The angle between the upper and lower lip of the hippocampal sulcus was measured. When this sulcus was closed, then the shape of the hippocampus was classified as non-oval or oval. The presence and orientation of the collateral sulcus was also recorded. If the angle between the collateral sulcus and the hippocampus was more than 70 degrees, it was defined as vertical.  

Two reviewers (DB and NCM) made independent evaluations and the interobserver agreement was calculated. In case of disagreement, the two radiologists re-evaluated the images and a consensus was achieved.

Differences in proportions were analyzed by the likelihood ratio $\chi^2$ method. $P$ values < .05 were considered significant. All tests were 2-tailed.

Figure 7.
Images of the hippocampal regions in coronal planes.
(a) Fetus of 25 GW. The arrows indicate the hippocampal sulci. The hippocampal sulcus is larger on the left side.
(b) Fetus of 29 GW. Closed hippocampal sulcus and a non-oval hippocampus with a vertical long axis bilaterally. The arrows indicate the hippocampal region and the stars the collateral sulcus.
(c) Fetus of 29 GW. Closed hippocampal sulcus and an oval hippocampus with a horizontal long axis bilaterally. The white arrows indicate the hippocampal region, the black arrow closed hippocampal sulcus and the star the collateral sulcus.
MRI Studies of the Fetal Brain and Cranium

Study III

The cochlea, vestibular apparatus, middle ear and external auditory canals were evaluated by one reader (NCM) that had no information about the gestational age of the fetuses.

Each ear was assessed individually and classified as follows:

Cochlea (fig. 8):

Level 0: Not clearly identifiable.
Level 1: Identifiable, but no individual turns are depicted.
Level 2: Turns are seen. Possibility to exclude cochlear aplasia, cystic cavity or severe hypoplasia.
Level 3: Most turns are identifiable. Possibility to exclude a classic Mondini malformation, but minor partition abnormalities cannot be ruled out.
Level 4: Complete cochlear identification.

Vestibule and semicircular canals (fig. 9):

Level 0: Not clearly identifiable.
Level 1: Identifiable and separated from the cochlea, but no detail.
Level 2: Well-defined vestibule, but LSC not totally delineated.
Level 3: LSC totally delineated.
Level 4: Complete delineation of the vestibule and the semicircular canals.

Middle ear (fig. 10):

Level 0: Middle ear not identified.
Level 1: Middle ear depicted but not enough detail as to identify ossicles.
Level 2: Ossicles depicted but not discriminated.
Level 3: Possibility to discriminate each ossicle.

External ear (fig. 11):

Level 0: EAC not depicted.
Level 1: EAC depicted unilaterally.
Level 2: EAC depicted bilaterally.

In the case of different scores arising between the left and right ear, the lower score was used for classification.

In order to test inter-observer variability of the in vivo group, a random sample of 25 fetuses was evaluated independently by another experienced neuroradiologist (JT), this one being also blind to the clinical outcome of the fetuses. For final analysis, only the scoring of the main reader was used.
Materials and Methods

Figure 8.
Examples of scoring levels for cochlear evaluation \textit{(in vivo} series):
\begin{itemize}
\item[a)] Fetus of 21 GW – level 1, (coronal plane).
\item[b)] Fetus of 28 GW – level 3, (coronal plane).
\end{itemize}

Figure 9.
Examples of scoring levels for vestibular evaluation \textit{(in vivo} series):
\begin{itemize}
\item[a)] Fetus of 22 GW – level 1, (axial plane).
\item[b)] Fetus of 28 GW – level 3, (axial plane).
\end{itemize}

Figure 10.
Examples of scoring levels for middle ear evaluation \textit{(in vivo} series):
\begin{itemize}
\item[a)] Fetus of 23 GW – level 1, (coronal plane).
\item[b)] Fetus of 34 GW – level 2, (coronal plane).
\end{itemize}

Figure 11.
Examples of scoring levels for external ear evaluation \textit{(in vivo} series):
\begin{itemize}
\item[a)] Fetus of 34 GW – level 1, (coronal plane).
\item[b)] Fetus of 32 GW – level 2, (axial plane).
\end{itemize}

Study IV

All the 60 studies were reviewed independently by two senior neuroradiologists (VR and JT). They were blinded to the aims and methodology of the study, including the presence or absence of pathological cases in the cohort. If there were any sequences specially directed for the face - as in the fetuses with a previous suspicion of a cleft at US - these were removed from review to prevent reading bias.

The readers were asked to grade the delineation and normality of the upper lip, primary palate, secondary palate and nasal septum of each fetus, according to the following scale:

\begin{itemize}
\item[1. Level 1:] Evidently normal.
\item[2. Level 2:] Probably normal, even if not completely delineated.
\item[3. Level 3:] Probably abnormal, even if not completely delineated.
\item[4. Level 4:] Evidently abnormal.
\end{itemize}

If pathology was suspected, the readers were also asked to make an assumptive diagnosis.
MRI Studies of the Fetal Brain and Cranium
Results

RESULTS

Study I

Seventy fetal MRI examinations were included in the study. 22 of them were performed in Sweden and 48 in Portugal. The planned measurements were obtainable at all studies.

