[Teresa Margarida da Fonseca Alves Loureiro]

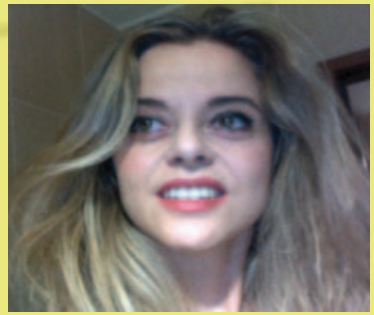
# DESCRIÇÃO DA ANATOMIA DO CÉREBRO FETAL NORMAL E ANÓMALO POR ECOGRAFIA TRIDIMENSIONAL NO PRIMEIRO TRIMESTRE DA GRAVIDEZ

# DESCRIPTIVE ANATOMY OF THE NORMAL AND ABNORMAL FETAL BRAIN BY THREE-DIMENSIONAL ULTRASOUND IN THE FIRST TRIMESTER OF PREGNANCY

DISSERTAÇÃO DE DOUTORAMENTO EM MEDICINA

PHD THESIS

2012



[Teresa Loureiro]

Teresa Loureiro was born in Coimbra, Portugal, on the 28th January 1972.

She concluded her pre-university education in 1989. In 1995, she graduated from the University of Coimbra Medical School. She worked as a junior resident for two years at University of Coimbra Hospital. She went on to complete her Obtetrics and Gynecology residency at S. João Hospital, Porto (1998-2004). Thereafter she worked as a staff specialist in Obstetrics and Gynaecology at Vale do Sousa Hospital, and since 2006, at S. João Hospital, Porto, where she works at the Ultrasound and Prenatal Diagnosis Unit. In addition to outpatient work, she has regular sessional work on delivery suite of S. João Hospital, Porto. In 2009-2010 she went on to a fellowship in Fetal Medicine at Harris Birthright Centre, King's College Hospital, London, UK.

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The present research was started in 2009, under the supervision of Prof. Nuno Montenegro and co-supervision of Prof. Kypros Nicolaides.

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Dissertação de candidatura ao grau de
Doutor em Medicina, na área de Obstetrícia,
submetida à Faculdade de Medicina
da Universidade do Porto


**Orientador:** Prof. Doutor Nuno Montenegro

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Os estudos sobre os quais se desenvolveu esta tese foram realizados no Serviço de Obstetrícia e Ginecologia do Centro Hospitalar de S. João, Porto, Portugal; no Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Londres, Reino Unido e Screening Unit, University College Hospital, Londres, Reino Unido.

The studies presented in this thesis were performed at the Department of Obstetrics and Gynecology, S. João Hospital, Medical School, University of Porto, Porto, Portugal; Harris Birthright Research Centre for Fetal Medicine, King's College Hospital and Screening Unit, University College Hospital, London, UK.

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| 2D US | Two-dimensional ultrasound |
| 3D US | Three-dimensional ultrasound |
| 3V | Third ventricle |
| 4V | Fourth ventricle |
| A | Aqueduct of Sylvius |
| ACC | Agenesis of the corpus callosum |
| AChE | acetylcholinesterase |
| AFP | Alphafetoprotein |
| AMA | Anterior membranous area |
| BPD | Biparietal diameter |
| BS | Brainstem |
| BSOB | Brain stem to occipital bone diameter |
| CLR | Choroid plexus area to lateral ventricle area ratio |
| CM | Cisterna magna |
| CM II | Chiari malformation II |
| CNS | Central nervous system |
| CRL | Crown-rump length |
| CSF | Cerebrospinal fluid |
| DWM | Dandy-Walker malformation |
| DWV | Dandy-Walker variant |
| eCSF | Embryonic cerebrospinal fluid |
| GA | Gestational age |
| HW | Hemispheric width |
| IT | Intracranial translucency |
| LOA | Limits of agreement |
| LV | Lateral ventricle |
| LVW | Lateral ventricle width |
| MMC | Myelomeningocele |
| N | Number of cases |
| NT | Nuchal translucency |
| NTD | Neural tube defects |
| PMA | Posterior membranous area |
| Sens | Sensitivity |
| Spec | Specificity |
| TA | Transabdominal scan |
| TV | Transvaginal scan |
| US | Ultrasound scan |
| VM | Ventriculomegaly |

***‘Measure what is measurable, and make measurable what is not so.’***

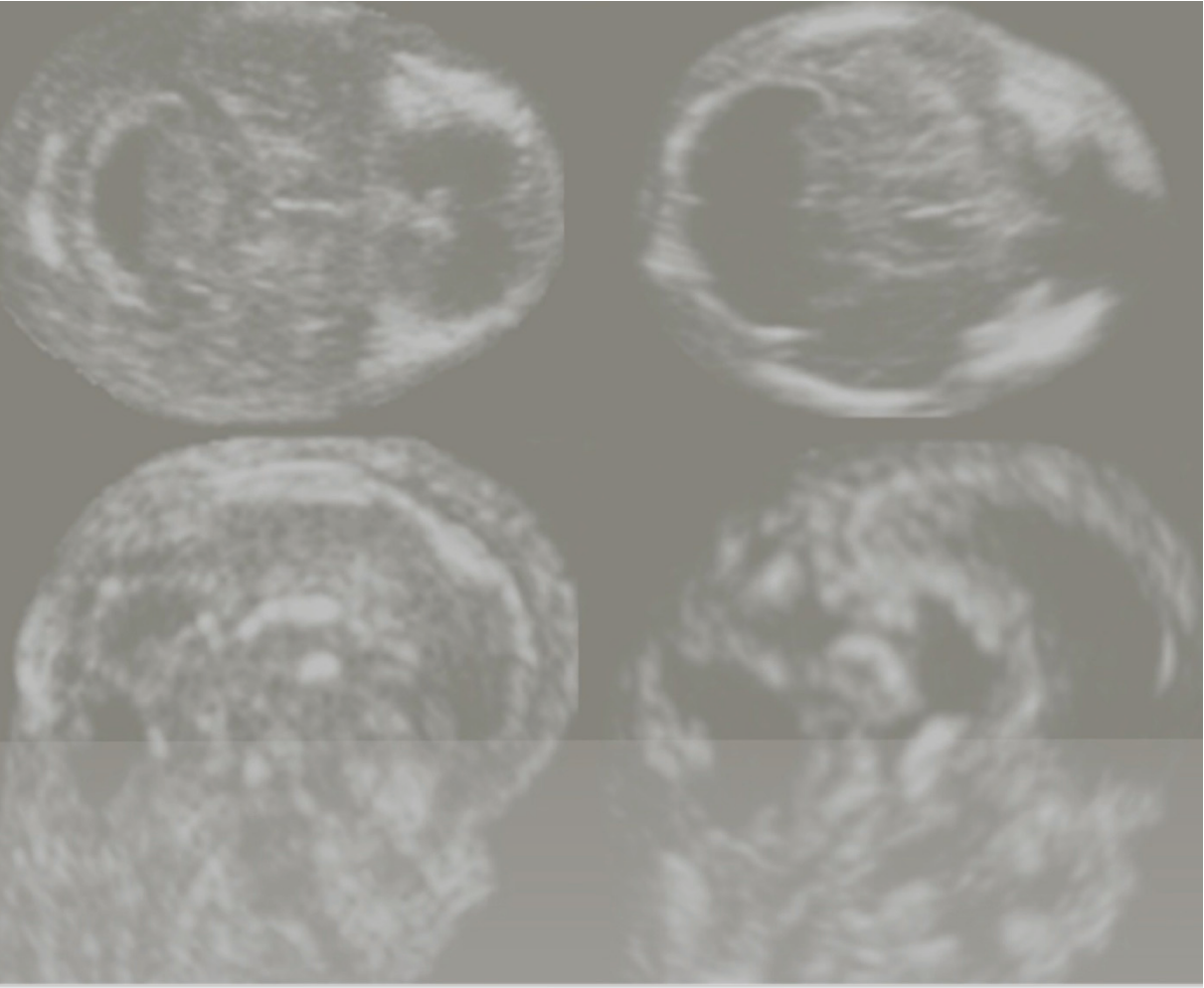
*Probably Galileo´s quotation, undoubtedly Nicolaides’ application.*

*Drawing of our scanning room in Ultrasound Unit of S. João Hospital made by a 4-year-old girl picturing me, her mother and a junior doctor working at the computer.*

*To my grandmother Teresa*

*To my parents António e Teresa*

*To my teachers*



# RESUMO

# SUMMARY

## RESUMO

O papel da ecografia na prática obstétrica acompanha o engenho da técnica, o desafio da clínica e o desígnio da grávida.

Desde os trabalhos realizados nos anos 90 até à data, é crescente a evidência na literatura da utilidade da ecografia realizada às 11-13 semanas como teste de rastreio de cromossomopatias fetais e complicações durante a gravidez, como a pré-eclâmpsia e o parto pré-termo. A excelente resolução da imagem que as novas sondas transabdominais e transvaginais de alta frequência possibilitam pode aumentar a eficácia diagnóstica do exame morfológico realizado durante o primeiro trimestre. Vários estudos sugerem a exequibilidade de uma avaliação mais detalhada da anatomia fetal no exame ecográfico do primeiro trimestre da gravidez. No entanto, o estudo do cérebro do feto coloca algumas dificuldades na gravidez inicial. Em contraste com outros órgãos fetais, o cérebro é a única estrutura que durante todo o seu processo de crescimento e maturação intrauterina modifica continuamente a sua anatomia. Durante a fase pós-embrionária, particularmente às 11-13 semanas de gravidez, a informação disponível na literatura versando o desenvolvimento cerebral fetal é escassa. A descrição ecográfica do desenvolvimento normal do cérebro durante o primeiro trimestre é fundamental para a compreensão e reconhecimento de padrões precoces de desenvolvimento atípico ou anómalo.

No **CAPÍTULO 1**, é discutido o papel geral da ecografia como teste de diagnóstico e de rastreio em Obstetrícia, bem como a aplicação dos ultrasons ao estudo do cérebro fetal. Adicionalmente, descrevem-se conceitos básicos relativos à neurosonografia fetal associada à tecnologia tri-dimensional (3D) e é feita referência aos principais trabalhos publicados nesta área. Relativamente à utilização da ecografia 3D na avaliação do cérebro fetal, existem algumas vantagens na realização deste exame durante o primeiro trimestre relacionadas com as menores dimensões do feto, a presença constante de líquido amniótico a rodear as estruturas fetais e as características das fontanelas, que nesta altura são proporcionalmente maiores, evitando a sombra acústica produzida pelos ossos do crânio na

avaliação do cérebro fetal. Um resumo do desenvolvimento inicial do sistema ventricular é descrito neste capítulo, bem como a formação do líquido cefalo-raquídeo (LCR) no embrião e feto. No segundo trimestre, há evidência de que anomalias cromossómicas e malformações fetais graves do sistema nervoso central, tais como defeitos do tubo neural e anomalias da fossa posterior, estão associadas a alterações do sistema ventricular cerebral. Neste contexto, coloca-se a hipótese de que variações nas dimensões das várias estruturas do sistema ventricular cerebral às 11-13 semanas possam ser úteis na identificação ou rastreio de desenvolvimento anómalo do cérebro fetal e aneuploidias. No CAPÍTULO 1 é feita uma revisão sumária da embriopatologia, etiologia e prognósticos relativos às anomalias da fossa posterior, ventriculomegalia e às doenças de tubo neural, focando com maior detalhe, neste último grupo, os defeitos abertos da coluna ou mielomeningocelo.

Esta tese tem como objectivo principal a descrição do sistema ventricular cerebral em fetos normais e em fetos afectados pelas aneuploidias mais frequentes (Trisomia 21, 18 e 13) ou por mielomeningocelo. Para conseguir este propósito, foram construídas curvas de referência para cada uma das estruturas do sistema ventricular cerebral fetal, que foram avaliadas através da medição dos respectivos diâmetros ou áreas. Nos fetos com aneuploidias e nos casos de mielomenogocelo, as medições obtidas foram comparadas com os valores de referência calculados para a população normal. É esperado que os marcadores ecográficos descritos possam ser clinicamente úteis na optimização do rastreio de fetos portadores das anomalias referidas (CAPÍTULO 2).

No CAPÍTULO 3 descreve-se de forma detalhada a metodologia da realização das medições do sistema ventricular cerebral em fetos normais às 11-13 semanas de gravidez. A área dos ventrículos laterais e dos plexos coroideus e o diâmetro do terceiro ventrículo aumentam com a idade gestacional, o diâmetro do aqueduto de Sílvius diminui e o diâmetro do quarto ventrículo mantém-se estável às 11-13 semanas. Foram realizados testes para a avaliar a variabilidade intraobservador e interobservador em 50 casos.

No CAPÍTULO 4, as diferenças relativas às dimensões do sistema ventricular entre fetos normais e afectados por mielomeningocelo são descritas. Às 11-13 semanas, em fetos portadores de mielomeningocelo, a quantidade de LCR intracraniano está significamente diminuída, reflectindo-se nos resultados obtidos pela medição da área do ventrículo lateral e diâmetros do aqueduto de Sílvius, terceiro e quarto ventrículos, cujos valores estão significamente diminuídos relativamente aos normais.

No CAPÍTULO 5, a medição do quarto ventrículo, que reflete o desenvolvimento da fossa posterior, foi avaliada em fetos com anomalias cromossómicas e comparada com os valores de referência relativos à população normal. O quarto ventrículo apresenta-se significativamente mais dilatado nos fetos com trissomia 18, 13 e triploidia, mas não nos casos de trissomia 21, relativamente aos fetos euploides.

No CAPÍTULO 6 é descrita a associação entre aneuploidias e ventriculomegalia detectada às 11-13 semanas. Os resultados mostram que na maioria dos fetos com trissomia 13 e em cerca de um terço dos fetos com trissomia 18 existe ventriculomegalia às 11-13 semanas, avaliada através do *ratio* entre a área dos plexos coroideus e a área dos ventrículos laterais.

Os resultados desta investigação demonstram que existem diferenças nas dimensões das estruturas do sistema ventricular cerebral fetal entre fetos euploides e com cromossomopatias e entre fetos normais e afectados por mielomeningocelo, que são estatisticamente significativas, e que podem ser detectadas às 11-13 semanas. A avaliação do sistema ventricular é exequível e variações do conteúdo líquido intraventricular fetal são notórias mesmo para observadores menos treinados. O desenvolvimento de um programa de *software* para identificar e quantificar estas regiões com conteúdo líquido, hipoecogénicas, em volumes 3D adquiridos em exames de rotina, possibilitando uma estimativa automatizada das suas dimensões absolutas, poderia reduzir a variabilidade interobservador. Um obstáculo no presente é a falta de acuidade das medições automatizadas em estruturas inferiores a 0.5 cm. No entanto, a evolução tecnológica, a imparável

melhoria da qualidade da imagem e o desenvolvimento de novos *software* e sistemas computorizados poderá tornar possível este desígnio nos próximos anos e permitir o uso generalizado de alguns destes marcadores cerebrais no rastreio, em especial relativamente ao rastreio do mielomeningocelo.

## SUMMARY

The part played by ultrasound in obstetric practice is galvanized by advances in technology, clinical challenges and patients' needs.

Ultrasound examination during the first-trimester has been shown to be useful in screening for aneuploidies but also in the diagnosis of fetal defects and in screening for pregnancy complications, such as preeclampsia and preterm delivery. Diagnosis of an increasing number of fetal defects has been made possible by the use of new high resolution transabdominal and high frequency transvaginal ultrasound probes. However, examination of the fetal brain and diagnosis of brain defects poses major challenges. In contrast to other organs, the brain is the only structure in the fetus that carries on modifying its anatomy during normal development. During post embryonic phase, particularly at 11 to 13 weeks' gestation, the available data in literature concerning brain development is limited. It is therefore essential to describe the normal developmental events of the early fetal brain to understand and describe early pathology.

In CHAPTER 1, the overall role of ultrasound as a diagnostic and screening test is discussed, as well as the application of ultrasound in the evaluation of the fetal brain. The use of three-dimensional (3D) ultrasound applied to fetal neurosonography is explored. The first trimester presents some advantageous features for application of 3D ultrasound in relation to later in pregnancy because of particularities related to the size of the fetus, the amount of amniotic fluid and to the presence of wide fontanels with consequent less shadowing from cranial bones. A summary of the initial development of the ventricular system is described as well as the formation of cerebrospinal fluid. Chromosomal abnormalities and severe fetal defects, like open spina bifida and posterior fossa abnormalities, have been linked to maldevelopment of the brain and cerebral ventricular system. Ventriculomegaly indicates the presence of a relatively excess of fluid in lateral ventricles. This chapter discusses the embryology, etiology and prognosis of these pathologies and provides a summary of the previous

studies aiming to assess these conditions during the first trimester of pregnancy.

CHAPTER 2 describes the main objective of this thesis which was to examine the cerebral ventricular system in normal fetuses and in those affected by aneuploidies (trisomy 21, trisomy 18, trisomy 13) and open spina bifida at 11-13 weeks' gestation. To achieve this target, comprehensive transvaginal ultrasonographic reference ranges of the different components of the cerebral ventricular system were calculated and the measurements obtained from abnormal fetuses were compared to normal ranges. The ultimate goal was to find brain markers more prevalent in aneuploid fetuses and in those with open spina bifida. It is expected that these new brain markers may ultimately prove themselves useful in improving screening for aneuploidies and open spina bifida.

CHAPTER 3 describes in detail the measurements of the different components of cerebral ventricular system in normal fetuses at 11-13 weeks' gestation and how they relate to biparietal diameter (BPD), that is gestational age. We found that the lateral ventricles area, choroid plexuses area and the third ventricle diameter increase with BPD, the width of the Aqueduct of Sylvius decreases and the anteroposterior diameter of the fourth ventricle is stable at this stage of gestation. This chapter also provides data on the reproducibility of measurements.

CHAPTER 4 reports the differences in size of the various components of the ventricular system in fetuses with open spina bifida. In fetuses with open spina at 11-13 weeks' gestation the intracranial collection of cerebrospinal fluid is substantially reduced which is reflected in the measurements of lateral ventricle area and diameters of the roof of the third ventricle, aqueduct of Sylvius and fourth ventricle which are all significantly decreased.

In CHAPTER 5, the fourth ventricle measurement, which is related to posterior fossa development, was measured in fetuses with aneuploidies and was compared to the reference normal ranges. We found that the fourth ventricle

diameter is significantly increased in fetuses with trisomy 18, trisomy 13 and triploidy but not in cases with trisomy 21 as compared to euploid fetuses.

CHAPTER 6 describes the association between aneuploidies and lateral cerebral ventriculomegaly at 11-13 weeks' of pregnancy. The results show that most fetuses with trisomy 13 and in one third of fetuses with trisomy 18 there is ventriculomegaly, assessed by the ratio between choroid plexus and lateral ventricle areas.

The results from this research show that there are differences in the measurements of the components of the cerebral ventricular system between normal fetuses and those with aneuploidy and open spina bifida and these differences can be detected as early as 11-13 weeks' gestation. Evaluation of the ventricular system is feasible and changes of fluid content are striking even to the untrained eye. With this thesis it is hoped to reduce the subjectivity in appreciation of the amount of fluid in the ventricular system. The development of software to identify and quantify hypoechoic regions within a 3D dataset, providing an automatic estimation of their absolute dimensions, would most probably reduce interobserver variability in cerebral ventricular system fluid analysis. One obstacle for the moment is the lack of accuracy of automatic volume assessments in measuring structures below 0.5 cm. Nevertheless, improvements in image quality of ultrasound machines as well as development of new software and computer-based systems will eventually assist this purpose.

# Chapter 1

# Background

## ABSTRACT

Routine antenatal ultrasound scanning is now extensively used in obstetric practice both as a diagnostic as well as a screening tool, aiming to date the pregnancy, identify the number of fetuses and type of placentation, detect fetal structural abnormalities, markers of fetal conditions or pregnancy complications and evaluate the growth and well-being of the fetus. Approximately 3% of newborns have a noticeable major anomaly and, in developed countries, congenital malformations play a significant part in perinatal mortality.

The provision of effective first-trimester screening and the preference of pregnant women to have screening performed in the first rather than in the second trimester have led to widespread uptake of first trimester ultrasound examination. This fact in association with a better resolution provided by transabdominal and high frequency transvaginal ultrasound probes motivated a more comprehensive look at fetal anatomy in early pregnancy. The first trimester presents some advantageous features for application of 3D ultrasound in relation to later in pregnancy as the fetus is small, has invariably amniotic fluid around it and the cranial bones produce minimal acoustic shadows.