Age distribution and the results for each GW are shown in table 4.

<table>
<thead>
<tr>
<th>GW</th>
<th>Measurement</th>
<th>Median mm</th>
<th>Range mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 (n=6)</td>
<td>FOD</td>
<td>49</td>
<td>45-49</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>32</td>
<td>31-35</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>16</td>
<td>15-17</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>7</td>
<td>6-8</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>4</td>
<td>4-5</td>
</tr>
<tr>
<td>18 (n=6)</td>
<td>FOD</td>
<td>49</td>
<td>43-54</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>33</td>
<td>32-35</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>17</td>
<td>15-17</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>7</td>
<td>6-8</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>5</td>
<td>4-6</td>
</tr>
<tr>
<td>19 (n=8)</td>
<td>FOD</td>
<td>54</td>
<td>45-63</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>39</td>
<td>37-42</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>20</td>
<td>18-21</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>9</td>
<td>7-11</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>6</td>
<td>4-9</td>
</tr>
<tr>
<td>20 (n=15)</td>
<td>FOD</td>
<td>59</td>
<td>49-65</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>42</td>
<td>38-47</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>21</td>
<td>20-23</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>9</td>
<td>7-12</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>7</td>
<td>6-9</td>
</tr>
<tr>
<td>21 (n=14)</td>
<td>FOD</td>
<td>62</td>
<td>57-70</td>
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<td></td>
<td>CBD</td>
<td>45</td>
<td>41-48</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>23</td>
<td>21-24</td>
</tr>
<tr>
<td></td>
<td>VH</td>
<td>11</td>
<td>9-12</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>7</td>
<td>6-9</td>
</tr>
<tr>
<td>22 (n=15)</td>
<td>FOD</td>
<td>64</td>
<td>60-66</td>
</tr>
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<td>CBD</td>
<td>46</td>
<td>44-47</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>25</td>
<td>24-27</td>
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<tr>
<td></td>
<td>VH</td>
<td>12</td>
<td>12-13</td>
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<tr>
<td></td>
<td>VAP</td>
<td>8</td>
<td>8-9</td>
</tr>
<tr>
<td>23 (n=6)</td>
<td>FOD</td>
<td>63</td>
<td>60-65</td>
</tr>
<tr>
<td></td>
<td>CBD</td>
<td>47</td>
<td>43-48</td>
</tr>
<tr>
<td></td>
<td>TCD</td>
<td>23</td>
<td>21-24</td>
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<tr>
<td></td>
<td>VH</td>
<td>13</td>
<td>13-14</td>
</tr>
<tr>
<td></td>
<td>VAP</td>
<td>9</td>
<td>8-9</td>
</tr>
</tbody>
</table>

Table 4.
Age distribution of the fetuses and measurements from 17 to 23 gestation weeks.
GW = Gestation week;
n = Number of fetuses;
FOD = Fronto-occipital diameter;
CBD = Cerebral biparietal diameter;
TCD = Transverse cerebellar diameter;
VH = Vermian cranio-caudal diameter;
VAP = Vermian anterio-posterior diameter.
Study II

The three *post mortem* examinations were performed at 17-18 GW and the 60 *in vivo* examinations at 19-36 GW. The inter-observer agreement for the hippocampus and collateral sulcus was 90% and 97%, respectively.

The hippocampal sulcus could be seen in all fetuses. From figure 12, it can be seen that it was open in 39 of the 51 fetuses that were examined from 17 GW until 32 GW, but not later. In 35 of these, the sulcus was open bilaterally but in 21 the angle between its upper and lower lip was already less than 90 degrees bilaterally. Asymmetry was observed in the closing process in 11 fetuses that had both hippocampal sulci still open; the narrower was found on the right side in 8 fetuses and on the left side in 3.

A closed hippocampal sulcus was found in 28 fetuses. In four of these, from 21 to 30 GW, the right sulcus was closed but the left one was still open.

Non-oval hippocampal shapes could be found bilaterally at 24-29 GW, and unilaterally still at 31-36 GW (fig. 12). In the 12 fetuses aged GW 33 or more, when all the hippocampal sulci were closed, the shape of the hippocampus was still asymmetric in 4 (33%), being non-oval on the left side and oval on the right.

The collateral sulcus (fig. 13) was detected at the earliest at 17 GW (*post mortem*) but in one subject this sulcus could not be seen even at 29 GW. A deep, well-defined, collateral sulcus was seen at the earliest at 26 GW on the right and at 27 GW on the left.

In 14 (58%) of the 24 fetuses that presented a bilateral deep collateral sulcus, there was a uni- or bilateral vertical orientation. The collateral sulcus was vertical in 5/26 fetuses (19%) on the right side and in 13/25 fetuses (52%) on the left side, a difference that was statistically significant (*p* = 0.014). In the nine fetuses that associated a deep collateral sulcus with a non-oval hippocampal shape, six had a vertical orientation of the collateral sulcus.

In total, asymmetric hippocampal development was found in 26/63 fetuses (41%). The development was faster on the right in 23 fetuses and on the left in 3.
Results

### Figure 12.
Closure of the hippocampal sulcus and the shapes of the hippocampi in fetuses with a closed hippocampal sulcus. Each square pair describes a fetus. R = right side, L = left side.

- □ = open hippocampal sulcus,
- □□ = non-oval shape of the inverted hippocampus,
- □□□ = oval shape of the inverted hippocampus, the long axis horizontal (complete inversion).

### Figure 13.
Collateral sulcus at different gestation ages. Each square pair describes a fetus. R = right side, L = left side.
Collateral sulcus: □ = not visible, □□ = shallow, □□□ = vertical, □□□□ = oblique or horizontal.
Study III

**Post mortem** group: All the structures of the inner and middle ear were normal and they could be visualised with the maximum detail on our scaling score (fig. 14). The outer margin of the lateral semicircular canals could only be delineated on axial slices in 2 of the 16 fetuses (12.5%) but its normality could always be confirmed at coronal slices.

The external auditory canals could be delineated in 12.5% (2/16) of the fetuses.

**In vivo** group: Inter-observer agreement was 84% for the cochlea (WK = 0.67), 65% for the vestibular apparatus (WK = 0.49), 80% for the middle ear (WK = 0.56) and 84% (WK = 0.83) for the external ear.

Of all 122 examinations, symmetry of the findings was observed in 96% of the cases for the cochlea, 93% for the vestibule and 97% for the middle ear.

The cochlea (fig. 15a) could be identified in all examinations after GW 21, although never with sufficient detail to visualise all its components (i.e. detail level 4). In only two examinations, at GW 27 and 38, was it possible to delineate all of the cochlear turns (level 3). Nevertheless, cochlear turns could be identified in 75% of all the examinations in our cohort. Before 25 GW cochlear turns were seen in 50% (21/42) of cases, and in 89% later on.

The vestibular apparatus (fig. 15b) could always be depicted after GW 23 but a complete identification of all its components could never be reached. It was possible to identify the vestibule and the lateral semicircular canals in 72% (88/122) of all the examinations, and in all of those above 33 GW. Before and after 25 GW, respectively, these structures could be delineated in 31% (13/42) and 93% (74/80) of cases. A complete delineation of the lateral semicircular canals (level 3) could only be achieved in 30 examinations (25%), all but one later than GW 24.

Ossicles (fig. 15c) could be identified in 70% of the examinations (86/122), and in one case as early as GW 21. The ability to identify the ossicles was only 33% (14/42) before 25 GW, but afterwards it reached 90% (72/80). The image detail was never sufficient to discriminate the malleus from the incus, or to identify the stapes.

---

**Figure 14.**

*Post mortem* series.

a) Fetus at 22 GW, coronal plane. All the cochlear turns are identifiable. The head of the malleus and the external auditory canal are also seen. Note the low signal of the EAC at this age, occluded by an ectodermal plug.

b) Fetus at 17 GW, axial plane. Inside the cochlea (arrow), it is possible to differentiate the scala vestibularis from the scala tympanica. The modiolus is also seen on the contralateral side.

c) Fetus at 18 GW, axial plane. The LSC is almost completely delineated, at the exception of its outer margin (arrow).

d) Fetus at 20 GW, coronal plane. The malleus head, long process of the incus and even the stapes are identified.
Results

Before 25 GW, the external auditory canals (fig. 15d) could only be seen in two examinations, and only on one side. Both EAC were identified at 26 GW at the earliest, and in 59% of cases after 29 GW (29/49 examinations). In one case, the EAC could not be identified as late as 34 GW.

Figure 15.
*In vivo* series (n=122).

Ability to identify components of the a) cochlea, b) vestibule / LSC, c) middle and d) external ear from 20 to 38 GW. The level of detail for each structure (see definitions in methods) is displayed on a gray scale. Level 4 detail was never reached in this group.
Study IV

The readers could interpret all the 60 examinations, and the results are summarized in table 5.

The interobserver agreement (weighted kappa) was 0.79 for the upper lip, 0.70 for the primary palate, 0.86 for the secondary palate and 0.90 for the nasal septum. By clustering scoring levels 1+2 and 3+4, i.e. clustering probable and evident normality on one hand, and probable and evident abnormality on the other, interobserver agreement (linear kappa) was 0.81, 0.78, 0.73 and 1 respectively.

There was no statistically significant differences in the scoring levels of the readers across gestational age (p ≥ 0.58).

Of the 55 normal upper lips, 53 (96%) were correctly identified by both readers when the scores of the levels 1 and 2 were combined. However, reader 1 incorrectly suspected an abnormal upper lip in two fetuses of 34 and 36 GW.

Of the 56 normal primary palates, 54 (96%) were correctly identified by both readers (levels 1 and 2 combined), but reader 1 also suspected abnormal primary palates in two fetuses with normal primary palates; these being the same 34 GW fetus referred to above and a 27-GW-old fetus that only had a cleft lip.

The secondary palate was normal in 58 fetuses. Of these, 54 (93%) were correctly identified by both readers when the scores of the levels 1 and 2 were combined. Reader 1 erroneously suspected an isolated cleft of the secondary palate (29 GW), and both readers suspected extensions to the secondary palate in three fetuses with clefts only involving the lip and primary palate (23, 27 and 37 GW).