The brain ventricular system is formed by cavities filled with cerebrospinal fluid (CSF) and appears on ultrasound as prominent related black spaces in the fetal brain at 11-13 weeks' gestation. Chromosomal abnormalities and severe fetal defects, like open spina bifida and posterior fossa abnormalities, have been linked to maldevelopment of the brain and the cerebral ventricular system. Making use of both high frequency ultrasound probes and 3D technology it is intended to achieve a better understanding of the normal and abnormal early cerebral ventricular system.

## 1.1. GENERAL VALUE OF ULTRASOUND IN PRENATAL DIAGNOSIS AND SCREENING OF FETAL ABNORMALITIES

Ian Donald first used ultrasound to visualize the fetus in 1957 (McNay MB et al, 1999) and since then ultrasound has made a major contribution to changes in obstetric practice. Routine antenatal ultrasound scanning is now widely adopted as a diagnostic tool to identify structural defects, but also as a screening test by recognizing defects or markers that indicate an increased risk of a disorder or pregnancy complications (Nicolaides KH, 2011).

The detection of fetal abnormalities is generally accepted by both patients and clinicians as beneficial. Approximately 3% of newborns have a noticeable major anomaly and, in developed countries, congenital malformations play a significant part in perinatal mortality (Nyberg DA, 2003). Nevertheless, a randomized study failed to show a statistically significant benefit of ultrasound in improving perinatal care (Crane JP et al, 1994). Ultrasound examination provides a general assessment of the fetus therefore sonographic findings may be seen in the absence of a true condition. It is subjected to many operator dependent variants like experience, time investment and equipment performance. A review by Levi concluded that the clinical value of routine ultrasound is determined by the standard of scanning that should be sufficiently high (Levi S, 2002).

Anomaly detection by US scan has been reported to vary from 13% to 82% (Levi S, 2002). A review of the results of 9 studies assessing the value of ultrasound for the detection of fetal anomalies showed that the reported prevalence of anomalies varied from 5.7 to 26.0 per 1000 fetuses examined and the detection rate varied from 3.1 to 11.0 per 1000 (Anderson et al, 1995). These very broad ranges reflect imprecise estimates of detection rates, disregard for the true prevalence of the condition and inexact false-positive rates. In contrast to the predictive value of a test, the sensitivity should be similar in any given population and be unaffected by the prevalence of the disease. Concerning ultrasonographic screening for malformations, the awareness of high risk factors may prompt a more

detailed examination of the fetus with consequent increase in efficacy. In addition, most of the major disorders are relatively rare and a positive result is often followed by termination of pregnancy, frequently without necropsy to determine diagnostic accuracy (Ramalho C et al, 2006, 2010). Differences in the criteria used for recording malformations make it difficult to compare the different studies. Some authors have excluded *a priori* abnormalities that are considered undetectable by ultrasonography, which may differ among centres. Overall, the detection rates are likely to be over-estimated and the false-positive rates under-estimated. A review including 19 studies on a combined total of more than 180,000 scanned pregnancies at 16-24 weeks' gestation reported 2,400 abnormalities diagnosed by ultrasound, with an overall detection rate of 13 per 1000 fetuses examined (Wald N et al, 2000). An overview of the results of published studies evaluating the validity of ultrasound scanning as a diagnostic tool in a routine setting are presented in Table I.

**Table I:** Validity of US as diagnostic tool in a routine setting by author and year of publication.

| Author, year | Type of study | N | GA (wks) | Results | Prevalence of anomalies in population |
|---|---|---|---|---|---|
| ***Chitty et al. 1991*** | Retrospective | 8342 | 18-20 | **Sensitivity:** 74.4% **Specificity:** 99.9% | 1.5% |
| ***Constantine et al. 1991*** | Prospective | 4984 | 16-18 | **Sensitivity:**63.0% | 0.98% |
| ***Luck et al.1992*** | Prospective | 8523 | 19 | **Sensitivity:**85.0% **Specificity:** 99.9% | 1.9% |
| ***Shirley et al. 1992*** | Retrospective&Prospective | 6183 | 19-22 | **Sensitivity:**73.0% **Specificity:** 99.9% | 1.4% |
| ***Crane et al. 1994 (RADIUS STUDY GROUP)*** | Prospective | 7327 | 15-23 | **Sensitivity:**35.0% | 2.3% |
| ***Levi et al. 1995*** | Prospective | 25 046 | < 23 | **Sensitivity:**64.0% **Specificity:**99.9% | 2.42 |
| ***Anderson et al. 1995*** | Prospective | 7880 | 16-20 | **Sensitivity:**60.0% | 1.98% |
| ***Papp et al. 1995*** | Prospective | 51 675 | 18-20 | **Sensitivity:**63.1% **Specificity:**100 % | 2.26% |
| ***Eurenius et al. 1999*** | Prospective | 8345 | 15-22 | **Sensitivity:**22.1% **Specificity:** 99.8% | 1.7% |
| ***Grandjean et al. 1999 (Eurofetus study)*** | Prospective | NR | 18-34 | **Sensitivity:**56.2% | NR |
| ***Tabor et al. 2003*** | Prospective | 7963 | 18-22 | **Sensitivity:**60.0% | 1.3% |
| ***Nakling et al. 2005*** | Retrospective | 18 181 | 18-22 | **Sensitivity:**39.0 % **Specificity:**99.9% | 1.5% |
| ***Pitukkijronnakorn S et al. 2009*** | Prospective | 29 839 | 18-22 | **Sensitivity:**45.6% **Specificity:** 99,9% | 1.06% |
| ***Fadda et al. 2009*** | Retrospective | 42 256 | 16-23 | **Sensitivity:**55.1% **Specificity:** 99.9% | 2.48% |

***GA**= gestational age; **NR** = not reported*

Ultrasound has also a well recognized clinical role as a screening test (Nicolaides KH, 2004, 2011). The identification of ultrasound markers associated with an increased risk for a specific disorder may indicate a more

detailed scan and/or an invasive diagnostic procedure, such as amniocentesis or chorionic villus sampling. Markers can be anatomical variations or specific measurements that are often transient, which differ between affected and normal fetuses in relation to their prevalence but they can also be structural abnormalities that are commonly found in a specific disorder.

Relatively frequent conditions associated with serious handicap like trisomy 21 (Down's syndrome), trisomy 18 (Edwards' syndrome), trisomy 13 (Patau's syndrome) and open spina bifida have been the focus of antenatal ultrasonographic screening. The confirmation in cases of suspected aneuploidy needs, at least for the moment, an invasive procedure and therefore the risk of the fetus being affected by a particular disorder must outweigh or be equivalent to the risk of the diagnostic test.

Central nervous system (CNS) malformations are commonly diagnosed on routine prenatal ultrasonography and are markers of chromosomal abnormalities. A study that aimed to evaluate the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common CNS malformations confirms that CNS structural malformations diagnosed on ultrasound are associated with and predictive of aneuploidy, especially trisomy 13 and 18. When these CNS markers are isolated, the strength of the association decreases but remains significant for ventriculomegaly, holoprosencephaly, agenesis of the corpus callosum and neural tube defects (Goetzinger et al, 2008).

## 1.2. EVOLUTION OF ULTRASOUND EXAMINATION FROM THE SECOND TO THE FIRST TRIMESTER

The second trimester scan aims to identify structural abnormalities, biometric inconsistencies and markers of chromosomal abnormalities. Each chromosomal defect has its own syndromal pattern of sonographically detectable abnormalities (Snidjers RJM, Nicolaides KH et al, 1995; Ramalho C et al, 2011). For example, trisomy 21 is associated with nasal hypoplasia,

increased nuchal fold thickness, cardiac defects, intracardiac echogenic foci, duodenal atresia and echogenic bowel, mild hydronephrosis, shortening of the femur and humerus, sandal gap, and clinodactyly or mid-phalanx hypoplasia of the fifth finger. In singleton pregnancies, screening for trisomy 21 by a combination of maternal age and second-trimester maternal serum biochemistry (quadruple test) detects 81% (95% CI 72-89) of cases with trisomy 21 to a high false-positive rate of 7% (Wald NJ et al, 2003). Another setback is that late screening is inevitably followed by a late diagnosis of fetal abnormalities and a late termination of pregnancy.

The provision of high-quality first-trimester screening (Nicolaides KH et al, 1992; Snidjers RJM et al, 1998; Kagan et al, 2008) and the preference of pregnant women to have screening performed in the first rather than in the second trimester (Mulvey S et al, 2000) have led to widespread uptake of first trimester ultrasound examination. Nuchal translucency measurement, which is the maximum distance of a fluid-filled space on the back of the fetal neck, is a useful marker for Down's syndrome and other aneuploidies and also for congenital abnormalities and genetic syndromes. The advantage of this marker is that it does not require a highly skilled operator in contrast to second trimester examination and it matches the essential step of accurate dating the pregnancy by crown-rump-length (CRL).

Although the primary aim of the first-trimester scan is the measurement of fetal CRL and NT it has become increasingly apparent that the examination can lead to the diagnosis of many serious defects (Souka AP et al, 2004; Syngelaki A et al., 2011; Montenegro N et al, 1996) and pregnancy complications (Nicolaides KH, 2011). Improvements in ultrasound technology with increasing spatial resolution and consequent better visualization of fetal structures have supported the diagnosis of head and brain defects including acrania, encephalocele, and holoprosencephaly in a routine setting (Syngelaki et al., 2011).

The introduction of high-frequency transvaginal ultrasound transducers, allowing a better resolution by reducing the scanning distance, led to several observational studies aiming the detection of congenital anomalies in the

first trimester (Cullen et al, 1990; Timor-Tritsch et al, 1992). Despite the fact these studies support the possibility of performing a more detailed anomaly evaluation after 13 weeks, the main limitation for this is the application of transvaginal ultrasound in screening of large populations, as this is not generally accepted and requires additional training and equipment.

## 1.3. NEUROSONOGRAPHY: FROM THE SECOND TO THE FIRST TRIMESTER

Central nervous system disorders are associated with serious disability and therefore evaluation of the head, brain and spine is part of the main structural fetal survey by ultrasound scan.

Routine examination of the fetal brain during the second-trimester scan consists of three axial basic planes through the fetal brain: the transventricular, the transthalamic and the posteriorly tilted transcerebellar plane (ISUOG Guidelines, 2007). These allow measurement of the head and evaluation of brain structures, such as the midline falx, thalami, cavum septi pellucidi, lateral ventricles, choroid plexuses, cerebellum and cisterna magna. The depiction of brain abnormalities is dependent on the gestational age at the time of examination, as fetal brain significantly and continuously changes its anatomy during its development. At 16-24 weeks' gestation the overall detection for anencephaly is 99% (80-100), for holoprosencephaly it is 92% (67-100) and for open spina bifida it is 84% (65-100). The reported detection rate for Dandy-Walker malformation, agenesis of the corpus callosum and microcephaly is variable, being 83% (0-100), 50% (0-75) and 35% (0-100), respectively. The reported false positive rate for CNS anomalies is 0-1.1% (Wald N et al, 2000).

Transvaginal neurosonography of the fetal brain was initially described in the 90's and applies a similar type of examination to the one used in neonatal brain scanning. It employs high-frequency ultrasound probes making use of fetal cranial fontanelles as an 'acoustic window'. Second and third trimester detailed neurosonography in high-risk cases for brain malformations, when

carried out by an expert examiner, has a greater diagnostic potential compared with the standard basic examination (Timor-Tritsch IE et al, 1996). The examination includes extended views like coronal as well as median and para-median planes and is of more elaborate understanding.

The better resolution provided by high frequency transvaginal ultrasound probes was soon applied to the evaluation of early embryonic development. Some observational studies reported by several authors proposed its use for assessing the sonoanatomic features of the developing embryonic and early fetal brain, including the dimensions of the cavities of the hemispheres, diencephalon, mesencephalon and rhombencephalon (Kushnir et al, 1989; Achiron et al, 1991, Timor-Tritsch et al, 1991, Blaas et al, 1994, 1995, 1998). However, none of these authors focused on the period of 11-13 weeks of gestation which is the time when first trimester screening is carried out. The clinical advantages and implications of an early detailed anatomical assessment of the fetus have not been estimated yet, but it is certain that the sonographer must not only have the knowledge of normal embryological development but also its corresponding ultrasonographic appearance at early stages.

## 1.4. APPLICATION OF THREE-DIMENSIONAL ULTRASOUND IN FETAL BRAIN ASSESSMENT

The use of three-dimensional ultrasound (3D US) in the evaluation of the fetal brain has been described by several groups (Monteagudo A et al, 2000; Timor-Tritsch IE et al, 2000; Kalache KD et al, 2006; Pilu G et al, 2006, Monteagudo A et al, 2009; Zalel Y et al, 2009). There is evidence from second-trimester neurosonography that 3D US is useful by providing the option for *off-line* assessment using multiple planes and allowing to evaluate simultaneously the same structures in three orthogonal planes. Furthermore this technique provides the option to revise and obtain additional images after the initial examination has been completed (Bornstein E et al 2010).The marker dot which is generated by the intersection of three planes is freely movable by the user and points the same spot within the volume, helping to

identify a specific structure within the three planes simultaneously. This allows an easier recognition of the anatomy in different planes, particularly when it looks disturbed. An additional advantage of 3D is that the saved volume can be used as a training or research tool. Nevertheless, there are a few limitations, as the quality of the volume is dependent on the quality of the acquisition plane and it is therefore essential to obtain a good quality two-dimensional (2D) image. The visualization rate is hampered by non cephalic presentations, fetal movements and maternal conditions like uterine position in the pelvis, fibroids and contractions.

The first trimester presents some advantageous features for application of 3D ultrasound in relation to the second and third trimesters, as the fetus is small and there is invariably amniotic fluid around the fetus. Although 3D ultrasound allows several alternative viewing modalities, the extent to which a given structure can be demonstrated is entirely dependent on the quality of the initial 2D image as well as on fetal position. Expertise is necessary both in acquisition as well as during manipulation and interpretation of the volumes (Dückelmann AM et al, 2010).

The initial description of 3D technology to assess the first trimester fetal brain was by Blaas and this was achieved by using purpose-built equipment (Blaas HG et al, 1995). Advances in technology allow the performance of more precise 3D fetal brain volume scans and in recent years several publications have described the normal and abnormal first trimester fetal brain using 3D ultrasound (Blaas HG et al, 2000; Kim MS et al, 2008; Timor-Tritsch IE, 2008; Egle et al, 2011).

## 1.5. THREE-DIMENSIONAL ULTRASOUND METHODOLOGY IN ASSESSING FIRST TRIMESTER FETAL BRAIN

The first step to any 3D ultrasound examination is to obtain a 2D image with the maximum possible quality. The goal is to get an optimal initial plane requiring minimal adjustments once the volume is obtained and for that it is sometimes necessary to perform a gentle manipulation of the fetus or

uterus. Secondly, the image is magnified as to occupy the entire screen and upon activation of the 3D mode, a render box appears on the screen that allows the selection of the volume of interest. This box may be modified regarding its position and size and should be placed in a way to contain only the fetal head. The speed of acquisition of a 3D volume depends on the position, dimensions of the render box and type of resolution chosen. For first trimester brain volume acquisition, the highest resolution is feasible and at the same time provides the best quality volumes. To minimize artefacts that occur with fetal movements, it is necessary to wait for fetal quiescence.

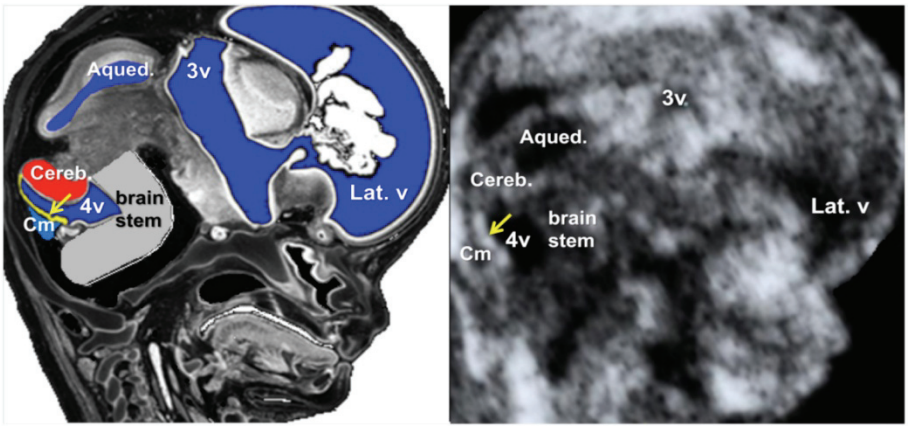
The acquired volume is displayed in three perpendicular planes that are shown simultaneously in the screen and this is called the multiplanar mode. The navigation through the different planes can be done 'off-line' using the ultrasound machine software or in a personal computer, using a specific 4D software (for example, *4D view software GE Medical System*). Each of the three planes can be rotated around the x, y and z axes. It is possible to use the marker dot which is generated by the intersection of the three planes to identify structures.

## 1.6. THE NORMAL BRAIN - THE VENTRICULAR SYSTEM AND CEREBROSPINAL FLUID

A complete understanding of the normal embryological development and its corresponding ultrasonographic appearance is mandatory when evaluating the early fetus and more particularly the early fetal brain. The brain ventricular system is formed by related cavities named ventricles filled with cerebrospinal fluid (CSF) and surrounded by neuroepithelium. At 6 weeks' gestation five brain regions are depicted: the telencephalon and the diencephalon that derive from the forebrain, the mesencephalon (midbrain) and rhombencephalon (hindbrain). The ventricular system begins its development with the closure of the caudal neuropore during stage 12 ($6^{th}$ week of gestational age) (O'Rahilly R et al, 1990) and expands itself significantly faster than the brain tissue during early brain development (Bayer SA et al, 1994). There are four connected cavities: two lateral

ventricles within the telencephalon, a third ventricle within the diencephalon and a fourth ventricle within the rhombencephalon, between the cerebellum and pons (Figure 1).

**Figure 1:** Sonographic image of a para-sagittal plane of the fetal head at 12 weeks' gestation obtained from a volume acquired by transvaginal (TV) ultrasound scan (on the right) and corresponding schematic drawing demonstrating the different components of the ventricular system shown in blue (on the left).




***Lat. v*** *= lateral ventricle;* ***3v*** *= third ventricle;* ***Aqued.*** *= aqueduct of Sylvius;* ***4v*** *= fourth ventricle;* ***Cereb.*** *= cerebellum;* ***Cm*** *= future cisterna magna (appearing light blue, on the left); the yellow arrow indicates the choroid plexus of the fourth ventricle (appearing in yellow, on the left).*

The lateral ventricles are connected to the third ventricle by the foramina of Monro, the third ventricle is linked to the fourth via the cerebral aqueduct and the fourth ventricle joins the spinal cord canal and the subarachnoid space that envelops the brain and future cistern magna by a medial foramen (Magendie) and two lateral foramina (Luschka). By ultrasound, the embryonic ventricular system appears as large hypoechogenic cavities in the brain and can be imaged with detail by using transvaginal scan from the 7$^{th}$ week of gestational age onward (Blaas et al, 2009).

CSF is produced mainly by the choroid plexuses (Lowery L et al, 2009),but before their development it is regulated by the neuroepithelium, that is the epithelium that covers the ventricles. There is evidence that the immature choroid plexus selectively transfers proteins from blood to CSF, so that in the early brain, CSF contains high concentrations of proteins including albumin, alpha-fetoprotein (AFP) and proteoglycans (Saunders et al, 1999; Zapaterra MD et al, 2007; Gato A et al, 2009). Results from animal studies in chick and rat embryos suggest that CSF is a promoter in shaping the ventricles and that proteoglycans also regulate fluid movement and size of brain ventricles (Alonso MI et al, 1999). Classic studies in chick embryos have suggested that intraluminal pressure resulting from the accumulation of embryonic CSF (eCSF) is necessary for normal brain development and consistently intubation of the chick embryonic hindbrain ventricle results in a collapse of the ventricles. There is a correlation between brain size and amount of neuronal cell proliferation and one proposed mechanism by which eCSF may regulate brain development is through creating pressure within the brain ventricles (Desmond ME et al, 2005).

There is evidence that during fetal life and until infancy, CSF dynamics are different from later on. In the adult, CSF is absorbed primarily through arachnoid villi and granulations into the venous sinuses of the brain. In the fetus, CSF absorption is mainly via the neuroependyma and the perineural space to the lymphatic system and capillaries (Oi S et al, 2006). Although the central nervous system parenchyma does not contain lymphatic vessels, CSF is traced along the subarachnoid space associated with several nerves to the cervical jugular lymphatic sac (Johnston M et al, 2002).