The nasal septum was considered normal (scoring levels 1 or 2) by both readers in all cases, at the exception of the two fetuses with confirmed unilateral primary palate clefts.

When summing the evaluations of both readers (i.e. 60×2 evaluations), incorrect suspicions of pathology existed in nine instances: four times a normal fetus was mislabeled as pathological and five times the degree of an existing cleft was over-rated. By anatomical structure, pathology was incorrectly presumed in 1.6% of the lips and primary palates, and in 5.8% of secondary palates.

In all fetuses that had confirmed cleft lips or cleft palates, both readers assessed the existence of orofacial pathology to be likely or evident on MRI.
Results

### Upper lip

<table>
<thead>
<tr>
<th>Score</th>
<th>Reader 1</th>
<th>Reader 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>43</td>
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<td>0</td>
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Table 5.
Scoring of readers 1 and 2 and interobserver agreement.
Scoring levels:
1) evidently normal,
2) probably normal,
3) probably abnormal
4) evidently abnormal.
True pathological cases in the series are marked with *.
For the nasal septum, cases with ** represent unilateral palate clefts.
MRI Studies of the Fetal Brain and Cranium

a. Vermian height vs. gestation weeks

b. Fronto-occipital diameter vs. gestation weeks

c. Cerebral biparietal diameter vs. gestation weeks

d. Transverse cerebellar diameter vs. gestation weeks

e. Vermis A-P diameter vs. gestation weeks

f. Vermian height vs. gestation weeks

Our cohort (n=70)
Parazzini et al (n=84)
DISCUSSION

In the studies of this thesis we have investigated the normal development of several cranio-cerebral structures that are not sufficiently covered in the fetal MRI literature, with special focus on the second trimester. The obtained results add to pre-existing knowledge and we believe they will be of benefit for evaluation of normality in these areas.

Our criteria to define “normality” and “normal” development must be clarified, since fetal MRI is a tertiary tool that is only performed when a disease is suspected. For the purpose of our project we used a normal postnatal clinical status for validation of the MRI examinations reported as “normal”, as it would not be ethical to perform post-natal control studies without any clinical indication.

Study I was the first published MRI series on fetal brain biometry that included fetuses below 20 GW. By using the same methodology as Parazzini et al, Tilea et al and Garel et al, our work contributed to a potential larger biometrical database spanning the period from 17 GW to full term (fig. 16 a-f).

CBD and cerebral FOD are both intended for evaluation of the brain growth, using roughly perpendicular axes. The CBD is a standard ultrasound measurement, but it is taken in a different manner than on MRI (axial plane, bone-to-bone), and therefore larger reference values are obtained. The FOD is difficult to assess by ultrasound but obtainable at MRI. Previous studies demonstrated a linear increase in FOD at the second trimester and we also found this pattern, even if with some scattering of the data.

The TCD is a standard measurement in fetal ultrasound. As expected, our data concurred completely with that method, since both US and MRI are able to evaluate this diameter in a precise way.

The vermis measurements showed the highest level of data dispersion in the series, which also was observed in Parazzini’s cohort. This suggests that MRI might not be accurate enough to evaluate vermian size at the 17-23 GW range.

Study II was the second in vivo fetal MRI study focused on the hippocampus and the first in which gyral development in the hippocampal region and the shape of the hippocampus was assessed. Our results show that there are wide individual variations, and a longer time for hippocampal closure than described before. We did also find some evidence that a non-oval shape of the hippocampus precedes a more mature oval shape and we demonstrated that there are significant differences in development between the left and right hippocampus.

The hippocampal region was well assessable in all post mortem examinations, but in vivo the image quality was much lower. In fact, 59% of the examinations available in the database were rejected for technical reasons. The youngest living fetus with a resolution good enough for hippocampal evaluation was examined at 19 GW.

The hippocampal sulcus was identifiable in all post mortem examinations, but in vivo the image quality was much lower. In fact, 59% of the examinations available in the database were rejected for technical reasons. The youngest living fetus with a resolution good enough for hippocampal evaluation was examined at 19 GW.

Concerning hippocampal shape, previous studies on formalin-fixed brain indicate that the hippocampus should assume an adult appearance by 21 GW, but we found a closed hippocampal sulcus only in one fetus at that age. Open hippocampal sulci could still be seen at 32 GW, thus reflecting a larger time variation for closure than previously thought.

When a closed hippocampal sulcus was seen, its
shape was classified as oval or non-oval, using the same criteria as in earlier studies on adults, children and premature neonates. As almost all the fetuses in our series with an oval hippocampus were at least 27 GW old and bilateral non-oval hippocampi were not observed after 29 GW, our data agree with the hypothesis that the non-oval shape precedes the oval shape in the hippocampal development.

The development of the hippocampus, including the hippocampal sulcus and the inverted hippocampal formation, was often asymmetric in our series (26/63 = 41%) and in a vast majority of these, the right side (23/26 = 88%) developed faster. This asymmetry was not observed in a previous prenatal MR study, where hippocampal infolding angles were measured at 20-37 GW, but another group that used the same methodology in infants and children found a left to right asymmetry and concluded for a slower development of the left hippocampus. The work by Chi et al, who evaluated photographs of 507 formalin-fixed brains from 10 to 44 GW also suggest a faster gyral development of the right cerebral hemisphere, the same being reported at a recent volumetric MR study that included the hippocampal sulcus.

The collateral sulcus also had a tendency to develop earlier on the right side and it was identifiable as early as at GW 17, even if still not visible in one fetus at GW 29. A vertical orientation was seen uni- or bilaterally in 58% of the fetuses in our cohort that presented a well-defined sulcus, a slightly larger number (p = 0.045) than the 36% described for children or adult populations. Also, this vertical orientation occurred more commonly on the left side, thus suggesting the possibility of a transitional phase towards a more mature horizontally oriented axis. However, this proposed hypothesis could not be confirmed within our own cohort, as we failed to find any significant association between the gestational age and the percentage of vertical collateral sulci.

Our study is limited by the small number of the fetuses, even if larger than earlier hippocampal studies on formalin-fixed brains. Repeated examinations of healthy fetuses would provide a better evaluation of the normal development, but this is not ethically acceptable.

**Study III** was the first publication on the development of the complete fetal ear in vivo. This study suffered from the expected difficulties when evaluating a very small organ, but it was possible to depict the components of the ear in more than 70% of the fetuses. However, by introducing a cut-off at the end of the second trimester, there was a significant difference in the scoring levels before and after that age, suggesting that MRI findings of the ear should be taken very cautiously before the third trimester.

The development of the ear begins at 4 GW and the labyrinth reaches a final adult shape and size by approximately 11 GW. The ossicles mature later, until 15-20 GW, and the tympanic cavity continues to enlarge until approximately GW 37. The external auditory canal only attains its final shape late in childhood.

Delineation of the small ear structures with fetal MRI is limited by movement, slice tilting and also by partial-volume averaging. Therefore, in order to better understand these limitations we decided to start our project by retrospectively analyzing a series of post mortem MRI examinations, to serve as a standard of what was possible to see in the ear using thin slices, absence of fetal motion and unlimited scan time.

The results of this post mortem group fully agree with the literature, with a near-perfect imaging quality being achieved for all organs. However, we encountered some difficulties upon assessing the lateral semicircular canals on axial views, as did Nemzek et al in their post mortem series. This might be related to the off-plane orientation of these brain-oriented axial slices, and also to a lack of T2 contrast with the surrounding non-osified mesenchyme at these early ages.

In the in vivo series, the symmetry in scoring for the inner and middle ear was always above 93%, attesting for an absence of significant slice tilting at the MRI studies we evaluated, but an identification of structures at a similar level as in the post mortem studies was never reached.
Discussion

The cochlea is known to be more prone to developmental malformations than the phylogenetically older vestibule \(^\text{114}\). Severe cochlear malformations are rare, but incomplete cochlear partitions are frequent \(^\text{115}\), being the second most common cause of congenital deafness after Sheibe’s membranous dysplasia \(^\text{116}\). In our series it was possible to obtain a detail that would be enough to exclude severe cochlear malformations in more than 70% of the fetuses, but a sufficient detail to rule out a simplification of the cochlea to 1.5 turns as described by Mondini (quoted in \(^\text{114}\)) could only be achieved in two cases (1.6%). To our surprise, the ability to identify the inner structure of the cochlea improved with gestational age, a feature we believe is related to a progressive gain of T2 contrast against the surrounding otic capsule as it ossifies.

The semicircular canals and remaining vestibule are also formed before 11 GW, with the exception of the vestibular sac and aqueduct that only reach full development in adolescence \(^\text{114}\). The lateral semicircular canals are the last ones to develop \(^\text{117}\) and they are particularly prone to malformations \(^\text{112}\). Indeed, it is very unusual for a malformation of the vestibular complex not to include the LSC \(^\text{114}\), and we postulate that their normality might be used as a hallmark for a normal vestibule as a whole. We were able to identify the LSC in more than 70% of the examinations in our cohort, but to see its entire contour was only possible in 24%, probably for the same reasons that disturbed its evaluation at the postmortem series. Again, the delineation of the LSC was only possible in 43% (18/42) of the studies before 25 GW, and we suppose this was related to the same lack of T2 contrast against the surrounding mesenchyme that affected the evaluation of the cochlea in young fetuses.

The visualization of the middle ear with fetal MRI has been the subject of a previous report \(^\text{91}\) concerning fetuses older than 25 GW. In that study the authors reported visualization of ossicles in 100% of the cases, but never with enough detail to discriminate each ossicle. In our study we were not able to identify ossicles in 30% of the fetuses, but most of these were younger than 25 GW. If we only take into account fetuses above 25 GW, as in the study referred above, ossicular identification was possible in 90% of the cases. In one fetus, however, the ossicles could not be identified as late as 33 GW.

The external auditory canals are normally only patent after 28 GW, after the progressive involution of an ectodermal plug that occludes them until then \(^\text{118}\). This absence of fluid inside the EAC surely contributed to the difficulty we had upon identifying this structure in our cohort, more often before 29 GW. Even afterwards, it could only be identified bilaterally with normal characteristics in 59% of the fetuses, thus suggesting MRI to be a suboptimal method for EAC evaluation before birth.