## 1.7. OPEN SPINA BIFIDA

Most open neural tube defects are the end result of defects in primary neurulation, that is, the formation of the neural tube, which later develops into the brain and spinal cord. The affected part does not become covered by the vertebral arches or calvarium, but remains inadequately protected and is affected by neuronal damage and degeneration, with loss of neural

tissue by the end of the pregnancy (Greene NDE et al, 2009).Neural tube defects affect 0.5-2 per 1000 pregnancies worldwide (Mitchell LE, 2005). In England and Wales the birth prevalence of neural tube defects in 1997 was 0.14 per 1000 births, which is less than 5% of the rate 25 years previously (Wald NJ, 2000). This reduction has been due to improvement in folate consumption as well as antenatal screening, diagnosis and selective termination.

The most common form of open spina bifida is myelomeningocele, identified by an open defect on the spine with protrusion of the spinal cord and meninges at any point along the spinal column. The term spina bifida refers to defects in the vertebral arches that go with open lesions. Except for the cervical myelomeningoceles which are covered at the base by skin and on the dome by thick epithelium overlying thick leptomeninges, in both thoracolumbar and lumbosacral a flattened caudal end of the spinal cord (placode) floats on top of the dome of the CSF sac covered by thin arachnoid and CSF leakage is common (Pang D et al, 1993).

### 1.7.1. ETIOLOGY

Both genetic and environmental factors are involved in the aetiology of neural tube defects. The risk of fetal neural tube defects is increased for siblings (2-5%), approximately 40-fold more than in the general population (Little J, 1992).It is now known that folate deficiency is a promoter of neural tube defects (MRC Vitamin 1991; Czeizel AE et al, 1992) and in about 75% these can be prevented by taking extra folic acid before and during early pregnancy (Wald NJ, 1991; 2000). Other factors linked to neural tube defects in human pregnancy are folate antagonists (carbamazepine, trimethoprin), hyperglycaemia, histone deacetylase inhibitors (valproic acid), hyperthermia in weeks 3-4 of pregnancy and deficiency in other micronutrients, including inositol, vitamin B12 and Zinc (Copp AJ et al, 2010).

### 1.7.2. CHIARI TYPE II MALFORMATION

It was in 1896 that Chiari published for the first time a thorough study of cerebellum malformations following hydrocephalus. In the type II malformation, Chiari reported displacement of the vermis, pons and medulla oblongata into the spinal canal. It was in 1907 that collaborators of Arnold described four cases of myelomeningocele and introduced the term Arnold-Chiari malformation (Bejjani G, 2001; Solt I, 2011) to designate Chiari II malformation.

Myelomeningocele is associated with Chiari II malformation of the cerebellum and hindbrain in 95% of cases (Swartwout et al, 2008). This condition is connected to several abnormalities of the posterior fossa. There is displacement of part of the cerebellum, vermis as well as brainstem (medulla and variably the pons) through the enlarged foramen magnum into the cervical spinal canal. The brainstem may become kinked and aqueduct gets obliterated. Additionally the superior vermis and cerebellar hemispheres are rotated cranially and lie within the middle fossa of the supratentorial compartment. Characteristic findings are: a small posterior fossa and a low lying tentorium. Chiari II is also associated with several abnormalities that involve the supratentorial compartment (Miller E et al, 2008). These include enlarged massa intermedia of the thalamus, small third ventricle, dysgenesis of the corpus callosum, beaking of quadrigeminal plate, cortical abnormalities and deformity of the frontal and parietal bones of the cranium (McLone D et al, 2003). The posterior fossa abnormalities and associated deformity of the skull are the basis of second trimester US markers for open spina bifida like the ‘banana’ and ‘lemon’ signs (Nicolaides KH et al, 1986).

McLone et al proposed a new theory (‘unified theory’) to explain the anomalies involved in Chiari II malformation that encompass all the brain (McLone et al, 1989). According to this author, the presence of an open neural tube allows leaking of CSF through the defect during embryonic and fetal life, disrupting the usual distension of the developing ventricles. During normal development of the human embryo, just before neural tube closure, there is a temporary period of spinal neurocele occlusion. The result of this

is that the CSF is held within the cerebral ventricular system under pressure with consequent expansion of the cranial ventricles and cavities. If, however, there is failure to maintain this intracranial pressure because of a neural tube defect, there is inadequate ventricular growth. The defective distension of the rhombencephalic and metencephalic vesicles translates into a decreased size of posterior fossa during cerebellar and brain stem development, resulting in crowding of these structures in a tight posterior fossa. In addition, the distension of the ventricular system is required for the development of the supratentorial brain structures and growth of frontal and parietal bones. When impaired the third ventricle is small and there is approximation of the thalami with a large massa intermedia and abnormal shape of the skull, referred on ultrasound as the 'lemon sign'.Also the normal CSF flow from the third to the fourth ventricle through the aqueduct may be reduced resulting in hydrocephalus. Therefore, hydrocephalus is an effect and not the cause of the Chiari II malformation (Mc Lone et al, 2003). The unified theory implies that the development of the Chiari II malformation and its consequences on brain growth begins in the embryo and continues throughout fetal development.

### 1.7.3. PROGNOSIS

Until a decade ago, surgical closure of the spinal canal at birth and long-term supportive care were the treatments offered to children affected by myelomenincocele. In the 1980' the prognosis of infants born with open spina bifida was poor with a 5-year survival rate of 36% when treated (Althouse, et al, 1980). Today, despite the aggressive intervention, nearly 14% of all spina bifida neonates do not survive past 5 years of age, with the mortality rising to 35% in those with symptoms of brainstem dysfunction secondary to the Chiari II malformation (Oakeshott P et al, 2003). More than two thirds of the patients have normal intelligence but only half are able to live independently (Adzick NS, 2010). Apart from motor and sensory deficits secondary to the level of spinal cord lesion, significant morbidity is related to Chiari II malformation and hydrocephalus and spinal cord tethering at the site of surgical repair.

It is the hydrocephalus and the other manifestations of Chiari II malformation that will determine the outcome in these children (Mc Lone, 2003). Myelomeningocele (MMC) is nearly always associated with Chiari II malformation and hydrocephalus occurs in more than 85% of the patients (Dias MS et al, 1993). On average 80% of patients need the placement of a shunt and nearly half of them have shunt related complications within the first year. Symptomatic CM II is a potentially life threatening condition in children, with up to 15% of patients dying by the age of 3 and nearly one third left with permanent neurological disability. The symptoms depend on the age of the child but are usually related to dysfunction of the cerebellum, medullary respiratory center and cranial nerves IX and X, as well as hydrocephalus. A common and serious symptom is inspiratory stridor caused by vocal cord dysfunction related to cranial nerve X compression. Gastrointestinal disturbances like neurogenic disphagia are nearly always progressive and are associated with neurological decline. Other symptoms and signs include para or quadriparesis, hypotonia, ocular abnormal movements, weak cry and developmental delay. In older children symptoms are more insidious and less frequently life threatening and reflect cervical myelopathy like upper extremity weakness (Stevenson KL et al, 2004). Surgical decompression of the posterior fossa for symptomatic CII is required in 8-17% of cases with MMC and usually leads to clinical improvement (McComb JG, 1997).

### 1.7.4. SECOND TRIMESTER SCREENING AND DIAGNOSTIC TESTS FOR OPEN NEURAL TUBE DEFECTS

#### SERUM AFP FOLLOWED BY AMNIOCENTESIS FOR ACETYLCHOLINESTERASE

The association of alphafetoprotein (AFP) with open neural tube defects was first described in 1972 and in the 1980s the main method of screening for open spina bifida was by the detection of this marker in maternal serum in increased amounts during early second trimester of pregnancy (Brock JH, 1976). AFP is mainly produced in fetal liver and placenta, and in open neural tube defect it leaks from CSF to amniotic fluid. The difference in maternal serum AFP levels between pregnancies with open spina bifida and

unaffected ones is greatest at 16-18 weeks of pregnancy (Brock DJ, 1981). At 17 weeks of gestation, using an AFP cut-off level of 2.5 MoM or greater, the detection rate for open spina bifida is 86% for a false-positive rate of 0.3 % (Wald NJ, 2000). High serum AFP may be also found if gestational age has been under-estimated, in multiple pregnancies and in cases associated with spontaneous fetal loss, low birth weight, abdominal wall defects, congenital nephrosis, pre-eclampsia and preterm delivery. Low serum AFP is associated with Down's syndrome and trisomy 18 (Wald NJ et al, 2003).

The main biochemical diagnostic test for open spina bifida is amniotic fluid acetylcholinesterase (AChE) measurement. The second report of the collaborative Acetylcholinesterase Study showed that AChE was better diagnostic test than amniotic fluid AFP, with a detection rate for open spina bifida of 99% at a false-positive rate of 0.34% (Wald NJ, 1989). For that reason, the main method of screening for open spina bifida in the 1980s was by maternal serum AFP at around 16 weeks and the method of diagnosis was by measurement of amniotic fluid AFP and acetyl cholinesterase. Although it was possible to diagnose the condition by US of the spine, the sensitivity was low (Roberts CJ et al, 1983).

### ULTRASOUND SCAN AS BOTH SCREENING AND DIAGNOSTIC TEST

Ultrasound scanning for neural tube defects is used as both screening and diagnostic tests. For instance, it allows the immediate diagnosis of anencephaly and by dating the pregnancy it improves the performance of AFP screening. Detection of spinal defects by ultrasound examination of the spine highly depends on the skill of the operator.

Two cranial signs have been described that are easily depicted on second trimester ultrasound scan and result from Chiari type II malformation: the 'lemon' and 'banana' signs (Nicolaides KH et al, 1986). Identification of any of these two cranial signs of spina bifida prompts the operator to check the spine thoroughly and find the defect. The 'lemon sign' refers to a retraction of the frontal region of the fetal skull that resembles the shape of a lemon. In

open spina bifida the thickness of the cerebellum is reduced as well as its transverse diameter and in some cases, with advance of gestation the cerebellum, is not seen at all. The two cerebellar hemispheres adopt a bow shape, the so called 'banana sign'.

The findings of a multicenter screening study including 61 972 singleton pregnancies demonstrated that the sensitivity for a routine scan at 16-22 weeks in the diagnosis of spina bifida is more than 95% (Sebire NJ et al, 1997). The easily checked 'lemon' and 'banana' signs have led to the replacement of biochemical assessment with second-trimester ultrasonography, both for screening and diagnosis, as it obviates the disadvantage of fetal loss due to amniocentesis which is 1% (Tabor A et al, 1986). An overview of the results of published studies evaluating the validity of cranial signs in the detection of open spina bifida is presented in Table II.

**Table II:** Validity of 'banana' and 'lemon' signs by author and year of publication.

| Author, year | Type of study | N | GA (wks) | 'Banana' Sign | | 'Lemon' sign | |
|---|---|---|---|---|---|---|---|
| | | | | DR | FPR | DR | FPR |
| ***Nicolaides et al.* 1986** | Retrospective | 70 | 16-23 | 95% | 0 | 100% | 0 |
| ***Campbell et al.* 1987** | Prospective | 26 | | 96% | 0 | 100% | 0 |
| ***Pilu et al.* 1988** | Prospective | 19 | | 100% | 0 | - | - |
| ***Nyberg et al.* 1988** | Retrospective & prospective | 27 | <24 | - | - | 89% | 1.3 |
| ***Goldstein et al.* 1989** | Retrospective | 20 | 17-38 | 100% | 10.6 | 70% | 2.1 |
| ***Thiagarajah et al.* 1990** | Retrospective | 16 | 16-24 | 100% | - | 100% | - |
| ***Van den Hof et al.* 1990** | Prospective | 130 | ≤24<br>>24 | 95% | 0% | 98%<br>13% | 0.6% |
| ***Ghi T et al.* 2006** | Retrospective | 53 | 16-34 | 100% | - | 100% | - |
| ***D'Addario et al.* 2008** | Prospective | 49 | 18-28 | 96% | - | 53% | - |

***GA** – gestational age; **Wks** – weeks; **DR** – detection rate; **FPR** – false positive rate*

## 1.7.5. FIRST TRIMESTER SCREENING FOR OPEN NEURAL TUBE DEFECTS

In the 1980s, the description of the cranial and cerebellar signs ('lemon' and 'banana', respectively) led to a major improvement in the second-trimester sonographic diagnosis of spina bifida (Nicolaides KH et al, 1986; Van den Hof MC et al, 1990). However, the incidence of these sonographic markers is gestational age-dependent (Van den Hof MC et al, 1990). Scalloping of the frontal bones may not occur before 12 weeks (Blumfeld, 1993). Where typical cranial signs of spina bifida are lacking or not searched for, the diagnosis of this defect can be easily missed.

In the 1990s, improvements in quality of ultrasound equipment have led to earlier diagnosis of a wide range of abnormalities. Small series of cases of spina bifida detected during first trimester have been reported (Blaas HG et al, 2000; Buisson O et al, 2002; Sebire NJ et al, 1997; Blumfeld et al, 1993). Some of these studies have described craniocerebral signs of spina bifida at the end of the first trimester. A small series of three cases of spina bifida detected from 12 to 14 weeks presented the 'lemon sign' (Sebire NJ et al, 1997). Blumfeld et al reported on the evolution of sonographic signs in a fetus with spina bifida in early pregnancy from 10 to 15 weeks. At 12 weeks an anterior curvature of the cerebellum was visible and at 15 weeks both lemon and banana signs together with mild ventriculomegaly were seen (Blumfeld et al, 1993). Buisson et al reviewed the first trimester ultrasound images of the fetal head from 12 consecutive cases of spina bifida. In two of the three cases of adequate first-trimester images there were abnormal cranial signs (retraction of the frontal bones, resulting in an acorn-shaped head), cerebral signs (cerebral peduncles appearing parallel) and displacement of the metencephalon that these authors related to open spinal defects (Buisson O et al, 2002).

### THE MID-SAGITTAL VIEW AND INTRACRANIAL TRANSLUCENCY (IT)

More consistent results have been reported in the last two years. There is now evidence that in the same mid-sagittal plane necessary for

measurement of fetal NT it is possible to suspect the presence of open spina bifida. In 2009, Chaoui et al described the intracranial translucency (IT) (Chaoui R et al, 2009; 2010).

In normal fetuses the fourth ventricle presents as an intracranial translucent space, parallel to NT. This space is defined by two lines: the border of the brainstem (anteriorly) and the choroid plexus of the fourth ventricle (posteriorly). Its measurement is similar to that of NT performed in its widest diameter. IT represents the fourth ventricle and therefore is anechoic (black). The brainstem appears hypochogenic (dark gray) and the choroid plexus looks like a bright line on transabdominal scan.

Chaoui et al proposed that in fetuses with open spina bifida at 11-13 weeks' gestation there is absence of IT (Chaoui R et al, 2009). However, in a few cases of open spina bifida some fluid can still be present in the posterior brain,but without the typical landmarks of a normal intracranial translucency (Chaoui R et al, 2011; Lachmann R et al, 2011).

In an analysis of 30 cases with open spina a shift of the posterior brain towards the occipital bone was observed and this was reflected by a thickened brainstem, a shortening of the distance between brainstem and occipital bone and an increase in the ratio of brainstem diameter to brainstem–occipital bone distance to more than 1 (Lachmann R et al, 2011; Chaoui R, Nicolaides KH, 2011).

A recent prospective study of 3D volumes of the fetal brain obtained by transabdominal approach from 10 normal fetuses and three fetuses with open spina bifida found that in fetuses with open spina bifida the cisterna magna was partially or completely obliterated (Scheier M et al, 2011).

The logical approach for using the mid-sagittal plane for examination of the posterior brain is that this view is obtained routinely at 11-13 weeks' gestation for screening for aneuploidies and the examiner can easily assess the structures of the posterior brain region without further expense of time or effort.

At the moment, the relevant issue is to determine the detection rate of open spina bifida using the described markers at the 11-13 weeks´s routine scan and before that to fully comprehend head features of spina bifida in early gestation.

An overview of published articles concerning the first trimester screening and diagnosis of open spina bifida are presented in Table III.

**Table III:** Overview of the results from studies using 2D and 3D ultrasound to assess first trimester brain in cases of spina bifida by author and year of publication.

| Author, year | GA (wks) | Plane | View | US marker | Results |
|---|---|---|---|---|---|
| ***Sebire et al. 1997*** | 12-14 | Axial | TA, 2D | *Lemon sign* | 3/3 |
| ***Blaas et al. 2000*** | 9 | Sagittal | TV, 2D-3D | *Irregular spine* | 3/3 |
| ***Buisson et al. 2002*** | 12 | Axial & Sagittal | TA, 2D | *Acorn-shaped head*<br>*Parallel cerebral peduncles*<br>*Flat occiciput* | 2/3 |
| ***Chaoui et al. 2009*** | 11-13 | Sagittal | TA, 2D | *Absent IT* | 4/4 |
| ***Lachmann et al. 2011*** | 11-13 | Sagittal | TA, 2D | *Increased BS*<br>*Decreased BSOB*<br>*Increased BS/BSOB* | 29/30<br>26/30<br>30/30 |
| ***Chaoui et al. 2011*** *(Prospective study)* | 11-13 | Sagittal | TA, 2D | *Absent IT*<br>*Increased BS/BSOB* | 2/6<br>6/6 |
| ***Solt et al. 2011*** | 11-13 | Sagittal | TA, 2D | *Absent IT* | 1/5 |
| ***Finn et al. 2011*** | 11-13 | Axial | TA, 2D | *Aqueduct of Sylvius to occiput distance* | 9/9 |
| ***Scheier et al. 2011*** *(Prospective study)* | 11-13 | Axial<br>Sagittal | TA, 3D | *Cisterna magna diameter*<br>*Fourth ventricle diameter*<br>*BS, BSOB, BS/BSOB* | 3/3 |

***GA** – gestational age; **TA** – transabdominal; **TV** – transvaginal; **IT –** intracranial translucency; **BS** – brain stem diameter; **BSOB** – Brain stem to occipital bone diameter*

# 1.8. POSTERIOR FOSSA ABNORMALITIES

## 1.8.1. EMBRYOLOGY OF THE POSTERIOR FOSSA

The rhombencephalon is the most caudal of the three primitive brain vesicles and gives rise to the medulla oblongata, pons and cerebellum. The roof of the rhombencephalon is thin and roughly rombus-shaped and is divided by the plica choroidea, a rudimentary choroid plexus, in two parts: the rostral or anterior membranous area (AMA) and the caudal or posterior membranous area (PMA). The AMA precedes the formation of the cerebellum: the failure results in a midline defect with an agenesis of most of the caudal vermis and the PMA forms the ventricular outlet: the failure results in a cystic dilatation of the fourth ventricle (Raybaud C, 2010).

The cerebellum is formed by growth of the primitive rhombic lips, with the entire vermis and cerebellar hemispheres being formed by the end of the 17th week (Babcook CJ et al, 1996). At 11 to 13 weeks' gestation there is a characteristic flexion of the brain stem (Figure 1, page 32) named the pontine flexure (Kollias SS et al, 1993).

The fourth ventricle extends caudally in a finger-like expansion of the PMA called the Blake's Pouch. This structure will later disappear, leaving a median opening, the Blake's metapore, considered the precursor of the foramen of Magendie, which connects the fourth ventricle to the cisterna magna. The choroid plexus of the fourth ventricle extends caudally to the Blake's metapore and into the cisterna magna through the foramen of Magendie. There is still no consensus about the exact timing of the formation of the opening of Magendie. Some authors date it around 16 weeks' gestation others around 10 or 12 weeks (Brocklehurst G, 1969; Friede RL, 1989). The process depends on the involution of the thin ependymal layer in the central part of the PMA although it has been reported that it can persist in normal fetuses of 20 weeks' gestation (Tortori-Donati P, 1996). The formation of the foramina de Luschka, which are openings of the lateral recessus of the fourth ventricle to the cisterna magna, occurs later

than 16 weeks' of gestation. Nevertheless, atresia of one or two cerebellar foramina is often detected in normal brains (Friede RL, 1989).

Understanding the embryology of cerebellar and fourth ventricle development is critical to the accurate interpretation of images of the fetal posterior fossa at 11 to 13 weeks' gestation scan. However, descriptions of the gross appearance of the cerebellum beyond the embryonic period are scarce and do not focus on the features important to prenatal diagnosis. At the 20 weeks' scan, normal measurements of cisterna magna and ventricular atrium confer a very high negative predictive value for abnormal CNS and spinal cord development (Filly RA et al, 1989). There is no information how equivalent structures perform during first trimester as to reassure normal brain development. Additionally there is normal physiological variation in time of structural development which still needs to be comprehensively described.