Overall, our goal to provide an insight into the development of the ear using in vivo fetal MRI proved to be a difficult task, which is reflected in the degree of interobserver agreement. The concordance of only 78% on average probably reflected our decision to use a rating system for structures that are at the limit of the spatial resolution of MRI, but blinding the second reviewer to the clinical outcome of the fetuses may also have been an important factor influencing the disagreement. Indeed, the main reader knew that a normal hearing of the subjects was part of the inclusion criteria, therefore introducing a reading bias due to expectation of normality.

We also realize that partial volume averaging did play a role in our results, as the 3-4 mm thick slices that are used in brain fetal MR studies represent a technical limitation that contributed to a suboptimal delineation of the very small structures we tried to evaluate.

Study IV is, to our knowledge the first MRI study to evaluate the lip and palate through all gestational ages, blinded to the results of previous ultrasound examinations. Our results support MRI as a reliable method for independent evaluation of these structures. One previous MRI report exists on the evaluation of the normal fetal profile including the lip and palate, but this report only included cases at 16 to 26 GW \(^\text{119}\).
The routine mid-trimester fetal ultrasound examination that is performed in most developed countries is accepted to have a sensitivity of 45-68% for the detection of cleft lips and a very low detection rate for isolated clefts of the palate. As it is then possible for facial anomalies to be missed on ultrasound and be later found on a fetal MRI that is referred for other purposes, it is important to have a notion of how well MRI independently evaluates the lip and palate on standard brain-targeted scans, out of its usual complementary role towards ultrasound.

In our study, it was possible to correctly delineate these structures in almost all of the fetuses with a “substantial” level of interobserver agreement, regardless of gestational age. We used quadratic weighted kappa as opposed to linear kappa to measure interobserver agreement for the four-grade scale, since the different levels in our scale did not carry a linear difference. Both readers correctly identified all the fetuses with clefts included in the series, but pathology was overdiagnosed.

The formation of the upper lip is completed at 7 GW, after the fusion of the paired medial nasal prominences and maxillary prominences. These medial nasal prominences also merge at a deeper level, forming the intermaxillary segment that includes the four incisor teeth and the triangle-shaped primary palate. The secondary palate is formed independently from two outgrowths of the maxillary prominences, the palatine shelves. The maxillary prominences also form the remaining of the upper alveolar ridge and the soft palate. Disturbances in the process may produce clefts, but these do not seem however to result merely from a mechanical mismatch in coupling, and local mesoderm penetration failure or a disturbance in migration/organization of neuroepithelial cells have been proposed as potential contributing factors. Isolated clefts of the secondary palate might be distinct embryological entities, as these are more common in females and not influenced by maternal age, as opposed to cleft lip / primary palate.

Visualizing the upper lip is a part of the checklist of the standard mid-trimester sonography. However, fetal age and motion, covering of the face with the hands or arms, maternal constitution or equipment-related limitations can preclude a correct identification of this area, thus justifying the relatively low accuracy 2D ultrasound has for detecting lip malformations. False positives, on the other hand, are extremely rare in low-risk populations when using this technique, without any single case being found by Offerdal et al. in a prospective series of 49,341 fetuses.

MRI examinations are likewise limited by a number of fetal and mother-related factors that may disturb the evaluation of the fetal facial profile. The lips may be obscured by the contact with the placenta or by superposing the arms and, in fact, it was a contact of the fetal face with the arm the cause of one falsely abnormal lip suspected by Reader 1 in our series (fig. 17). This represents a pitfall of MRI by not being a method as interactive as ultrasound, where a delay of just a few seconds may help to clarify any posture-related false images.

On the contrary to the facial profile, both the primary and secondary palate of fetuses are very difficult to investigate with ultrasound, especially if there is no suspicion of an associated cleft lip. Even in its presence, a 25% error can be expected to exist when evaluating the degree of palatal involvement by this method. The detection of isolated palate abnormalities by ultrasound can be as low as 0 to 7%, but the accuracy increases for high-risk populations seen at tertiary care units, or when volume acquisitions for the face are used.

MRI allows a clearer visualization of the palate (fig. 18), but fetal motion, young fetal age, or the absence of amniotic fluid between the palate and the tongue may limit its accuracy. This lack of T2 contrast between the tongue and the palate has been held responsible for a 16% failure rate for palate delineation in a previous series and the same probably explained the incorrect suspicions both readers in our work had of posterior extensions of primary palate clefts to the secondary palate (fig. 19).
Discussion

Figure 17.
The arm of this 36 GW fetus is seen touching the lip and hiding its contours. Lip pathology was erroneously suspected (scoring level 3) by one reader.

Figure 18.
Normal anatomy of the palate and lips. Fetus of 27 GW.

a) Sagittal plane. The posterior limit of the primary palate (arrow), the secondary palate (short dashed arrow) and upper alveolar ridge (long dashed arrow) are pointed.

b) Axial plane. The bone of the hard palate (dashed arrow) is seen, with a lower signal than the posteriorly located soft palate (arrowhead).

Figure 19.
A cleft primary palate can be seen (arrow). The absence of fluid between the tongue and palate - compare with figure 17 - lead both readers to a false suspicion of a cleft secondary palate in this 37 GW fetus (scoring level 3).
It is known that the nasal septum tends to be deviated to the homolateral side when a unilateral cleft palate is present. In fact, in our series deviated septa were only found in such instances, thus suggesting this finding to be a valuable indirect sign of an unilateral cleft palate.

Our study was intended to be descriptive, with the aim to assess how well experienced neuroradiologists could delineate the normal anatomy of the lip and palate on fetal MRI, blinded to previous ultrasound findings. The detection of the abnormal fetuses and their assumptive diagnosis was only a secondary aim, because the amount of pathological cases in our database was too small to draw conclusions about this group. Furthermore, a selection bias for this particular material could not be excluded, since all of them had been earlier suspected on US and diagnosed on MRI. Our reason to include these pathological cases at the series was mainly to induce some incertitude in the readers and to avoid a potential bias towards expecting normality, as it is known that only 1/700 fetuses have malformations in this area. However, a reading bias in the reverse sense (i.e. towards over-scoring pathology) might have influenced the results, since the readers were asked to focus on the palate and lips, which is not a special area of attention when performing brain fetal MRI in daily practice.
Conclusions and Future Directions

CONCLUSIONS

This MRI project focused on the normal development of the fetal brain and skull.

We provided:

a) New MRI reference data for brain measurements in young fetuses.

b) New data on the fetal hippocampus, that seems to develop later and more asymmetrically than previously thought.

c) The first research on the development of the ear *in vivo*.

d) The notion that brain-targeted MRI is reliable for independent evaluation of the lip and palate across gestation.

FUTURE DIRECTIONS

I do not dare to predict the future of fetal MRI (or of any other medical area, for that matter…). However, it seems clear that the method is nowadays waiting for “ethical clearance” to move into higher field strengths that may provide a higher spatial resolution and a better access to more “physiological” approaches to the fetal development, as functional MRI or MR spectroscopy.

At the pace permitted by electronics and software, the quality of imaging will surely improve in the future, including methods to overcome the limitations that result from fetal and maternal motion.

But MRI is just a tool, and it needs skilful operators in order to be useful. Therefore, I hope for the future development of a broader network of knowledge in fetal MRI, spanning as many centres as possible, and also for an international “teaching core” for the medical training in this field.

For myself, I would like to know more about the functional aspects of the fetal development. Although probably difficult in practice, research on “resting-state” functional MRI seems to be worth a try, as has been recently shown by the Vienna group 127.

We shall see….
MRI Studies of the Fetal Brain and Cranium

RESUMO EM PORTUGUÊS

A ultrassonografia é o método imagiológico padrão para estudo fetal, mas a RM tem hoje um papel complementar importante, uma vez que muitas vezes revela alterações que alteram a gestão da gravidez de um modo significativo.

A RM tem sido também usada para estudar o desenvolvimento fetal normal, mas há falta de conhecimento em algumas áreas clinicamente relevantes, tais como padrões para as dimensões normais do cérebro ao longo da gestação, o desenvolvimento normal do hipocampo ou do crânio fetais.

O objetivo desta tese foi colmatar algumas desaﬁos, focando no período antes do fim do segundo trimestre, prazo limite para interrupção de gravidez por causa fetal em muitos países (incluindo Portugal). Procurámos a obtenção de dados biométricos para normalidade antes de 24 semanas de gestação, estudámos o desenvolvimento do hipocampo e do ouvido fetais, e tentámos ainda avaliar o valor da ressonância magnética para avaliar o lábio e palato fetais.

Para isso, foi analisada retrospectivamente uma base de dados com aproximadamente 450 RM fetais in vivo e 21 post mortem.

O estudo I avaliou uma série de 70 exames de RM fetais in vivo normais. Uma tabela de medidas normais do cérebro entre as 17e 23 semanas de gestação foi construída, a primeira na literatura que inclui idades abaixo de 20 semanas de gestação.

O estudo II centrou-se na evolução do hipocampo a partir das 18 semanas de gestação, avaliando 3 RM post mortem e 60 in vivo. Este estudo sugere que o desenvolvimento do hipocampo fetal se faz mais tarde e de um modo mais assimétrico do que previamente descrito.

O estudo III foi analisada uma série de 122 RM fetais in vivo normais e 16 post mortem. Descrevemos pela primeira vez na literatura o desenvolvimento do ouvido fetal in vivo, dando-nos conta que a RM fetal é limitada pelo tamanho das estruturas avaliadas, sobretudo até ao fim do segundo trimestre de gestação.

No estudo IV, os lábios e palatos de 55 fetos normais e 5 fetos com fendas orofaciais foram analisados de modo cego por dois revisores. Os nossos resultados sugerem uma alta precisão da RM na avaliação desta área, independentemente da idade fetal ou de dados ultrassonográficos anteriores.

Este trabalho traz portanto novos conhecimentos sobre o desenvolvimento normal do cérebro e crânio fetais.

SAMMANFATTNING PÅ SVENSKA

Ultraljudsundersökning är första-handsmetod för fosteravbildning men magnetisk resonanstomograﬁ (MRT) kan vara ett värdefullt komplement. MRT kan ofta ge tilläggsinformation som kan påverka rådgivning och handläggning och har gett ökad förståelse för hjärnans normala utveckling.