## 1.8.2. DANDY-WALKER MALFORMATION

Dandy-Walker malformation (DWM) is part of a heterogeneous group of defects of the posterior fossa that includes other conditions like vermian hypogenesis/hypoplasia (VH) and mega cisterna magna (MCM). Overall, posterior fossa anomalies are found in approximately 1 in every 5,000 live births (Patek KJ et al, 2012). Dandy-Walker malformation shows the most severe abnormalities of the spectrum of posterior fossa defects. The classic DWM is a rare congenital malformation, with an incidence of 1 in 25,000 to 1 in 35,000 births (Boddaert N et al, 2003).

Dandy-Walker Malformation is diagnosed on the basis of the classic triad: complete or partial agenesis of the vermis, dilatation of the fourth ventricle and cisterna magna and upward displacement of the venous sinus (lateral and torcular) and cerebellar tentorium, which is an extension of the dura mater that separates the cerebellum from the occipital lobes. The entire vermis is absent in 25% of patients and the rest have partial aplasia, always involving the inferior vermis. The small vermis is rotated superiorly, attached

to the tentorium which maintains a 'Y' configuration, similar to the early fetus (Kollias et al, 1993). Hydrocephalus is a common complication (Boddaert et al, 2003). Other abnormalities of CNS are present in 68% cases (Kollias et al, 1993) such as dysplasia of the brain stem, dysgenesis of corpus callosum and abnormalities of the cortex. Extracranial common anomalies associated include congenital heart defects (38%), abnormalities of the limbs (28%), kidneys (28%), and face (26%) (Ecker JL et al, 2000).

One theory proposed to explain the pathogenesis of Dandy-Walker malformation is a developmental arrest in the rhombencephalon, with lack of fusion of the cerebellum in the midline. This results in the persistence of the anterior membranous area, which expands posteriorly between the hypoplastic vermis and the choroid plexus. The distended fourth ventricle leads to the high position of the tentorium seen in DWM (Friede RL, 1989; Kollias et al, 1993).

### TERMINOLOGY – THE DANDY-WALKER SPECTRUM/COMPLEX

Several terminologies have been applied by different authors. One traditionally used by prenatal ultrasound literature subdivides the posterior fossa abnormalities into two groups: the Dandy Walker malformation (DWM) and the Dandy Walker variant (DWV). The differences between them are based on the severity of the cerebellar vermis defect, the presence or absence of an elevated tentorium and the finding or absence of a dilated cisterna magna (Estroff JA, 1992; Nyberg DA, 2003).

Pediatric and radiology literature traditionally categorizes posterior fossa abnormalities as a spectrum or a complex of disease from mild (isolated mega cisterna magna), to moderate DW complex (mild hypoplasia of the vermis and communication of the cisterna magna with an enlarged fourth ventricle), and severe DW complex (marked dilatation of the cisterna magna and fourth ventricle with agenesis of the cerebellar vermis) (Castillo SMS, 1996).

More recently and taking into account the information provided by fetal MRI, the focus has moved to a detailed description of vermian characteristics like size and foliation (Garel, 2004; Robinson AJ et al, 2007). The term vermian hypoplasia is referred to the presence of a small vermis with normal foliation (Patek KJ et al, 2012).

### DANDY-WALKER MALFORMATION: ASSOCIATION WITH ANEUPLOIDY AND OTHER BRAIN ABNORMALITIES

Abnormal karyotypes are found in half of fetuses with antenatal diagnosis of DWM. The commonest chromosomal defects underlying DWM are T18, triploidy and T13 (Ulm B et al, 1997). Frequently associated brain abnormalities that are relevant for the outcome are agenesis of corpus callosum, ventriculomegaly and hydrocephaly (Goetzinger RK, 2008).

### PROGNOSIS

The lack of evidence-based outcome data for fetuses diagnosed with posterior fossa abnormalities makes it difficult to provide accurate prognostic information (Patek KJ et al, 2012).

The prognosis in relation to the degree of developmental delay seems to be associated with vermis anatomy and the presence of other brain malformations like agenesis of corpus callosum, brainstem and cortical abnormalities (Boddaert N et al, 2003). The identification of additional intracranial anomalies or extracranial anomalies correlates with a worse outcome regardless of the type of posterior fossa abnormality (Patek KJ et al, 2012).

Some brain abnormalities that are relevant for the final outcome are not depicted on ultrasound like brainstem and cortical abnormalities. In a study describing the follow up of 15 cases with Dandy-Walker complex (DWC) associated with brainstem abnormality, nine (60%) died and all six survivors

(40%) had abnormal development compared with 35 cases with a normal brainstem where 14 (40%) were normal, 17 (49%) were abnormal, and four (11%) died (Patek KJ et al, 2012). The same study describes the neurodevelopmental outcome of six surviving cases (2-55 months of age) with Dandy–Walker malformation and all except one where it was not possible to assess development based on age, had developmental delay. In the group of 21 surviving cases (1-79 months) with hypogenesis/hypoplasia of the vermis, half of them had developmental delay, in 4 it was unknown and in one third the development was normal.

### INCONSISTENCIES IN DANDY-WALKER MALFORMATION DIAGNOSIS

Several studies have shown that the correlation between prenatal ultrasound diagnosis of Dandy-Walker malformation complex and autopsy or post-natal MRI is poor at 50% (Phillips JJ et al, 2006; Harper T et al, 2007, Carroll SG et al, 2000).

Almost 20 years ago Bromley et al published a study on the timing of 'closure' of the vermis; the study involved 897 participants and they concluded that prenatal diagnosis of Dandy-Walker variant should not be made before 18 weeks gestation as the development of the cerebellar vermis may be incomplete before this time (Bromley at al, 1994). However, it is occasionally possible to find a normal fetus with a slight delay in the development of the vermis to beyond 18 weeks (Ecker et al, 2000). This raises the possibility of false positive diagnoses as full anatomic scans by ultrasound are usually completed between 18 and 22 weeks' of gestation. In a recent study where all cases had prenatal MRI diagnosis, postnatal brain imaging confirmed the diagnosis in 85%. Nevertheless, 50% of the cases of vermis hypoplasia diagnosed by MRI before 24 week's gestation were unconfirmed, raising the question of increased false positive results until 24 weeks (Patek KJ et al, 2012).

## 1.8.3. PRENATAL ULTRASOUND EVALUATION OF THE FOURTH VENTRICLE

Not many studies have concerned the measurement of fetal fourth ventricle and only a small number address it during first trimester.

Blaas et al in 1995 examined 29 normal cases between 7-12 weeks and measured the fourth ventricle, the cerebellum and the choroid plexus using transvaginal US scan. They found that the fourth ventricle is visible by ultrasound from 7 weeks onwards. The antero-posterior diameter mean was 1.5 mm at 7 weeks and 3.2 mm at 12 weeks (Blaas et al, 1995). A prospective cross-sectional study measured the fourth ventricle in 299 normal fetuses at 13-40 weeks' gestation using either transabdominal or transvaginal route. The fourth ventricle increased linearly with biparietal diameter (BPD). At $13^{+0}$ to $13^{+6}$ weeks the $10^{th}$, $50^{th}$ and $90^{th}$ centile were 2.1 mm, 2.5 mm and 3.0 mm, respectively (Goldstein I et al, 2002).

Chaoui R et al in 2009 measured the fourth ventricle by transabdominal route in the mid-sagittal plane in 200 normal fetuses. The fourth ventricle was always visible and the median anteroposterior diameter increased from 1.5 mm at a crown–rump length (CRL) of 45 mm to 2.5 mm at a CRL of 84 mm (Chaoui R et al, 2009). Another prospective study including 502 fetuses measured the fourth ventricle in an axial view by transabdominal scan, and found a linear correlation between CRL and fourth ventricle. The fourth ventricle increased from 1.7 mm at a CRL of 45 mm to 2.5 mm at a CRL of 84 mm (Eagle et al, 2011). Similar results were found by a recent study using the transabdominal route and the mid-sagittal plane (Papastefanou I et al, 2011).

### THE MEANING OF AN ISOLATED ENLARGED FOURTH VENTRICLE

A study published by Bronshtein et al reported 21 fetuses with an enlarged fourth ventricle as an isolated finding that were detected by ultrasound at 14–16 weeks' gestation. No other central nervous system anomalies were

observed and a normal size fourth ventricle was noted in all cases on follow-up scans at 22–23 weeks' gestation. These authors concluded that an isolated enlarged fourth ventricle might be a physiological variant (Bronshtein M et al, 1998). In 2002, Goldstein et al described 12 cases of dilated fourth ventricle at 14-16 weeks and all were normal at the 20 weeks' scan (Goldstein I et al, 2002). It was suggested that an isolated enlarged fourth ventricle should be followed, but no decisions should be made solely on this isolated finding.

A similar question is raised during first trimester, although the depiction of other malformations is less accurate and therefore to conclude that an enlarged fourth ventricle is an isolated finding is imprecise. One interesting issue would be to ascertain the negative predictive value of normal fourth ventricle in the detection of posterior fossa abnormalities.

#### THE FOURTH VENTRICLE AND ANEUPLOIDIES

A study investigated the possible association between aneuploidies and the diameter of the fourth ventricle measured in the midsagittal view of the fetal profile at 11-14 weeks' gestation (Papastefanou I et al, 2011). In 17 aneuploid fetuses, including nine with trisomy 21, three with trisomy 18, three with trisomy 13, one with trisomy 20 and one with triploidy, the mean diameter of the fourth ventricle, corrected for CRL, was significantly increased. The authors concluded that intracranial translucency appears to have a significant association with chromosomal abnormalities.

## 1.9. VENTRICULOMEGALY

### 1.9.1. DEFINITION AND INCIDENCE

Ventriculomegaly or dilatation of the lateral ventricles of the brain is diagnosed in around 1-2 per 1000 pregnancies (Achiron R et al 1993; Nicolaides KH et al, 1990) and has been defined as a width of the atrium of

the lateral ventricles greater than 10 mm (Cardoza JD, 1988). Several classifications of ventriculomegaly exist. Some authors define mild ventriculomegaly as a diameter of the atrium between 10 and 15 mm, whereas others define mild or ‘‘borderline’’ ventriculomegaly as between 10 and 11.9 mm and moderate ventriculomegaly as between 12 and 15 mm. Measurement of the atrium above 15 mm is called severe ventriculomegaly (Signorelli M et al, 2004, Gaglioti P et al, 2009). The detection of mildly enlarged ventricles in the absence of other fetal abnormalities is designated isolated ventriculomegaly and is observed in 0.7% of pregnancies (Gilmore JH et al, 2008).

Ventriculomegaly is among the most common false positive diagnosis at US screening (Martinez-Zamora MA et al, 2007). There are no data on diagnostic accuracy for borderline ventriculomegaly, but some overestimation of the ventricular width is due to regression of ventriculomegaly during fetal life (Gaglioti P et al, 2005; Falip C et al, 2007) or measurement variability (Gaglioti P et al, 2009). Levine D et al found that in 10% of 200 cases there was disagreement among four experienced radiologist, basically in cases concerning mild ventriculomegaly (Levine D et al, 2008).

### 1.9.2. ETIOLOGY

Ventriculomegaly indicates the presence of a relatively excess of fluid in lateral ventricles. It can result from obstruction within the ventricular system or between this and the subarachnoid space, can be secondary to development disorders or destructive brain lesions.

Obstructive lesions can be seen at the level of the natural narrowings of the ventricular system, such as the aqueduct of Sylvius. This can be the consequence of genetic conditions (Hydrocephalus X-linked recessive trait), cerebral malformation (DWM, Chiari type II), inflammatory process (cytomegalovirus), hemorrhage and tumours. Non-obstructive lesions like choroid plexus papilloma are rare in the fetus. Ventriculomegaly can also be

secondary to disorders of neuronal proliferation, as in microcephaly and megalencephaly or linked to neuronal migration abnormalities as in lissencephaly. Malformations such as agenesis of corpus callosum and holoprosencephaly are commonly associated with ventriculomegaly. Chromosomal abnormalities (trisomy 13, trisomy 18 and trisomy 21) as well as genetic syndromes are related with fetal ventriculomegaly. The same occurs with destructive lesions in the brain secondary to infections and vascular insults (Gaglioti et al, 2009; Mahony BS et al, 1988; Bromley B et al, 1991).

Little is known about the cause of isolated ventriculomegaly except that it is associated with older maternal age, lower gestational age at birth (Gilmore JH et al, 1998) and high levels of interferon in the cord blood indicating prenatal exposure to infection (Dommergues M et al, 1996).

### 1.9.3. PROGNOSIS

Mild ventriculomegaly (10-15 mm) in the absence of other structural or chromosomal abnormalities is associated with a 7-10% risk of learning disabilities (Gaglioti P et al, 2005; Vergani P et al, 1998) and the cognitive or motor delay is predominantly mild (Gaglioti P et al, 2005).

Some studies have evaluated the outcome of mild (10-11.9 mm) and moderate (12-15 mm) ventriculomegaly. A prospective study including 101 fetuses with isolated ventriculomegaly found 6% of developmental disorders in the subgroup of 10-11.9 mm, which is still twice the proportion found in the general population, and 15% in the subgroup of 12–15 mm. In the same study, 14 of 58 (24%) children who had postnatal MRI showed white-matter abnormalities and these included two thirds of the infants with poor outcome (Falip C et al, 2007). A retrospective study including 101 children with prenatal isolated mild ventriculomegaly were assessed at a mean age 55 months. Poor neurological outcome was more often associated with atrial width greater than or equal to 12 mm, asymmetrical bilateral enlargement,

and progression of the ventriculomegaly (Ouahba J et al, 2006) and this is in agreement with a previous study (Signorelli et al, 2004).

A recent systematic review aiming to evaluate the prognosis of isolated mild to moderate cerebral ventriculomegaly included 28 studies. The overall rate of infection and chromosomal abnormality was 1.5% and 5%, respectively. The risk of neurological abnormality regardless of karyotype or infection screen was 14% (95% CI 10-18) and this reduced to 12% (95% CI 9-15) when both chromosomes and infection screens were normal. The risk of neurological abnormality was significantly lower in stable compared to progressive ventriculomegaly (Devaseelan P et al, 2010).

### 1.9.4. FIRST TRIMESTER ASSESSMENT OF LATERAL VENTRICLES

A few previous studies have assessed lateral ventricles during the first trimester by applying the same methodology described for the second and third trimesters of pregnancy.

In 1989, Kushnir et al published normal values for fetal intracranial structures that were studied by high resolution transvaginal ultrasound scan at 12 -14 weeks' gestation in 50 cases. The data included the hemispheric width (HW), the lateral ventricle width (LVW) and the LVW/HW ratio. The HW increased with gestational age from a mean of 8.6 mm at 12 weeks to 11.5 mm at 14 weeks. The LVW/HW ratio decreased with gestational age from 72% to 61% (Kushnir U et al, 1989). Blaas et al in 1994 examined 29 normal cases between 7-12 weeks and measured the lateral ventricles using transvaginal US scan. At 12 weeks' gestation, the mean width of the lateral ventricles was 6.1 mm (Blaas HG et al, 1994).Von Kaisenberg CS et al published in 2002 nomograms for fetal transabdominal biometry at 11 to 14 weeks' of gestation. The anterior horn, the posterior horn, the hemisphere as well as other head measurements including BPD were assessed in 167 fetuses. All fetal biometric parameters showed an increase with gestational age: the mean distance of anterior horn and posterior horn was 3.3 mm and 3.1 mm, respectively at 11 weeks and 5.7 mm and 5.9 mm at 14 weeks.

These authors found that the ratio between the anterior horn and the hemisphere and between the posterior horn and the hemisphere remained constant throughout first trimester with a mean of 0.5 for both (von Kaisenberg et al, 2002).

The results reported by studies concerning the evaluation of lateral ventricles during first trimester have not been found clinically useful for the detection of first trimester ventriculomegaly. Several obstacles make it difficult to achieve objectivity in lateral ventricle assessment. In the first trimester the brain cortex is very thin and it is expected that in ventriculomegaly the width of the ventricle may not be significantly increased. Any possible distortion in shape is not accurately reflected in measurements by using the same methodology described for the second and third trimesters of pregnancy. Furthermore, transient findings are frequent. All these questions raise the need to further investigate the early fetal brain development, by using ultrasound scan and the new technologies available, aiming for a more comprehensive understanding of the normal and pathological brain at 11 to 13 weeks' gestation.

## REFERENCES

*Achiron R, Achiron A.* Transvaginal ultrasonic assessment of the early fetal brain. Ultrasound Obstet Gynecol 1991; I: 336-44.

*Achiron R, Schimmel M, Achiron A, Mashiach S.*Fetal mild idiopathic lateral ventriculomegaly: is there a correlation with fetal trisomy?Ultrasound Obstet Gynecol 1993; 3: 89-92.

*Adzick NS.* Fetal myelomeningocele: Natural history, pathophysiology, and in-utero intervention. Semin Fetal Neonatal Med. 2010; 15 :9-14.

*Alonso MI, Gato A, Moro JA, Martin P, Barbosa E.*Involvement of sulfated proteoglycans in embryonic brain expansion at earliest stages of development in rat embryos.Cells Tissues Organs1999;165:1-9.

*Althouse R, Wald N.* Survival and handicap of infants with spina bifida. Arch Dis Child 1980; 55: 845-50.

*Anderson N, Boswell O, Duff G.* Prenatal sonography for the detection of fetal anomalies: results of a prospective study and comparison with prior series. AJR Am J Roentgenol 1995; 165: 943-50.

*Babcook CJ, Chong BW, Salamat MS, Ellis WG, Goldstein RB.*Sonographic anatomy of the developing cerebellum: normal embryology can resemble pathology. AJR Am J Roentgenol 1996; 166:427-33.

*Bayer SA, Zhang X, Russo RJ, Altman J.*Three-dimensional reconstructions of the developing forebrain in rat embryos. Neuroimage 1994 ;1: 296-307.

*Bejjani GK.* Definition of the adult Chiari malformation: a brief historical overview. Neurosurg Focus 2001; 11: 1-8.

*Blaas HG, Eik-Nes SH, Berg S, Torp H*. In-vivo three-dimensional ultrasound reconstructions of embryos and early fetuses. Lancet 1998; 352: 1182-6.

*Blaas HG, Eik-Nes SH, Kiserud T, Berg S, Angelsen B, Olstad B.* Three-dimensional imaging of the fetal brain cavities in human embryos. Ultrasound Obstet Gynecol 1995; 5: 228-232.

*Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR.* Early development of the forebrain and midbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol 1994; 4: 183-192.

*Blaas, HG, Eik-Nes SH, Isaksen CV*. The detection of spina bifida before 10 gestational weeks using two and three-dimensional ultrasound. Ultrasound Obstet Gynecol 2000; 16: 25-29.

*Blass H-GK, Eik-Nes SH.* Sonoembriology and early prenatal diagnosis of neural anomalies. Prenat Diagn 2009; 312-25.

*Blumenfeld Z, Siegler E, Bronstein M.* The early diagnosis of neural tube defects. Prenat Diagn 1993; 13: 863-71.

*Boddaert N, Klein O, Ferguson N, Sonigo P, Parisot D, Hertz-Pannier L, Baraton J, Emond S, Simon I, Chigot V, Schmit P, Pierre-Kahn A, Brunelle F.* Intellectual prognosis of the Dandy-Walker malformation in children: the importance of vermian lobulation. Neuroradiology 2003;45: 320-4.

*Bornstein E, Monteagudo A, Santos R, Strock I, Tsymbal T, Lenchner E, Timor-Tritsch IE.* Basic as well detailed neurosonograms can be performed by offline analysis of three-dimensional fetal brain volumes. Ultrasound Obstet Gynecol 2010; 36: 20-25.

*Brock DJ.* The use of amniotic fluid AFP action limits in diagnosing open neural tube defects. Prenat Diagn 1981;1:11-6.

*Brock JH.* Alphafetoprotein and neural tube defects. J Clin Pathol Suppl (R Coll Pathol) 1976;10:157-64.

*Brocklehurst G*. The development of the human cerebrospinal fluid pathway with particular reference to the roof of the fourth ventricle. J Anat 1969;105: 467-75.

*Bromley B, Frigoletto FD, Benacerraf BR*. Mild fetal lateral cerebral ventriculomegaly: clinical course and outcome. Am J Obstet Gynecol 1991; 164: 863-7.

*Bromley B, Nadel AS, Pauker S, Estroff JA, Benacerraf BR*. Closure of the cerebellar vermis: evaluation with second trimester US. Radiology 1994;193:761-3.

*Bronshtein M, Zimmer EZ, Blazer S.*Isolated large fourth ventricle in early pregnancy--a possible benign transient phenomenon. Prenat Diagn 1998; 18: 997-1000.