Inom vissa kliniskt relevanta områden är kunskapen dock bristfällig, t.ex. avseende normalinterval för olika hjärnmått, hippocampus utveckling och bedömningen av öra och ansikte. Syftet med studierna i denna avhandling var därför att med MRT samla normaldata för olika hjärnmått i tidig graviditet, studera utvecklingen av hippocampus, undersöka hur väl olika strukturer i fosterörat kan bedömas med MRT och undersöka hur väl fostrets överläpp och gom kan bedömas med MRT.

För att rätt kunna bedöma resultatet av en MR-undersökning av fosterhjärnan är det viktigt att känna till normalvariationen. Referensdata för olika mått inom hjärnan finns sedan tidigare för ultraljud. En del av de mått som kan göras med MRT dock kan inte utföras med ultraljud och vissa mått mäts på olika sätt med ultraljud och MRT. Separata referensmaterial för MRT behövs därför. För MRT finns endast ett fåtal studier med normaldata publicerade och endast efter graviditetsvecka 19. I 70 MR-undersökningar av foster vid 17-23 graviditetssveckor uppmättes olika mått i storhjärna och lillhjärna. Alla mätningar kunde
göras. En tabell med normalintervall från graviditetsvecka 17 till 23 skapades. För de av mått-
ten som kan mätas med ultraljud överensstämmer resultaten väl med ultraljudsreferensdata. För de
graviditetsveckor där det finns tidigare MR-stu-
dier överensstämmer resultaten väl med dessa.

Hippokampus är en struktur i tinninglobens
inre del som tidigare studier av vuxna visat ofta ha asymmetrisk form. In vivo-studier av hippo-
kampusform hos foster med MRT saknas. I 60
normala in vivo och 3 post mortem MR-undersök-
ingar studerades förekomst och slutning av
sulcus hippocampalis, hippokampusform och förekomst och orientering av sulcus collateralis.
Sulcus hippocampalis sågs i alla foster. Den var
oftast sluten efter 28 gestationsveckor och aldrig
öppen efter 32 gestationsveckor. Icke-ovala hip-
pokampusformer sågs bilateralt vid 24-29 gesta-
tionsveckor och unilateralalt fortfarande vid 31-36
graviditetsveckor. Asymmetrisk hippokampussut-
veckling sågs i 41% av fostren och snabbare på
höger sida i 88% av dem.

Missbildningar inom inneröra, mellanöra eller
hörselgång är inte ovanliga och kan ibland vara
del i ett missbildningssyndrom. Bedömningen
av dessa strukturer med ultraljud är svår. Det har
inte systematiskt utvärderats hur väl man kan be-
döma fosterörat vid olika graviditetsländer med
MRT. Bedömbarhet med MRT in vivo av snäcka,
båggångsapparat, hörselben och yttre hörselgång
studerades hos 122 foster vid 20-38 graviditets-
veckor. Samtliga hade normal postnatal uppfölj-
ing. Samma strukturer studerades dessutom hos
16 foster undersökt post mortem vid 15-22 gra-
viditetsveckor. Snäckans vindlingar, laterala båg-
gången och hörselbenen sågs med MRT in vivo i
50/30/33% och 89/93/90% före respektive efter 25
graviditetsveckor. Skillnaden i bedömning av ör-
ats strukturer före resp. efter 25 graviditetsveckor
tolkas bero på ökad kontrast mellan olika struktu-
er med ökande grad av förbening. Med undantag
av yttre hörselgången kunde alla studerade struk-
turer i örat avgränsas i 100% av post mortem-un-
dersökningarna, där detaljupplösningen var bättre
pga. att undersökningsstiden inte var begränsad.
Detta talar för att bedömningen av fosterörat med
MRT in vivo kan förbättras om tekniken utveck-
las.

Läpp-käk-gomspalt (LKG-spalt) ses i 1/700 föds-
lar och kan vara svår att diagnostera intrauterint
med ultraljud, särskilt vid isolerad gomspalt. Det
har inte tidigare studerats hur väl överläpp och
gom kan bedömas med MRT vid olika graviditets-
längder. Överläpp och gom studerades av två obe-
roende undersökningsgrupper vid 60 MR-undersök-
ingar vid 20-38 gestationsveckor. Bedömare 1 och 2 iden-
tifierade 100% av fall med LKG-spalt, 96-100% av
normala överläppar och primära gomanlag och
93-100% av normala sekundära gomanlag. Be-
dömbarheten skiljde sig inte signifikant med gra-
viditetsländen. Resultaten tyder på att överläpp
och gom är väl bedömbare med MRT, oberoende
av graviditetslängd. Bedömning av dessa struktu-
er bör därför ingå som rutin vid MRT av foster
och kan leda till upptäckt av annars odagnostice-
rade LKG-spalter.

Avhandlingen tillför: referensvärden för hjärn-
mått i tidig graviditet med MRT, kunskap om ut-
vecklingen av hippokampus och kunskap om be-
dömbarheten av fosteröra, överläpp och gom med
MRT vid olika graviditetsländer.
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