*Buisson O, De Keersmaecker B, Senat MV, Bernard JP, Moscoso G, Ville Y.* Sonographic diagnosis of spina bifida at 12 weeks: heading towards indirect signs. Ultrasound Obstet Gynecol 2002; 19: 290-292.

*Campbell J, Gilbert WM, Nicolaides KH, Campbell S.* Ultrasound screening for spina bifida: cranial and cerebellar signs in a high-risk population. Obstet Gynecol 1987;70:247-50.

*Cardoza JD, Goldstein RB, Filly RA.* Exclusion of fetal ventriculomegaly with a single measurement: the width of the lateral ventricular atrium. Radiology 1988; 169: 711-4.

*Carroll SG, Porter H, Abdel-Fattah S, Kyle PM, Soothill PW.* Correlation of prenatal ultrasound diagnosis and pathological findings in fetal brain abnormalities. Ultrasound Obstet Gynecol 2000; 16: 149-53.

*Castillo MSM:* Imaging of the Pediatric Head, Neck and Spine. Philadelphia, Lippincott- Raven, 1996.

*Chaoui R, Benoit B, Heling KS, Kagan KO, Pietzsch V, Sarut Lopez A, Tekesin I, Karl K.* Prospective detection of open spina bifida at 11-13 weeks by assessing intracranial translucency and posterior brain. Ultrasound Obstet Gynecol 2011; 38:722-6.

*Chaoui R, Benoit B, Mitkowska-Wozniak H, Heling KS, Nicolaides KH.* Assessment of intracranial translucency (IT) in the detection of spina bifida at the 11-13-week scan. Ultrasound Obstet Gynecol 2009 ;34:249-52.

*Chaoui R, Nicolaides KH*. Detecting open spina bifida at the 11-13-week scan by assessing intracranial translucency and the posterior brain region: mid-sagittal or axial plane? Ultrasound Obstet Gynecol 2011;38:609-12.

*Chaoui R, Nicolaides KH.* From nuchal translucency to intracranial translucency: towards the early detection of spina bifida. Ultrasound Obstet Gynecol 2010; 133-38.

*Chitty LS, Hunt GH, Moore J, Lobb MO.* Effectiveness of routine ultrasonography in detecting fetal structural abnormalities in a low risk population. BMJ 1991; 303:1165-9.

*Constantine G, McCormack J.* Comparative audit of booking and mid-trimester ultrasound scans in the prenatal diagnosis of congenital anomalies. Prenat Diagn 1991;11:905-14.

*Copp AJ, Greene NDE.* Genetics and development of neural tube defects. J Pathol 2010; 220: 217-30.

*Crane JP, LeFevre ML, Winborn RC, Evans JK, Ewigman BG, Bain RP, Frigoletto FD, McNellis D.* A randomized trial of prenatal ultrasonographic screening: impact on the detection, management, and outcome of anomalous fetuses. The RADIUS Study Group. Am J Obstet Gynecol 1994; 171:392-9.

*Cullen MT, Green J, Wheatham J, Salafia C, Gabrielli S, Hobbins JC.* Transvaginal ultrasonographic detection of congenital anomalies in the first trimester. Am J Obstet Gynecol 1990; 163: 446-76.

*Czeizel AE, Dudas I*. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Eng J Med 1992; 327: 1832-5.

*D'Addario V, Rossi AC, Pinto V, Pintucci A, Di Cagno L.* Comparison of six sonographic signs in the prenatal diagnosis of spina bifida. J Perinat Med 2008; 36: 330-4.

*Desmond ME, Levitan ML, Haas AR.* Internal luminal pressure during early chick embryonic brain growth: descriptive and empirical observations. Anat Rec A Discov Mol Cell Evol Biol 2005;285: 737-47.

*Devaseelan P, Cardwell C, Bell B, Ong S*. Prognosis of isolated mild to moderate fetal cerebral ventriculomegaly: a systematic review. J Perinat Med 2010; 38: 401-9.

*Dias MS, McLone DG.* Hydrocephalus in the child with dysraphism. Neurosurg Clin N Am 1993;4:715–26.

*Dommergues M, Mahieu-Caputo D, Fallet-Bianco C, Mirlesse V, Aubry MC, Delezoide AL, Dumez Y, Lebon P.* Fetal serum interferon-alpha suggests viral infection as the aetiology of unexplained lateral cerebral ventriculomegaly. Prenat Diagn 1996; 16: 883-92.

*Dückelmann AM, Kalache K*. Three-dimensional ultrasound in evaluating the fetus. Prenat Diagn 2010; 30: 631-38.

*Ecker JL, Shipp TD, Bromley B, Benacerraf B.* The sonographic diagnosis of Dandy-Walker and Dandy-Walker variant: associated findings and outcomes. Prenat Diagn 2000; 20: 328–32.

*Egle D, Strobl I, Weskopf-Scwendinger V, Grubinger E, Kraxner F, Mutz-Dehbalaie IS, Strasak A, Scheier M.* Appearance of the fetal posterior fossa at 11 + 3 to 13 + 6 gestational weeks on transabdominal ultrasound examination. Ultrasound Obstet Gynecol 2011; 38: 620–24.

*Estroff JA, Scott MR, Benacerraf BR*. Dandy-Walker variant: prenatal sonographic features and clinical outcome. Radiology 1992; 185: 755–8.

*Eurenius K, Axelsson O, Cnattingius S, Eriksson L, Norsted T.* Second trimester ultrasound screening performed by midwives; sensitivity for detection of fetal anomalies. Acta Obstet Gynecol Scand 1999;78:98-104.

*Fadda GM, Capobianco G, Balata A, Litta P, Ambrosini G, D'Antona D, Cosmi E, Dessole S*. Routine second trimester ultrasound screening for prenatal detection of

fetal malformations in Sassari University Hospital, Italy: 23 years of experience in 42,256 pregnancies. Eur J Obstet Gynecol Reprod Biol 2009; 144:110-4.

*Falip C, Blanc N, Maes E, Zaccaria I, Oury JF, Sebag G, Garel C.* Postnatal clinical and imaging follow-up of infants with prenatal isolated mild ventriculomegaly: a series of 101 cases. Pediatr Radiol 2007; 37: 981-9.

*Filly RA, Cardoza JD, Goldstein RB, Barkovich AJ.* Detection of fetal central nervous system anomalies: a practical level of effort for a routine sonogram. Radiology 1989; 172: 403-8.

*Finn M, Sutton D, Atkinson S, Ransome K, Sujenthiran P, Ditcham V, Wakefield P, Meagher S.* The aqueduct of Sylvius: a sonographic landmark for neural tube defects in the first trimester. Ultrasound Obstet Gynecol 2011; 38: 640-5.

*Friede RL*. Developmental neuropathology. 2nd ed. Berlin, Germany: Springer-Verlag 1989; 347-371.

*Gaglioti P, Danelon D, Bontempo S, Mombrò M, Cardaropoli S, Todros T.*Fetal cerebral ventriculomegaly: outcome in 176 cases. Ultrasound Obstet Gynecol 2005; 25: 372-7.

*Gaglioti P, Oberto M, Todros T.* The significance of fetal ventriculomegaly: etiology, short- and long-term outcomes. Prenat Diagn 2009; 29: 381-8.

*Garel C.* MR of the Fetal Brain: Normal Development and Cerebral Pathologies, Springer-Verlag: Berlin 2004.

*Gato A, Desmond ME.* Why the embryo still matters: CSF and neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis. Dev Biol 2009; 327: 263-72.

*Ghi T, Pilu G, Falco P, Segata M, Carletti A, Cocchi G, Santini D, Bonasoni P, Tani G, Rizzo N.* Prenatal diagnosis of open and closed spina bifida. Ultrasound Obstet Gynecol 2006; 28: 899-903.

*Gilmore JH, Smith LC, Wolfe HM, Hertzberg BS, Smith JK, Chescheir NC, Evans DD, Kang C, Hamer RM, Lin W, Gerig G.* Prenatal mild ventriculomegaly predicts abnormal development of the neonatal brain. Biol Psychiatry 2008; 64: 1069-76.

*Gilmore JH, van Tol J, Kliewer MA, Silva SG, Cohen SB, Hertzberg BS, Chescheir NC*. Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia. Schizophr Res 1998; 33: 133-40.

*Goetzinger RK, Stamilio DM, Dicke JM, Macones GA, Odibo AO*. Evaluating the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common central nervous system malformations. Am J Obstet Gynecol 2008; 199:285.e1-285.e6.

*Goldstein I, Makhoul IR, Tamir A, Rajamim BS, Nisman D.* Ultrasonographic nomograms of the fetal fourth ventricle: additional tool for detecting abnormalities of the posterior fossa. J Ultrasound Med 2002; 21: 849-56.

*Goldstein RB, Podrasky AE, Filly RA, Callen PW.* Effacement of the fetal cisterna magna in association with myelomeningocele. Radiology 1989;172:409-13.

*Grandjean H, Larroque D, Levi S*. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus Study. Am J Obstet Gynecol 1999; 181:446-54.

*Greene NDE, Coop AJ.* Development of the vertebrate central nervous system: formation of the neural tube. Prenat Diagn 2009; 29: 303-11.

*Harper T, Fordham LA, Wolfe HM.* The fetal Dandy Walker complex: associated anomalies, perinatal outcome and postnatal imaging. Fetal Diagn Ther 2007; 22: 277-81.

*ISUOG Guidelines*: Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram'. Ultrasound Obstet Gynecol 2007; 29: 109–116.

*Johnston M, Papaiconomou C.* Cerebrospinal fluid transport: a lymphatic perspective. News Physiol Sci 2002;17:227-30.

*Juranek J, Salman MS.* Anomalous development of brain structure and function in spina bifida myelomeningocele. Dev Disabil Res Dev 2010;16: 23-30.

*Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH.* Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free b-hCG and pregnancy-associated plasma protein-A. Hum Reprod 2008; 23: 1968-75.

Kalache KD, Eder K, Esser T, Proquitté H, Stoltenburg-Didinger G, Hartung JP, Bamberg C. Three dimensional ultrasonographic reslicing of the fetal brain to assist prenatal diagnosis of central nervous system anomalies. J Ultrasound Med 2006; 25 :509-14.

*Kim MS, Jeanty P, Turner C, Benoit B.* Three-dimensional sonographic evaluations of embryonic brain development. J Ultrasound Med 2008; 27: 119-24.

*Kollias SS, Ball WS Jr, Prenger EC*. Cystic malformations of the posterior fossa: differential diagnosis clarified through embryologic analysis. Radiographics 1993;13:1211-1231.

*Kushnir U, Shalev J, Bronstein M, Bider D, Lipitz S, Nebel L, Mashiach S, Ben-Rafael Z.* Fetal intracranial anatomy in the first trimester of pregnancy: transvaginal ultrasonographic evaluation. Neuroradiology 1989; 31: 222-5.

*Lachmann R, Chaoui R, Moratalla J, Picciarelli G, Nicolaides KH.* Posterior brain in fetuses with open spina bifida at 11 to 13 weeks. Prenat Diagn 2011;31:103-6.

*Levi S, Schaaps JP, De Havay P, Coulon R, Defoort P*. End-result of routine ultrasound screening for congenital anomalies: the Belgian Multicentric Study 1984-92. Ultrasound Obstet Gynecol 1995; 5: 366-71.

*Levi S.* Ultrasound in prenatal diagnosis: polemics around routine ultrasound screening for second trimester fetal malformations. Prenat Diagn 2002; 22: 285-95.

*Levine D, Feldman HA, Tannus JF, Estroff JA, Magnino M, Robson CD, Poussaint TY, Barnewolt CE, Mehta TS, Robertson RL.* Frequency and cause of disagreements in diagnoses for fetuses referred for ventriculomegaly. Radiology 2008; 247: 516-27.

*Little J.* Risks in siblings and other relatives. In Epidemiology and control of neural tube defects (ed JM Elwood, J Little and JH Elwood) 604-76. Oxford University Press, 1992.

Lowery LA, Sive H. Totally tubular: the mystery behind function and origin of the brain ventricular system. Bioessays 2009; 31: 446-58.

*Luck CA.* Value of routine ultrasound scanning at 19 weeks: a four year study of 8849 deliveries. BMJ 1992; 304: 1474-8.

*Mahony BS, Nyberg DA, Hirsch JH, Petty CN, Hendricks SK, Mack LA*. Mild idiopathic lateral cerebral ventriculomegaly: clinical course and outcome. Radiology 1988; 169: 715-21.

*Martinez-Zamora MA, Borrell A, Borobio V, Gonce A, Perez M, Botet F, Nadal A, Albert A, Puerto B, Fortuny A*. False positive in the prenatal ultrasound screening of fetal structural anomalies. Prenat Diagn 2007; 27: 18-22.

*McComb JG.* Spinal and cranial neural tube defects. Semin Pediatr Neurol 1997;4:156–66.

*McLone DG, Dias MS.* The Chiari II malformation: cause and impact. Childs Nerv Syst 2003; 19: 540-50.

*McLone DG, Knepper PA.* The cause of Chiari II malformation: a unified theory. Pediatr Neuros 1989; 15: 1 12.

*McNay MB, Fleming JEE.* Forty years of obstetric ultrasound 1957-1997: from a-scope to three dimensions. Ultrasound Med Biol 1999; 25: 3–56.

*Miller E, Widjaja E, Blaser S. Dennis MM. Raybaud C.* The old and the new: supratentorial MR findings in Chiari II malformation. Childs Nerv Syst 2008; 24: 563-75.

*Mitchell LE.* Epidemiology of neural tube defects. Am J Med Genet C Sem Med Genet 2005; 135: 88-94.

*Monteagudo A, Timor-Tritsch IE, Mayberry P.*Three-dimensional transvaginal neurosonography of the fetal brain: 'navigating' in the volume scan. Ultrasound Obstet Gynecol 2000;16: 307-13.

*Monteagudo A, Timor-Tritsch IE.* Normal sonographic development of the central nervous system from the second trimester onwards using 2D, 3D and transvaginal sonography. Prenat Diagn 2009; 29: 326-339.

*Montenegro N, Matias A*. First trimester ultrasound. In: Textbook of Perinatal Medicine, 2nd Ed. Parthenon Publishing Group 2006: pp1053-63.

*MRC Vitamin Study Research Group.* Prevention of neural tube defects: results of the M. R. C. Vitamin Study. Lancet 1991; 338: 132-7.

*Mulvey S, Wallace EM*. Women's knowledge of and attitudes to first and second trimester screening for Down's syndrome. BJOG 2000; 107: 1302-5.

*Nakling J, Backe B.* Routine ultrasound screening and detection of congenital anomalies outside a university setting. Acta Obstet Gynecol Scand 2005; 84:1042-8.

*Nicolaides KH, Campbell S, Gabbe SG, Guidetti R.* Ultrasound screening for spina bifida: cranial and cerebellar signs. Lancet 1986;2:72-4.

*Nicolaides KH, Berry S, Snijders RJ, Thorpe-Beeston JG, Gosden C*. Fetal lateral cerebral ventriculomegaly: associated malformations and chromosomal defects. Fetal Diagn Ther 1990; 5: 5-14.

*Nicolaides KH, Azar GB, Byrne D, Mansur CA, Marks K.* Nuchal translucency: ultrasound screening for chromosomal defects in the first trimester of pregnancy. BMJ 1992; 304:867-869.

*Nicolaides KH.* Nuchal translucency and other first trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004; 191: 45-67.

*Nicolaides KH.*Turning the pyramid of prenatal care. Fetal Diagn Ther 2011; 29:183-196.

*Nyberg DA, Mack LA, Hirsch J, Mahony BS.* Abnormalities of fetal cranial contour in sonographic detection of spina bifida: evaluation of the "lemon" sign. Radiology 1988;167:387-92.

*Nyberg DA, Pretorius DH, Pilu G*. Diagnostic Imaging of Fetal Anomalies. Philadelphia, Lippincott Williams & Wilkins, 2003, pp 13-14.

*Oakeshott P, Hunt GM.* Long-term outcome in open spina bifida. Br J Gen Pract 2003;53:632–6.

*Oi S, Di Rocco C.* Proposal of 'evolution theory in cerebrospinal fluid dynamics' and minor pathway hydrocephalus in developin immature brain. Childs Nerv Syst 2006; 22: 662-9.

*O'Rahilly R, Müller F.* Ventricular system and choroid plexuses of the human brain during the embryonic period proper. Am J Anat 1990;189 :285-302.

*Ouahba J, Luton D, Vuillard E, Garel C, Gressens P, Blanc N, Elmaleh M, Evrard P, Oury JF*. Prenatal isolated mild ventriculomegaly: outcome in 167 cases. BJOG 2006;113:1072-9.

*Pang D, Dias M.* Cervical myelomeningoceles. Neurosurgery 1993; 33: 363-73.

*Papastefanou I, Souka AP, Pilalis A, Panagopoulos P, Kassanos D*. Fetal intracranial translucency and cisterna magna at 11 to 14 weeks: reference ranges and correlation with chromosomal abnormalities. Prenat Diagn 2011; 31: 1189-92.

*Papp Z, Tóth-Pál E, Papp C, Tóth Z, Szabó M, Veress L, Török O*. Impact of prenatal mid-trimester screening on the prevalence of fetal structural anomalies: a prospective epidemiological study. Ultrasound Obstet Gynecol 1995; 6:320-6.

*Patek KJ, Kline-Fath BM, Hopkin RJ, Pilipenko VV, Crombleholme TM, Spaeth CG.* Posterior fossa anomalies diagnosed with fetal MRI: associated anomalies and neurodevelopmental outcomes. Prenat Diagn 2012; 32:75-82.

*Phillips JJ, Mahony B, Siebert JR*, *Lalani T, Fligner CL, Kapur RP*. Dandy-Walker malformation complex: correlation between prenatal ultrasonographic diagnosis and post-mortem neuropathology. Obstet Gynecol 2006; 107: 685-93.

*Pilu G, Romero R, Reece EA, Goldstein I, Hobbins JC, Bovicelli L.* Subnormal cerebellum in fetuses with spina bifida. Am J Obstet Gynecol 1988;158:1052-6.

*Pilu G, Segata M, Ghi T, Carletti A, Perolo A, Santini D, Bonasoni P, Tani G, Rizzo N.* Diagnosis of midline anomalies of the fetal brain with the three-dimensional median view. Ultrasound Obstet Gynecol 2006; 27:522-9.

*Pitukkijronnakorn S, Chittacharoen A, Jetsawangsri T, Panburana P, Jaovisidha A, Roungsipragarn R, Saropala N, Herabutya Y.* The value of mid-trimester routine ultrasonographic screening in antenatal detection of congenital malformations. J Med Assoc Thai 2009;92:748-53.

*Ramalho C, Matias A, Brandão O, Montenegro N*. Critical evaluation of elective termination of pregnancy in a tertiary fetal medicine center during 43 months: correlation of prenatal diagnosis findings and postmortem examination. Prenat Diagn 2006;26:1084-8.

*Ramalho C, Brandão O, Matias A, Montenegro N.* Systematic fetal autopsy in cases of medical termination of pregnancy due to chromosomal abnormalities: improving the quality of perinatal care?Prenat Diagn. 2010; 30:1000-4.

*Ramalho C, Brandão O, Matias A, Montenegro N.* Phenotypic variability in fetuses with Down syndrome: a case-control pathological evaluation. Fetal Diagn Ther 2011; 30:207-14.

*Raybaud C.*Comment to the paper (CNS-09-0420) "The clinical spectrum of Blake's pouch cyst: report of 6 illustrative cases". Childs Nerv Syst 2010; 26:1065-1066.

*Roberts CJ, Evans KT, Hibbard BM, Laurence KM, Roberts EE, Robertson IB*. Diagnostic effectiveness of ultrasound in detection of neural tube defect. The South Wales experience of 2509 scans (1977-1982) in high-risk mothers. Lancet 1983; 2:1068-9.

*Robinson AJ, Blaser S, Toi A, Chitayat D, Halliday W, Pantazi S, Gundogan M, Laughlin S, Ryan G.* The fetal cerebellar vermis: assessment for abnormal development by ultrasonography and magnetic resonance imaging. Ultrasound Q 2007; 23: 211–23.

*Saunders NR, Habgood MD, Dziegielewska KM.* Barrier mechanisms in the brain, II. Immature brain. Clin Exp Pharmacol Physiol 1999; 26: 85-91.

*Scheier M, Lachmann R, Pětroš M, Nicolaides KH.* Three-dimensional sonography of the posterior fossa in fetuses with open spina bifida at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2011; 38: 625-9.

*Sebire NJ, Noble PL, Thorpe-Beeston JG, Snidjers RJM, Nicolaides KH.* Presence of 'lemon sign' in foetuses with spina bifida at the 10-14 week scan. Ultrasound Obstet Gynecol 1997; 10: 406-9.

*Shirley IM, Bottomley F, Robinson VP.* Routine radiographer screening for fetal abnormalities by ultrasound in an unselected low risk population. Br J Radiol. 1992; 65 :564-9.

*Signorelli M, Tiberti A, Valseriati D, Molin E, Cerri V, Groli C, Bianchi UA.* Width of the fetal lateral ventricular atrium between 10 and 12 mm: a simple variation of the norm? Ultrasound Obstet Gynecol 2004; 23:14-8.

*Snijders RJM, Farrias M, von Kaisenberg C, Nicolaides KH.* Chromosomal defects in fetal abnormalities. In Snijders RJ, Nicolaides KH (eds): Ultrasound Screening for Chromosomal Defects. Parthenon Publishing Group 1995: pp7-9.

*Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH.* UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998; 352: 343-346.

*Solt I, Acuna JG, Adeniji BA, Mirocha J, Kim MJ, Rotmensch S.* First-trimester visualization of the fourth ventricle in fetuses with and without spina bifida. J Ultrasound Med 2011; 30: 1643-7.

*Solt I.* Chiari malformation eponym – time for historical justice. Ultrasound Obstet Gynecol 2011; 37: 250-1.

*Souka AP, Pilalis A, Kavalakis Y, Kosmas Y, Antsaklis P, Antsaklis A*. Assessment of fetal anatomy at the 11–14-week ultrasound examination. *Ultrasound Obstet Gynecol* 2004; 24: 730–734.

*Stevenson KL.* Chiari type II malformation: past, present and future. Neurosurg Focus 2004; 16: E5.

*Swartwout MD, Cirino PT, Hampson AW, Fletcher JM.* Sustained attention in children with two etiologies of early hydrocephalus. Neuropsychology 2008; 22: 765-75.

*Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH.* Challenges in the diagnosis of fetal non chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011; 31: 90-102.

*Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pederson B.* Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; i: 1287-93.

*Tabor A, Zdravkovic M, Perslev A, Møller LK, Pedersen BL.* Screening for congenital malformations by ultrasonography in the general population of pregnant women: factors affecting the efficacy. Acta Obstet Gynecol Scand 2003; 82:1092-8.

*Thiagarajah S, Henke J, Hogge WA, Abbitt PL, Breeden N, Ferguson JE.* Early diagnosis of spina bifida: the value of cranial ultrasound markers. Obstet Gynecol 1990; 76: 54-7.

*Timor-Tritsch IE, Monteagudo A, Warren WB*. Transvaginal ultrasonographic definition of the central nervous system in the first and early second trimesters. Am J Obstet Gynecol 1991;164:497-503.

*Timor-Tritsch IE, Monteagudo A, Peisner DB.* High frequency transvaginal sonographic examination for the potential malformation assessment of the 9-week to 14-week fetus. J Clin Ultrasound 1992; 20: 231-8.

*Timor-Tritsch IE, Monteagudo A, Mayberry P.* Three-dimensional ultrasound evaluation of the fetal brain: the three horn view. Ultrasound Obstet Gynecol 2000; 16 :302-6.

*Timor-Tritsch IE, Monteagudo A, Santos R.* Three-dimensional inversion rendering in the first- and early second-trimester fetal brain: its use in holoprosencephaly. Ultrasound Obstet Gynecol 2008; 32 :744-50.

*Timor-Tritsch IE, Monteagudo A.* Transvaginal fetal neurosonography: standardization of the planes and sections by anatomic landmarks. Ultrasound Obstet Gynecol 1996; 8: 42-7.

*Tortori-Donati P, Fondelli MP, Rossi A, Carini S*. Cystic malformations of the posterior cranial fossa originating from a defect of the posterior membranous area. Mega cisterna magna and persisting Blake's pouch: two separate entities. Childs Nerv Syst 1996;12:303-8.

*Ulm B, Ulm MR, Deutinger J, Bernaschek G.*Dandy-Walker malformation diagnosed before 21 weeks of gestation: associated malformations and chromosomal abnormalities. Ultrasound Obstet Gynecol 1997; 10: 167-70.

*Van den Hof MC, Nicolaides KH, Campbell J, Campbell S.* Evaluation of the lemon and banana signs in one hundred thirty fetuses with open spina bifida. Am J Obstet Gynecol 1990; 162: 322-7.

*Vergani P, Locatelli A, Strobelt N, Cavallone M, Ceruti P, Paterlini G, Ghidini A*. Clinical outcome of mild fetal ventriculomegaly. Am J Obstet Gynecol 1998; 178: 218-22.

*von Kaisenberg CS, Fritzer E, Kühling H, Jonat W.* Fetal transabdominal biometry at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2002; 20: 564-74.

*Wald NJ, Cuckle HS, Nanchahal K.* Amniotic fluid acetylcholinesterase measurement in the prenatal diagnosis of open neural tube defects. Second report of the collaborative acetylcholinesterase study. Pren Diagn 1989; 9: 813-29.

*Wald NJ, Sneddon J, Densem J, Frost C, Stone R.* MRC Vitamin Study Res Group. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. Lancet 1991; 338: 131-37.

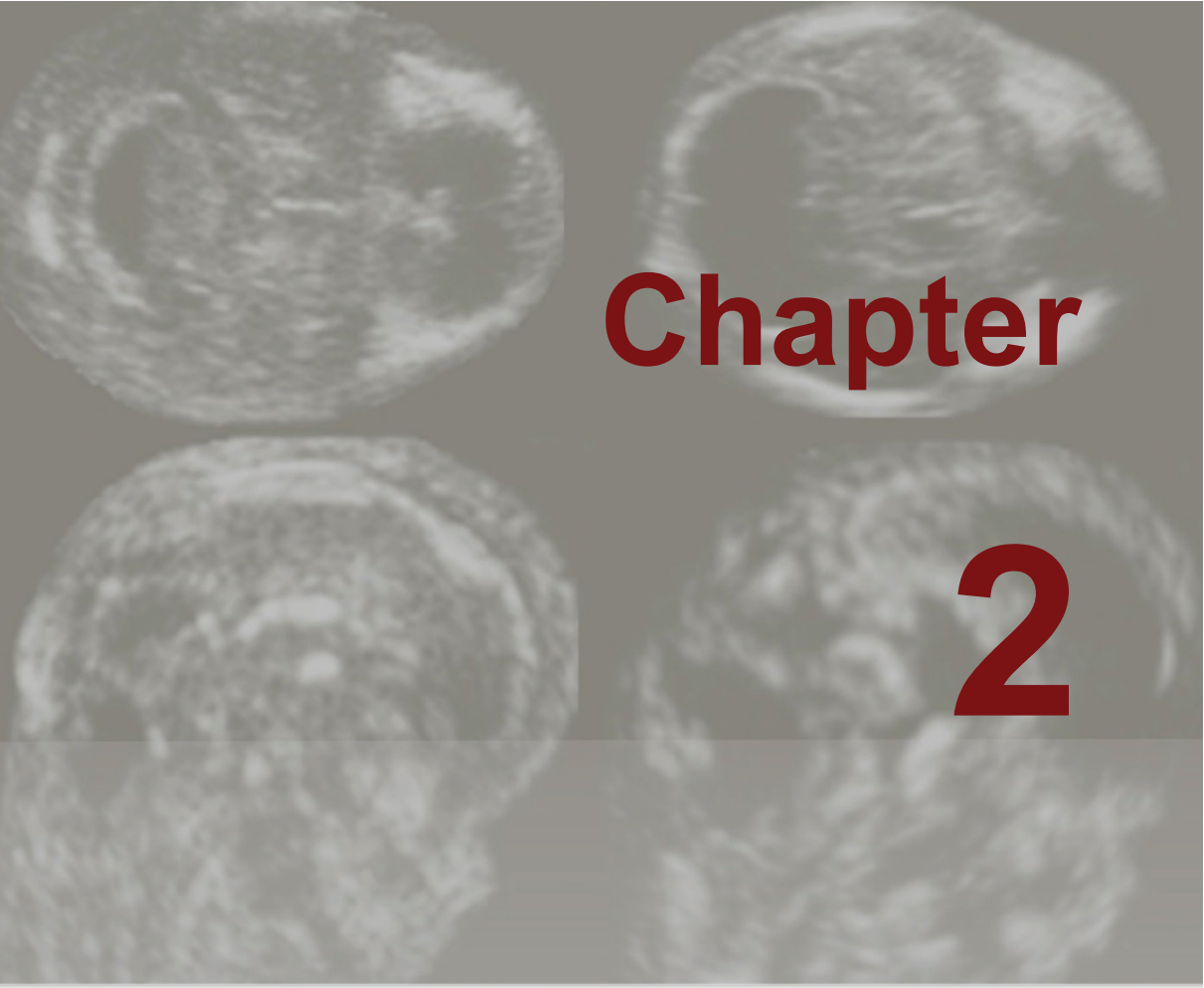
*Wald NJ, Kennard A, Donnenfeld A, Leck I.* Ultrasound scanning for congenital abnormalities. Antenatal & Neonatal screening, 2nd edition. Oxford University Press, 2000. Chapter 18: pp441-69.

*Wald NJ.* Neural tube defects, Antenatal & Neonatal screening, 2nd edition. Oxford University Press, 2000. Chapter 3: pp.61-84.

*Wald NJ, Huttly WJ, Hackshaw AK.* Antenatal screening for Down's syndrome with the quadruple test. Lancet 2003; 361: 835-6.

*Zalel Y, Yagel S, Achiron R, Kivilevich Z, Gindes L*. Three-dimensional ultrasonography of the fetal vermis at 18 to 26 weeks' gestation: time of appearance of the primary fissure. J Ultrasound Med 2009; 28: 1-8.

*Zappaterra MD, Lisgo SN, Lindsay S, Gygi SP, Walsh CA, Ballif BA.* A comparative proteomic analysis of human and rat embryonic cerebrospinal fluid. J Proteome Res. 2007; 6: 3537-48.



# Chapter 2

## AIM AND OUTLINE OF THE THESIS

## 2.1. INTRODUCTION

Congenital anomalies of the fetal central nervous system occur with an incidence of approximately 1 in 1 000 pregnancies, with neural tube defects being among the most common (Verity C et al, 2003).

Several factors limit the early fetal neuroscan such as the size of the brain and cerebral structures, the incomplete understanding of developmental stages and especially the fact that most of the common congenital brain abnormalities cannot be correctly depicted ahead of the time of full development, that is, 20 to 24 weeks' of gestation (Bromley B et al, 1994; Babcook CJ et al, 1996). For these reasons, the results reported by studies concerning the evaluation of first trimester fetal head and brain biometry have been inconsistent with providing clinical guidance for the detection of brain abnormalities (Kushnir U et al, 1989, Blaas HG et al, 1994, von Kaisenberg et al, 2002). It has become increasingly apparent that first trimester brain examination in a routine setting can lead to the diagnosis of some severe defects including acrania, encephalocele, and holoprosencephaly (Syngelaki et al., 2011). But apart from these serious conditions, only a few other steps have been made in describing ultrasonographic developmental milestones and abnormal patterns of brain development (Chaoui R et al, 2009, 2010, 2011; Lachmann R et al, 2011).

The ventricular system is normally prominent during first trimester and appears as large sonolucent free spaces (Blaas HG et al, 1995; Timor-Tritsch IE et al, 2008). At this stage the brain cortex is very thin and it is expected that atypical development of the ventricular system may not be only traduced in terms of differences in dimensions of the spaces but also as a change in configuration. For example, any possible distortion in the shape of the lateral ventricles in early brain is not expected to be accurately reflected in measurements if the same methodology described for the second and third trimesters of pregnancy is used. Furthermore, transient findings are frequent, as previously reported (Bronshtein M et al, 1998; Goldstein I et al, 2002).

By taking advantage of the improved quality of image provided by high frequency ultrasound transvaginal probes and of recent advances in 3D ultrasound signal acquisition, processing and display, it is aimed for a more comprehensive understanding of the normal and pathological brain at 11 to 13 weeks' gestation.

## 2.2. AIM AND OUTLINE OF THE THESIS

The main objective of this thesis was the evaluation of the cerebral ventricular system at 11-13 weeks' gestation both in normal fetuses and those affected by aneuploidy or spina bifida. The ultimate goal is to find brain markers in frequent conditions associated with serious handicap like trisomy 21 (Down's syndrome), trisomy 18 (Edwards' syndrome), trisomy 13 (Patau's syndrome) and spina bifida that may be useful in screening.In order to accomplish this aim, the following research plan phases were established:

1. Describe the normal ventricular system in euploid fetuses and test the reproducibility of measurements **– CHAPTER 3.**

2. To determine if in fetuses with open spina bifida at 11-13 weeks' gestation there are alterations in the cerebral ventricular system **– CHAPTER 4.**

3. To determine if in fetuses with aneuploidies the diameter of the fourth cerebral ventricle at 11-13 weeks' gestation is different from euploid fetuses **– CHAPTER 5.**

4. To examine the possible association between aneuploidies and lateral cerebral ventriculomegaly in the first trimester of pregnancy **– CHAPTER 6.**

## REFERENCES

*Babcook CJ, Chong BW, Salamat MS, Ellis WG, Goldstein RB.* Sonographic anatomy of the developing cerebellum: normal embryology can resemble pathology. AJR Am J Roentgenol 1996; 166:427-33.

*Blaas HG, Eik-Nes SH, Kiserud T, Berg S, Angelsen B, Olstad B.* Three-dimensional imaging of the fetal brain cavities in human embryos. Ultrasound Obstet Gynecol 1995; 5: 228-232.

*Bromley B, Nadel AS, Pauker S, Estroff JA, Benacerraf BR*. Closure of the cerebellar vermis: evaluation with second trimester US. Radiology 1994;193:761-3.

*Bronshtein M, Zimmer EZ, Blazer S* .Isolated large fourth ventricle in early pregnancy--a possible benign transient phenomenon. Prenat Diagn 1998; 18: 997-1000.

*Goldstein I, Makhoul IR, Tamir A, Rajamim BS, Nisman D.* Ultrasonographic nomograms of the fetal fourth ventricle: additional tool for detecting abnormalities of the posterior fossa. J Ultrasound Med 2002; 21: 849-56.

*Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH.* Challenges in the diagnosis of fetal non chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011; 31: 90-102.

*Timor-Tritsch IE, Monteagudo A, Santos R.* Three-dimensional inversion rendering in the first- and early second-trimester fetal brain: its use in holoprosencephaly. Ultrasound Obstet Gynecol 2008; 32: 744–50.

*Verity C, Firth H, French-Constant C*. Congenital abnormalities of the central nervous system. J Neurol Neurosurg Psychiatry 2003;74:i3-i8.

# Chapter 3

## CEREBRAL VENTRICULAR SYSTEM IN NORMAL FETUSES AT 11-13 WEEKS' GESTATION

***This chapter contents, namely tables and figures, reproduce some of the results present in the following articles:***

*Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH. Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012; 39: 620-4.*

*Loureiro T, Ushakov F, Nerea M, Montenegro N, Nicolaides KH. Lateral ventricles in aneuploidies at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012; 40: 282-7.*

## ABSTRACT

**OBJECTIVE:** to establish reference ranges of different components of the cerebral ventricular system at 11-13 weeks' gestation by using three-dimensional (3D) ultrasound; to assess the intra- and interobserver reproducibility of the measurements performed.

**METHODS:** Measurements of lateral ventricles area, choroid plexuses area, third ventricle, aqueduct and fourth ventricle diameters were taken in a series of transverse views assessed in 410 volumes acquired by 3D transvaginal scan at 11-13 weeks' gestation in euploid fetuses. The ratio between choroid plexus and lateral ventricle areas was calculated. Reference ranges were built and the mean, 5$^{th}$ and 95$^{th}$ centiles were plotted against biparietal diameter (BPD). Mean difference and 95% limits of agreement between paired measurements of the different components of the ventricular system by the same and by two different sonographers were determined.

**RESULTS:** In normal fetuses the area of the lateral ventricles and choroid plexuses increased, whereas the choroid plexus to lateral ventricle ratio decreased with fetal BPD. The third ventricle diameter increased with fetal BPD, the fourth ventricle diameter did not change significantly with BPD and aqueduct diameter decreased with gestational age. In 95% of the cases, the difference between paired measurements of third ventricle by the same sonographer was between -0.29 mm and 0.18 mm, being between -0.2 mm and 0.18 mm and -0.4 mm and -0.39 mm for the aqueduct and fourth ventricle, respectively. The difference in measurements between two sonographers was between −0.38 mm and 0.53 mm for third ventricle, -0.39 mm and 0.29 mm for the aqueduct of Sylvius and -0.75 mm and 0.64 mm for the fourth ventricle. In 95% of the cases, the lateral ventricles area and the choroid plexus area differences between paired measurements were both within ±0.06 cm$^2$ by the same sonographer and also between two sonographers.

**CONCLUSIONS:** Measurement of the components of the cerebral ventricular system is feasible. The evaluation of lateral ventricles and choroid plexus is highly reproducible concerning both intraobserver and interobserver variability. Smaller size structures like third and fourth ventricles and aqueduct of Sylvius present higher deviations concerning interobserver assessment.

## 3.1. INTRODUCTION

The brain is the only structure in the fetus that changes its anatomy during its normal development. Extensive information about the early development of the nervous system in the human embryo is available in the literature. Nevertheless, during post embryonic phase particularly at 11-13 weeks' gestation the data is sparse and usually limited to basic investigation. A staging system is not available for the fetal period. To describe the developmental of the normal early fetal brain is essential to fully comprehend early pathology.

Several studies utilizing high frequency transvaginal ultrasound probes have reported the sonoanatomic features of the developing embryonic and early fetal brain, including the dimensions of the cavities of the hemispheres, diencephalon, mesencephalon and rhombencephalon (Kushnir *et al.,* 1989; Blaas *et al*., 1994; 1995; 1998; Kim *et al*. 2008). There is evidence from second-trimester neurosonography that three-dimensional (3D) ultrasound is useful because it provides the option for *off-line* assessment of small structures without the inconvenience of the frequent fetal movements (Monteagudo A et al, 2009; Bornstein E et al, 2010, Dückelmann AM). Furthermore, volume acquisition allows a correct alignment of small and tiny structures in the early brain and therefore could potentially constitute a more precise tool for making measurements.

The aim of this observational study was to describe the development of the fetal cerebral ventricular system at 11-13 weeks' gestation by 3D ultrasound.

## 3.2. METHODS

The data used to calculate the normal population was collected in three hospitals between October 2009 and June 2011. Each fetus was assessed once during the period of 11–13 weeks' gestation (CRL: 45–84 mm). Fetuses which subsequently miscarried, those with chromosomal abnormalities or malformations were excluded from the calculation of normal reference ranges. Four hundred and ten normal singleton pregnancies resulting in the delivery of phenotypically normal neonates were included. Transvaginal sonography was carried out and the fetal head was systematically assessed in transverse views from crown to neck. Brain volumes were acquired (transvaginal 5-9L probe, GE Voluson Expert 750, GE Voluson E8 or GE Voluson E6, GE Medical Systems, Zipf, Austria) by one of three doctors with experience in this technique. The fetal head image was magnified until filling 75% of the screen. The 3D sample box was placed in a way to contain only the fetal head and the acquisition plane was set at the level of the thalamus and mesencephalon, in a transverse view. The angle of acquisition was 35-55$^{o}$ depending on the distance between the transducer and the fetal head. Volume acquisition was during fetal quiescence and took 3 seconds to complete. The resulting 3D volume, which included the whole fetal head from crown to neck, was stored and studied *off-line*, using 4D view software (GE Medical System, version 6.0). This allowed the display of brain structures in three orthogonal planes simultaneously (Figure 1) that compose the multiplanar mode of the 3D image.

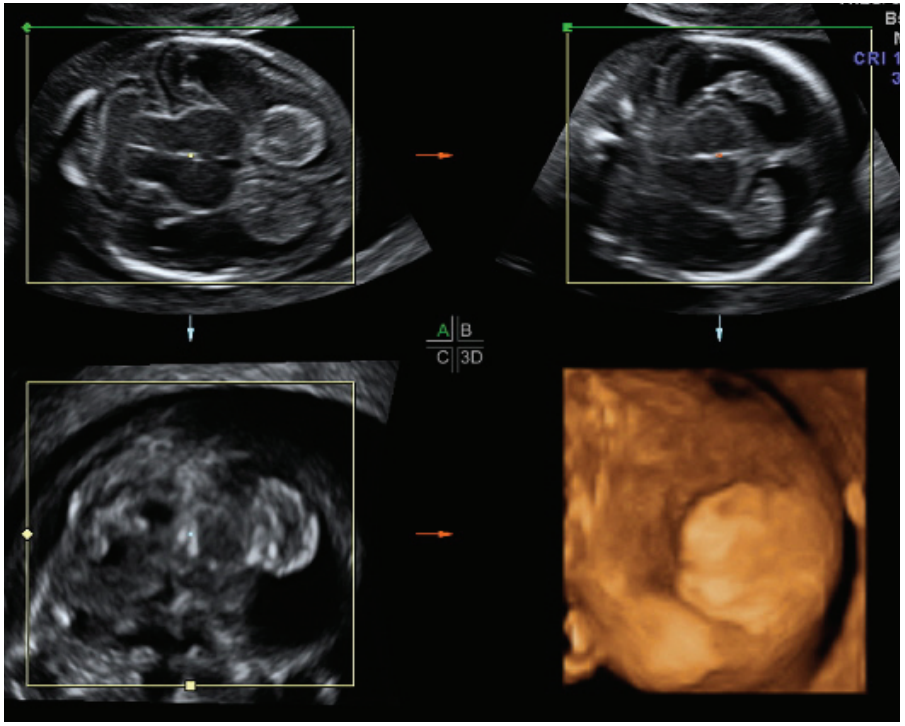



**Figure 1 –** Display of the fetal brain volume in three orthogonal planes (multiplanar mode).
The axial view is shown in plane A, the coronal view in plane B and the sagittal view in plane C. The 3D render mode is displayed in the lower right image. The acquisition view is plane A (axial).

During *off-line* manipulation, the position of the brain was adjusted to obtain an exact sagittal view in plain C. Different post-processing features to improve the quality of image were used, like candle chrome map and speckle-reduction imaging (SRI) feature.

The navigation through the different planes was done 'off-line' in a personal computer, using 4D view software (GE Medical System, version 6.0). The advantage of this 'off-line' processing is that each of the three planes can be rotated around the x, y and z axes and it is possible to use the marker dot

which is generated by the intersection of the three planes to identify structures. Four different views of the brain were obtained where measurements were performed: lateral-third ventricle view (Figure 2), the lateral ventricles view (Figure 3), the transthalamic view, (Figure 4) and fourth ventricle view (Figure 5).

A perpendicular sagittal view was obtained in plane C and the marker dot was moved to the upper brain so that in the transverse view in plane A the butterfly image of the two chroid plexuses with the third ventricle in the middle could be visualized. In this lateral third ventricle view we measured the BPD and third ventricle (Figure 2). The lateral ventricles and the choroid plexuses were assessed in a transverse view, just above the roof of the third ventricle and the caudate nucleus (Figure 3). The area of each lateral ventricle and of each choroid plexus was measured by tracing the border of these structures and the average of the two measurements was considered. The ratio between choroid plexus and lateral ventricle areas was then calculated. The transthalamic view was obtained by tilting the head anteriorly by 22-28$^{o}$ so that the thalamus, midbrain and aqueduct can be seen. In this view we measured the diameter of the aqueduct in its wider portion, by placing the callipers on the hyperechogenic lines that correspond to the aqueduct walls (Figure 4). Parallel and caudally to the transthalamic view and just below the cerebellum, the semilunar fourth ventricle is depicted in plane A and in this view the largest antero-posterior diameter was measured (Figure 5).

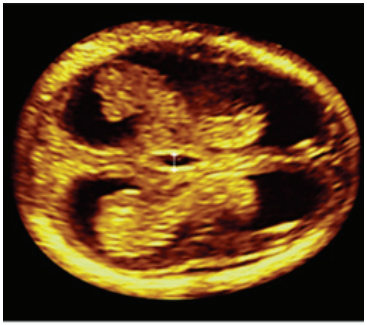


**Figure 2 –** Lateral-third ventricle view where the diameter of the third ventricle was measured.

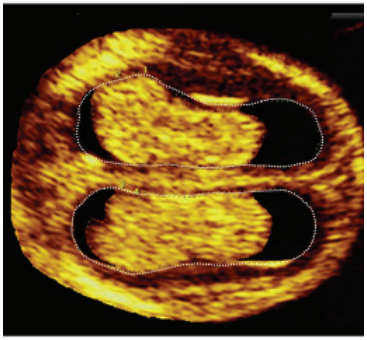


**Figure 3 –** Lateral ventricles view where the areas of the lateral ventricles and choroid plexuses were measured.

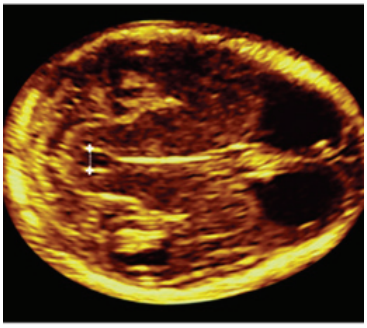


**Figure 4 –** The transthalamic view where the diameter ofthe aqueduct of Sylvius was measured.

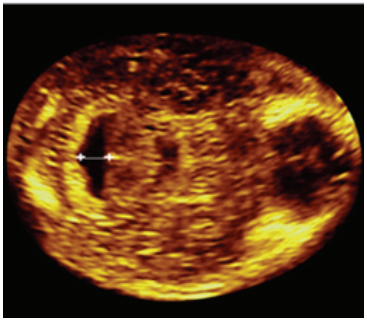


**Figure 5 –** The suboccipitobregmatic view, below the cerebellum, where the largest antero-posterior diameter of the fourth ventricle was measured.

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination. The outcome was assessed by second trimester ultrasound and postnatal examination. In this study we report the findings in normal singleton pregnancies resulting in the delivery of phenotypically normal neonates.

### STATISTICAL ANALYSIS

Regression analysis was used to examine whether each measurement changed with the BPD and whether the relationship was linear or non-linear. A reference range (5th, 50th and 95th centiles) for each measurement was constructed based on the relationship with BPD.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL) was used for data analyses.

### INTRAOBSERVER AND INTEROBSERVER STUDIES

In 50 volumes selected at random all the measurements were recorded by two different observers and twice by one of the observers with more than one month interval. Limits of agreement were calculated. Bland–Altman analysis was used to compare the measurement agreement and bias for a single examiner and between different examiners (Bland JM et al, 1986). Mean difference in lateral ventricles area, choroid plexuses area, third ventricle diameter, aqueduct of Sylvius diameter and fourth ventricle diameter and the 95% limits of agreement (LOA) with their 95% CI between 50 paired measurements by the same sonographer and between 50 paired measurements by two sonographers in the chromosomally normal fetuses are shown in table 3. The statistical software package MedCalc 12 was used for data analysis.

## 3.3. RESULTS

The demographic and pregnancy characteristics of the study population of 410 euploid cases are summarized in Table 1.

**Table 1:** Demographic and pregnancy characteristics of the study population

| Characteristic | Normal outcome (n=410) |
|---|---|
| Maternal age in years | 31.5 (28.0-35.0) |
| Gestational age in days | 87.0 (84.0-90.0) |
| Fetal biparietal diameter in mm | 21.0 (19.1-23.4) |
| Fetal crown rump length in mm | 60.8 (55.5-66.3) |
| Fetal nuchal translucency in mm | 1.9 (1.6-2.3) |

*Adapted from Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH. Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012.*

**Table 2:** Results of regression analysis demonstrating the relationship of various measurements of the cerebral ventricular system in normal fetuses with biparietal diameter. In the case of aqueduct of Sylvius diameter the relationship is quadratic.

| Measurement | Coefficient (95% CI) | Standard error | P value |
|---|---|---|---|
| Third ventricle diameter | 0.029 (0.020 to 0.038) | 0.005 | <0.0001 |
| Aqueduct of Sylvius diameter | | | |
| Biparietal diameter | 0.080 (0.015 to 0.146) | 0.033 | 0.016 |
| (Biparietal diameter)$^2$ | -0.003 (-0.004 to -0.001) | 0.001 | <0.0001 |
| Fourth ventricle diameter | 0.009 (-0.002 to 0.020) | 0.006 | 0.124 |

*Adapted from Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH. Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012.*

***CI** = confidence interval*

The scatter plots with reference ranges (5th, 50th and 95th centiles) of the various measurements with fetal BPD are shown in Figures 6-7.

**Table 3.** Mean difference of lateral ventricles area, choroid plexuses area, third ventricle, aqueduct and fourth ventricle diameters and the 95% limits of agreement (LOA) with their 95% CI between 50 paired measurements by the same sonographer and between 50 paired measurements by two sonographers.

| Measurement | Mean difference (95% LOA) [95% CI] |
|---|---|
| **INTRAOBSERVER A** | **INTRAOBSERVER A** |
| Lateral ventricle area ($cm^2$) | 0.00 (-0.06[-0.07; -0.04], 0.06 [0.04, 0.03]) |
| Choroid plexuses area ($cm^2$) | 0.00 (-0.03[-0.04; -0.02], 0.04 [0.03, 0.05]) |
| Third ventricle diameter (mm) | -0.05 (-0.29[-0.35; -0.23], 0.18 [0.12, 0.24]) |
| Aqueduct of Sylvius diameter (mm) | -0.01 (-0.20[-0.25; -0.15], 0.18 [0.13, 0.22]) |
| Fourth ventricle diameter (mm) | -0.02 (-0.43[-0.53; -0.33], 0.39 [0.28, 0.49]) |
| **INTEROBSERVER** | **INTEROBSERVER** |
| Lateral ventricle area ($cm^2$) | 0.00 (-0.04[-0.05; -0.03], 0.05 [0.04, 0.06]) |
| Choroid plexuses area ($cm^2$) | 0.00 (-0.05[-0.06; -0.03], 0.05 [0.04, 0.06]) |
| Third ventricle diameter (mm) | 0.07 (-0.38[-0.50; -0.27], 0.53 [0.42, 0.64]) |
| Aqueduct of Sylvius diameter (mm) | -0.05 (-0.39[-0.47; -0.30], 0.29 [0.21, 0.38]) |
| Fourth ventricle diameter (mm) | -0.05 (-0.75[-0.92; -0.57], 0.64 [0.47, 0.81]) |

***LOA** = limits of agreement*

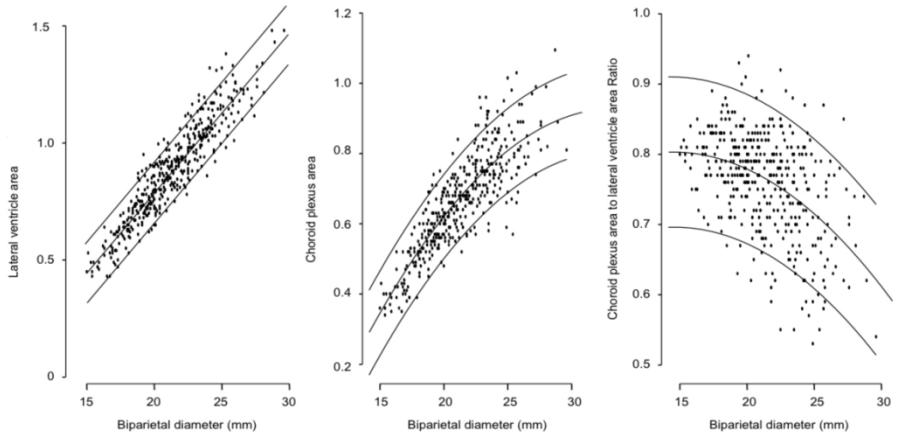



**Figure 6 –** Reference ranges of lateral ventricle area ($cm^2$), choroid plexus area ($cm^2$) and choroid plexus to lateral ventricle area ratio with biparietal diameter in euploid fetuses at 11-13 weeks' gestation ($5^{th}$, $50^{th}$ and $95^{th}$centiles). From *Loureiro T, Ushakov F, Nerea M Montenegro N, Nicolaides KH.* Lateral ventricles in aneuploidies at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012.

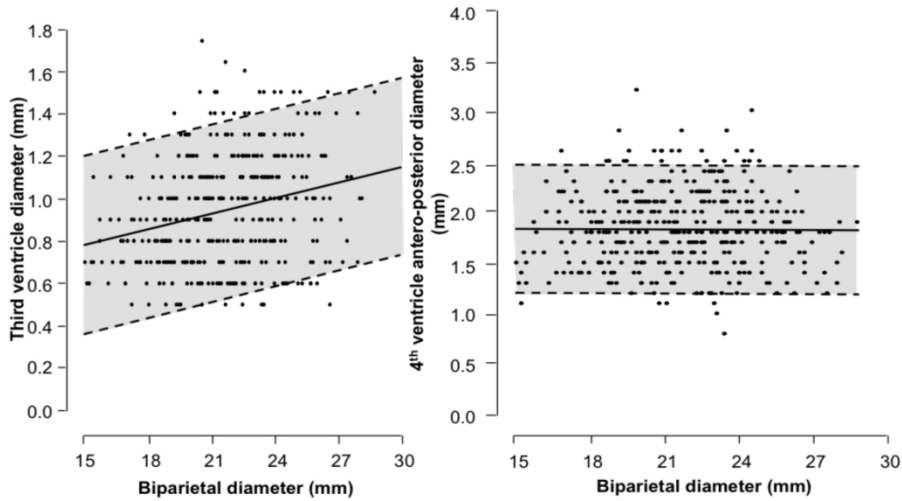



**Figure 7 –** Reference ranges of third and fourth ventricles diameter with biparietal diameter in euploid fetuses at 11-13 weeks' gestation ($5^{th}$, $50^{th}$ and $95^{th}$centiles).

## 3.4. DISCUSSION

Brain biometric parameters such as the lateral ventricle area, the choroid plexus area and the third ventricle diameter increased with gestional age, the choroid plexus to lateral ventricle area ratio decreased as well as the aqueduct of Sylvius diameter and the measurement of the fourth ventricle remained stable at 11 to 13 weeks' of gestation.

Not many studies have assessed the ventricular system during first trimester. Blaas et al in 1995 examined 29 normal cases between 7-12 weeks and measured the fourth ventricle and the choroid plexus using transvaginal ultrasound (US) scan. This author concluded that the fourth ventricle is visible by ultrasound from 7 weeks onwards and increases with gestational age from an antero-posterior diameter mean of 1.5 mm at 7 weeks to 3.2 mm at 12 weeks (Blaas et al, 1995). In another study that aimed to measure the fourth ventricle in 299 normal fetuses at 13-40 weeks' gestation, the fourth ventricle increased linearly with biparietal diameter (BPD). At $13^{+0}$ to $13^{+6}$ weeks the $10^{th}$, $50^{th}$ and $90^{th}$ centile were 2.1 mm, 2.5 mm and 3.0 mm, respectively (Goldstein I et al, 2002). Chaoui R et al in 2009 measured the fourth ventricle by transabdominal route in the mid-sagittal plane in 200 normal fetuses. The fourth ventricle was always visible and the median anteroposterior diameter increased from 1.5 mm at a crown–rump length (CRL) of 45 mm to 2.5 mm at a CRL of 84 mm (Chaoui R et al, 2009).

Another prospective study including 502 fetuses measured the fourth ventricle in an axial view by transabdominal scan, and found a linear correlation between CRL and fourth ventricle. The fourth ventricle increased from 1.7 mm at a CRL of 45 mm to 2.5 mm at a CRL of 84 mm (Eagle et al, 2011). Similar results were found by a recent study using the transabdominal route and the mid-sagittal plane (Papastefanou I et al, 2011).

In this study we have used a different methodology to measure the cerebral ventricular system components. Only good quality volumes acquired by high frequency transvaginal probes and where all the measurements could be

clearly performed were selected. We have used a tilted axial view below the plane that includes the cerebellum to measure the fourth ventricle. In this plane it is possible to see the semilunar fourth ventricle that appears as a black space well defined in its borders by two lines: the edge of the brainstem (anteriorly) and the choroid plexus of the fourth ventricle (posteriorly) (Figure 5). Our data show that the fourth ventricle measurement was stable at 11 to 13 weeks' of gestation being the 50$^{th}$ and 95$^{th}$ centile 1.9 mm and 2.5 mm, respectively. There are no prior studies reporting reference ranges for the area of the lateral ventricles, choroid plexus area and ratio of choroid plexus to lateral ventricle area at 11 to 13 weeks' gestation, nor evaluating the diameter of the aqueduct of Sylvius or third ventricle.

Previous studies at 14–40 weeks' gestation have established likelihood ratios for fetal chromosomal defects using specific alterations of fetal biometry such as ventriculomegaly (Va/HEM or Vp/HEM > 95th centile) (Snijders RJM et al,1996).The establishment of comprehensive reference ranges for first-trimester cerebral ventricular system may allow the interpretation of measurements in chromosomally abnormal fetuses and brain anomalies.

The data demonstrate that the measurements of the lateral ventricles and choroid plexuses area are highly reproducible. For the third ventricle, aqueduct and fourth ventricle diameter in about 95% of cases the differences between two measurements by the same observer are within 0.4 mm and by different observers are within 0.7 mm of each other. These structures present higher deviations concerning interobserver assessment which can be improved by training or eventually by the development of automatic software of estimation their absolute dimensions.

## 3.5. ACKNOWLEDGMENTS

I am grateful to Dr. Fred Ushakov, Dr. João Pedro Neves and Dr. Ranjit Akolekar for their contributions.

## REFERENCES

*Blaas HG, Eik-Nes SH, Berg S, Torp H*. In-vivo three-dimensional ultrasound reconstructions of embryos and early fetuses. Lancet 1998; 352: 1182–86.

*Blaas HG, Eik-Nes SH, Kiserud T, Berg S, Angelsen B, Olstad B.* Three-dimensional imaging of the fetal brain cavities in human embryos. Ultrasound Obstet Gynecol 1995; 5: 228-232.

*Blaas HG, Eik-Nes SH, Kiserud T, Hellevik LR.* Early development of the forebrain and midbrain: a longitudinal ultrasound study from 7 to 12 weeks of gestation. Ultrasound Obstet Gynecol 1994; 4: 183-192.

Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; 1: 307–310.

*Bornstein E, Monteagudo A, Santos R, Strock I, Tsymbal T, Lenchner E, Timor-Tritsch IE.* Basic as well detailed neurosonograms can be performed by offline analysis of three-dimensional fetal brain volumes. Ultrasound Obstet Gynecol 2010; 36: 20-25.

*Dückelmann AM, Kalache K.* Three-dimensional ultrasound in evaluating the fetus. Prenat Diagn 2010; 30: 631-38.

*Goldstein I, Makhoul IR, Tamir A, Rajamim BS, Nisman D.* Ultrasonographic nomograms of the fetal fourth ventricle: additional tool for detecting abnormalities of the posterior fossa. J Ultrasound Med 2002; 21: 849-56.

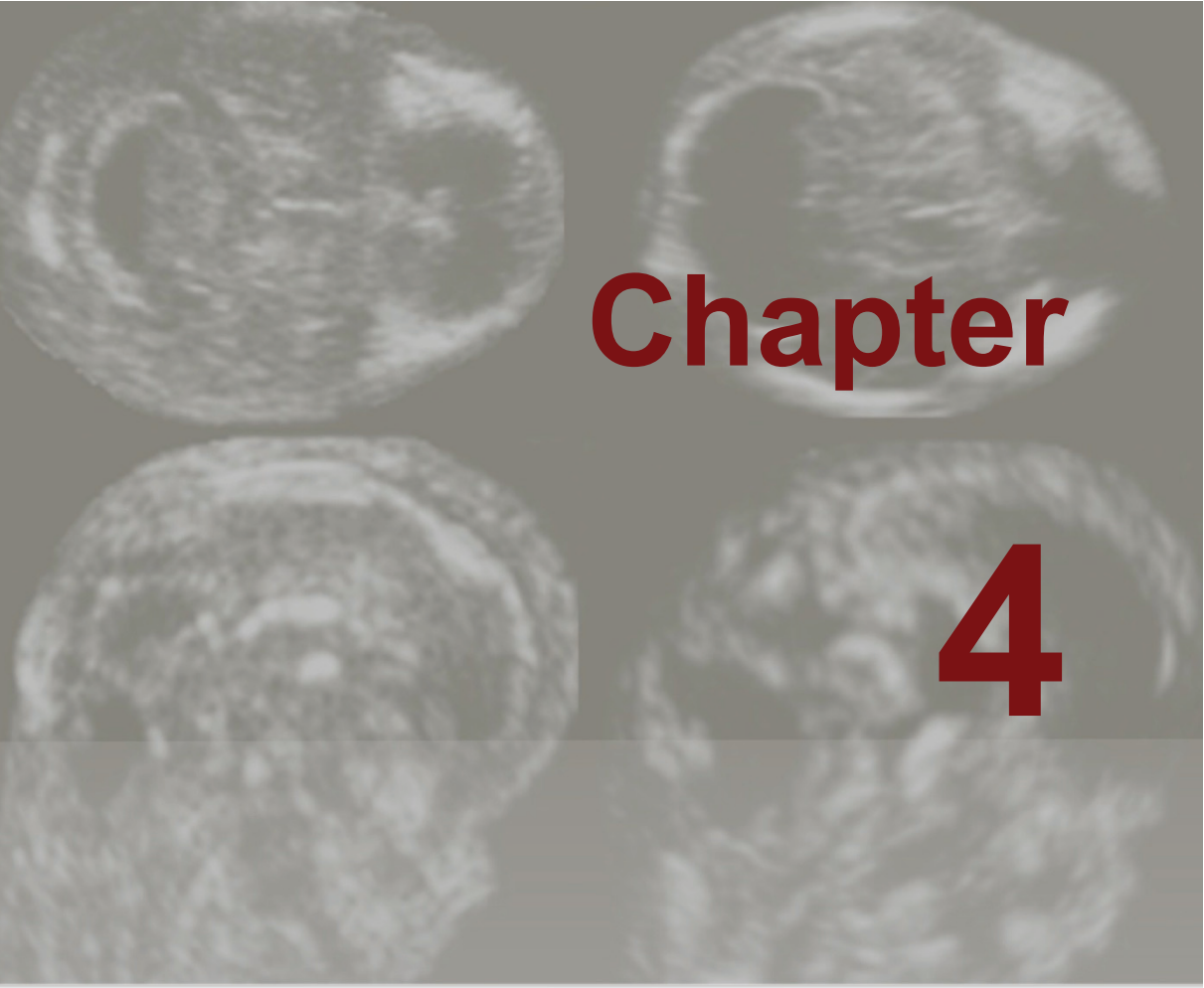
*Kim MS, Jeanty P, Turner C, Benoit B.* Three-dimensional sonographic evaluations of embryonic brain development. J Ultrasound Med 2008; 27: 119-24.

*Kushnir U, Shalev J, Bronstein M, Bider D, Lipitz S, Nebel L, Mashiach S, Ben-Rafael Z.* Fetal intracranial anatomy in the first trimester of pregnancy: transvaginal ultrasonographic evaluation. Neuroradiology 1989; 31: 222-5.

*Monteagudo A, Timor-Tritsch IE.* Normal sonographic development of the central nervous system from thesecond trimester onwards using 2D, 3D and transvaginal sonography. Prenat Diagn 2009; 29: 326-339.

*Papastefanou I, Souka AP, Pilalis A, Panagopoulos P, Kassanos D*. Fetal intracranial translucency and cisterna magna at 11 to 14 weeks: reference ranges and correlation with chromosomal abnormalities. Prenat Diagn 2011; 31: 1189-92.

*Snijders RJM, Nicolaides KH.* Ultrasound Markers for Fetal Chromosomal Defects. The Parthenon Publishing Group, 1996.



# Chapter 4

Ultrasound Obstet Gynecol 2012; 39: 620-4.

## CEREBRAL VENTRICULAR SYSTEM IN FETUSES WITH OPEN SPINA BIFIDA AT 11-13 WEEKS' GESTATION

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## ABSTRACT

**OBJECTIVE:** To determine if in fetuses with open spina bifida at 11-13 weeks' gestation there are alterations in the cerebral ventricular system.

**METHODS:** In this study we selected 10 cases of open spina bifida and 410 normal singleton pregnancies which subsequently resulted in the delivery of phenotypically normal neonates. In all cases transvaginal sonography was carried out at 11-13 weeks' gestation and 3D brain volumes were acquired. The fetal head was systematically assessed in a series of transverse views and measurements were obtained of the area of the lateral ventricles, the diameters of roof of the third ventricle, aqueduct of Sylvius and fourth ventricle. The measurements on the normal and affected fetuses were compared.

**RESULTS:** In normal fetuses all diameters increased with fetal biparietal diameter (BPD), except for the diameter of the fourth ventricle which did not change significantly with BPD. In open spina bifida, compared to the normal fetuses, the measurement of lateral ventricle area, the diameters of the roof of the third ventricle, aqueduct of Sylvius and fourth ventricle were significantly decreased ($p<0.0001$).

**CONCLUSIONS:** In fetuses with open spina at 11-13 weeks the intracranial collection of cerebrospinal fluid is substantially reduced.

## 4.1. INTRODUCTION

Open spina bifida is associated with the Chiari II malformation which is thought to be the consequence of leakage of cerebrospinal fluid (CSF) into the amniotic cavity, hypotension in the cerebral ventricular system and subarachnoid spaces and caudal displacement of the hindbrain (McLone DG et al, 1989, 2003).

In the second-trimester of pregnancy the sonographically detectable manifestations of the Chiari II malformation are the lemon and banana signs, which are visible in the axial planes used for measurement of the biparietal diameter (BPD) and assessment of cerebellum and cisterna magna, respectively (Nicolaides KH et al, 1986; Van den Hof MC et al, 1990; Ghi T et al, 2006). In the first-trimester the reported manifestations of the Chiari II malformation are decrease in the diameter of the fourth ventricle and/or cisterna magna and increase in the diameter of the brain stem, which are visible in the same mid-sagittal plane of the head as for measurement of nuchal translucency thickness and assessment of the nasal bone (Chaoui R et al, 2009; Lachmann R et al, 2011).

The aim of this study is to explore further possible alterations in the cerebral ventricular system of first trimester fetuses with open spina bifida. We establish reference ranges of the lateral ventricles, roof of the third ventricle, aqueduct of Sylvius and fourth ventricle and investigate possible differences in these measurements in fetuses with open spina bifida at 11-13 weeks' gestation.

## 4.2. METHODS

The study was conducted in three university hospitals between October 2009 and June 2011. The patients were undergoing first trimester combined screening for aneuploidies and in the three participating centers fetal anatomical survey is part of routine examination. In this study we selected 10 cases of open spina bifida and 410 normal singleton pregnancies resulting in the delivery of phenotypically normal neonates. The diagnosis of

open spina bifida was made by visualization of a myelomeningocele in nine cases at 11-13 weeks' gestation and in one case at 20 weeks.

Transvaginal sonography was carried out and the fetal head was systematically assessed in a series of transverse views. Brain volumes were acquired (transvaginal 5 -9L probe, GE Voluson Expert 730, GE Voluson E8 or GE Voluson E6, GE Medical Systems, Zipf, Austria) by one of three doctors with experience in this technique. The fetal head image was magnified to occupy 75% of the screen. The 3D sample box was placed in a way to contain only the fetal head and the acquisition plane was set at the level of the thalamus and mesencephalon, in a transverse view.

The angle of acquisition was 40-55$^{o}$ depending on the distance between the transducer and the fetal head. Volume acquisition was during fetal quiescence and took 3 seconds to complete. The resulting 3D volume, which included the whole fetal head from crown to neck, was stored and studied *off-line*, using 4D view software (GE Medical System, version 6.0). This allowed a correct alignment of brain structures in three orthogonal planes simultaneously that compose the multiplanar mode of the 3D image. During *off-line* manipulation, the position of the brain was adjusted to obtain an exact mid-sagittal view in plane C. Different post-processing features to improve the quality of image were used, like candle chrome map and speckle-reduction imaging (SRI) feature.

Three different planes of the brain were obtained where measurements were performed: lateral third ventricle plane, transthalamic plane and fourth ventricle plane. A perpendicular sagittal view was obtained in plane C and the reference dot was moved to the upper brain so that in the transverse view in plane A the butterfly image of the two chroroid plexuses with the roof of the third ventricle in the middle could be visualized (Figure 1a). In this lateral third ventricle plane we measured the BPD, the area of the lateral ventricles and the roof of the third ventricle. The area of each lateral ventricle was measured by tracing the border of this structure, which included the choroid plexus and the average of the two measurements was considered.

The transthalamic plane was obtained by tilting the head anteriorly by 22-28$^{o}$ so that the thalamus, midbrain, inter-thalamic part of the third ventricle and aqueduct can be seen (Figure 2a). In this plane we measured the transverse diameter of the aqueduct in its wider portion. The plane used to assess the fourth ventricle was the suboccipitobregmatic one, below the plane of the cerebellum, where the largest anteroposterior diameter of the fourth ventricle was measured (Figure 3a).

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination.

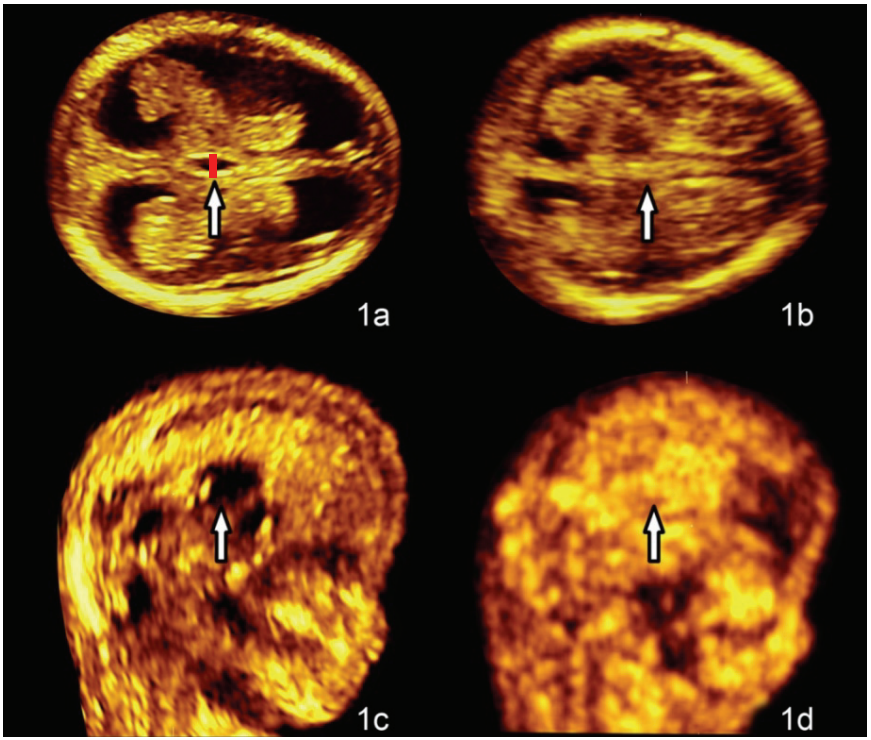


**Figure 1 –** Lateral third ventricle view in transverse plane (1a and 1b) and corresponding sagittal plane (1c and 1d) in a normal fetus (left) and one with open spina bifida (right). The arrows demonstrate the roof of the third ventricle and the red line the measurement of the diameter.

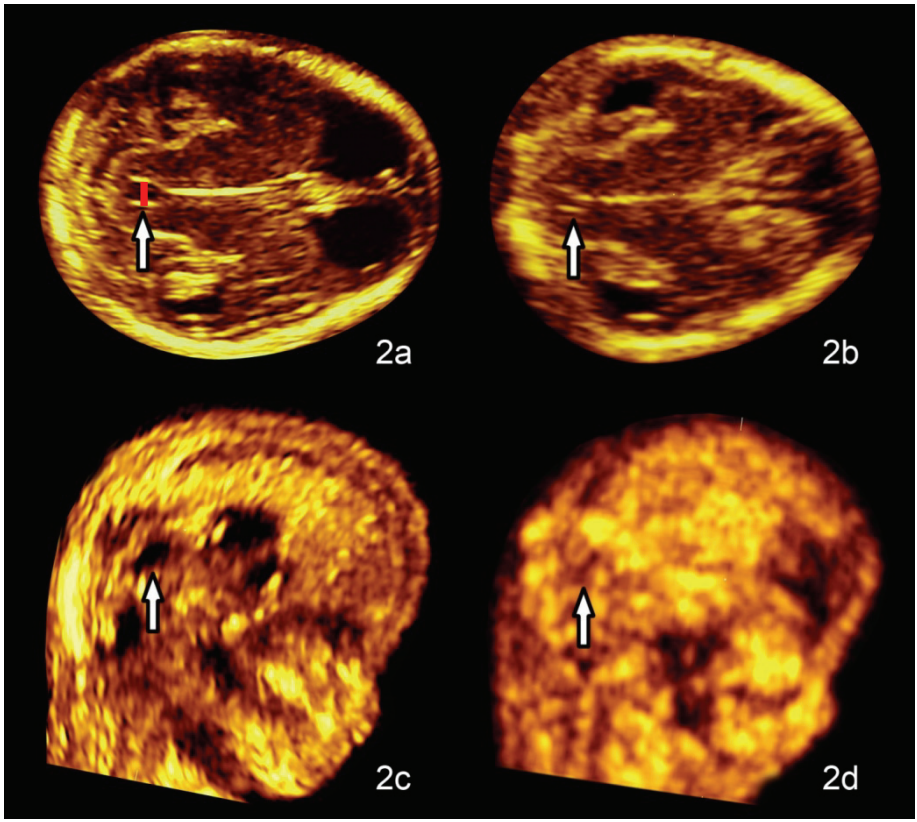


**Figure 2 –** Transthalamic view in transverse plane (2a and 2b) and corresponding sagittal plane (2c and 2d) in a normal fetus (left) and one with open spina bifida (right). The arrows demonstrate the aqueduct of Sylvius and the red line the measurement of the diameter.

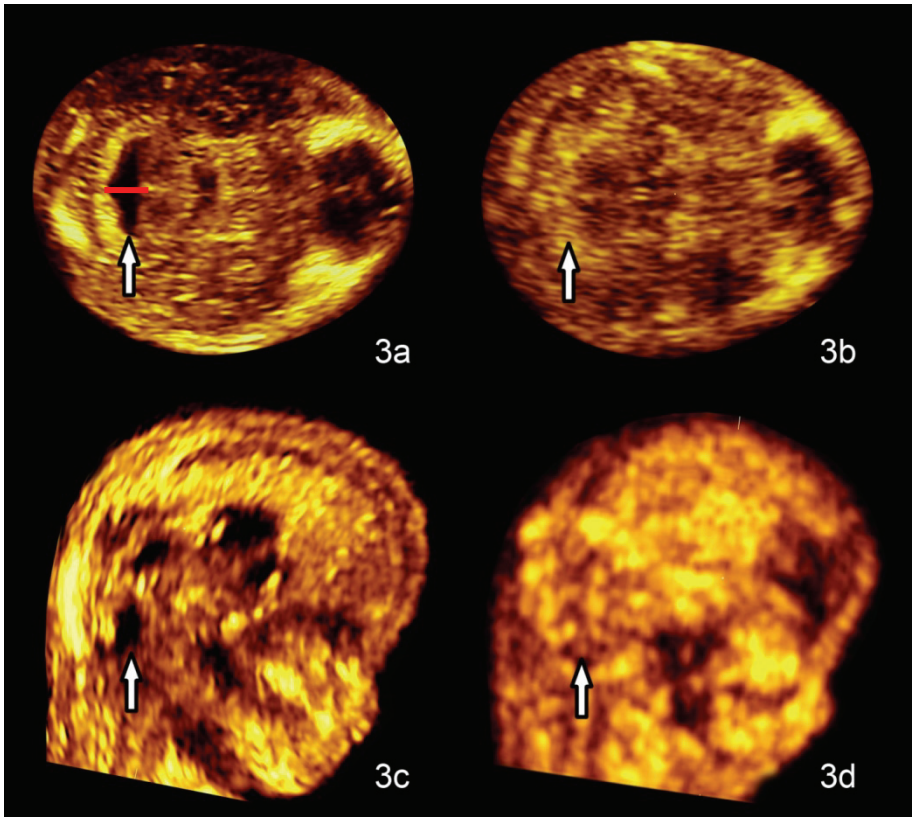


**Figure 3 –** Fourth ventricle view in transverse plane (3a and 3b) and corresponding sagittal plane (3c and 3d) in a normal fetus (left) and one with open spina bifida (right). The arrows demonstrate the fourth ventricle and the red line the measurement of the diameter.

## STATISTICAL ANALYSIS

Continuous and categorical variables were compared using Mann-Whitney U-test and 2-square test or Fisher's exact test, respectively. The distribution of measurements of the lateral ventricles, the roof of the third ventricle, aqueduct of Sylvius and fourth ventricle were found to be normal using

histograms and probability plots after exclusion of outliers outside three standard deviations (total of five measurements). In the normal fetuses regression analysis was used to examine whether each measurement changed with the BPD and whether the relationship was linear or non-linear. A reference range (5th, 50th and 95th centiles) for each measurement was constructed based on the relationship with BPD.

The measurements in the normal fetuses and in those with open spina bifida were expressed as a difference from expected normal mean for BPD (delta value). In the case of the fourth ventricle the measurements did not change with BPD and the values were expressed as a multiple of the normal median (MoM). Mann-Whitney U-test was used to examine the significance of difference between normal fetuses and those with open spina bifida.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL) was used for data analyses.

## 4.3. RESULTS

The demographic and pregnancy characteristics of the study population are summarized in Table 1.

**Table 1:** Demographic and pregnancy characteristics of the study population.

| Characteristic | Normal outcome (n=410) | Spina bifida (n=10) | P value |
|---|---|---|---|
| Maternal age in years | 31.5 (28.0-35.0) | 36.5 (31.0-40.0) | 0.018 |
| Gestational age in days | 87.0 (84.0-90.0) | 91.0 (84.0-97.0) | 0.149 |
| Fetal biparietal diameter in mm | 21.0 (19.1-23.4) | 19.8 (13.8-23.5) | 0.692 |
| Fetal crown rump length in mm | 60.8 (55.5-66.3) | 60.0 (47.9-70.2) | 0.396 |
| Fetal nuchal translucency in mm | 1.9 (1.6-2.3) | 1.9 (1.7-2.0) | 0.212 |

In normal fetuses the area of lateral ventricles and the diameter of the roof of the third ventricle increased, the diameter of the aqueduct of Sylvius decreased and the diameter of the fourth ventricle did not change significantly with BPD (Table 2, Figure 4).

**Table 2.** Results of regression analysis demonstrating the relationship of various measurements of the cerebral ventricular system in normal fetuses with biparietal diameter. In the case of aqueduct of Sylvius diameter the relationship is quadratic.

| Measurement | Coefficient (95% CI) | Standard error | P value |
|---|---|---|---|
| Lateral ventricle area | 0.078 (0.075 to 0.081) | 0.001 | <0.0001 |
| Third ventricle diameter | 0.029 (0.020 to 0.038) | 0.005 | <0.0001 |
| Aqueduct of Sylvius diameter | 0.080 (0.015 to 0.146) | 0.033 | 0.016 |
| Biparietal diameter | -0.003 (-0.004 to -0.001) | 0.001 | <0.0001 |
| (Biparietal diameter)$^2$ | | | |
| Fourth ventricle diameter | 0.009 (-0.002 to 0.020) | 0.006 | 0.124 |

The characteristics of the individual cases of open spina bifida are summarized in Table 3.

**Table 3:** Characteristics of the fetuses with open spina bifida.

| Case | CRL (mm) | BPD (mm) | Defects | Karyotype | Outcome |
|---|---|---|---|---|---|
| 1 | 43.0 | 12.5 | Spina bifida | Normal | Termination |
| 2 | 52.3 | 17.3 | Spina bifida | Normal | Miscarriage |
| 3 | 70.9 | 21.8 | Spina bifida | Normal | Termination |
| 4 | 67.6 | 20.3 | None at 12 wks, spina bifida diagnosed at 20 wks | Normal | Termination |
| 5 | 49.5 | 19.2 | Spina bifida, exomphalos | Normal | Termination |
| 6 | 38.4 | 14.2 | Spina bifida, exomphalos, clenched hands | Trisomy 18 | Termination |
| 7 | 70.0 | 23.1 | Spina bifida, cardiac defect, clenched hands | Trisomy 18 | Termination |
| 8 | 69.7 | 24.6 | Spina bifida, cardiac defect, clenched hands | Trisomy 18 | Termination |
| 9 | 50.2 | 12 | Spina bifida, holoprosencephaly, cardiac defect, polydactyly | Trisomy 13 | Termination |
| 10 | 79.7 | 25.4 | Spina bifida, ventriculomegaly, clenched hands | Triploidy | Termination |

In the case of spina bifida with holoprosencephaly the measurements recorded were for the aqueduct of Sylvius and fourth ventricle, because the lateral and third ventricles were malformed. In open spina bifida, compared to the normal fetuses, the area of lateral ventricles (P=0.009), diameters of the third ventricle, aqueduct of Sylvius and fourth ventricle were significantly decreased (P<0.0001) (Table 4 and Figure 4).

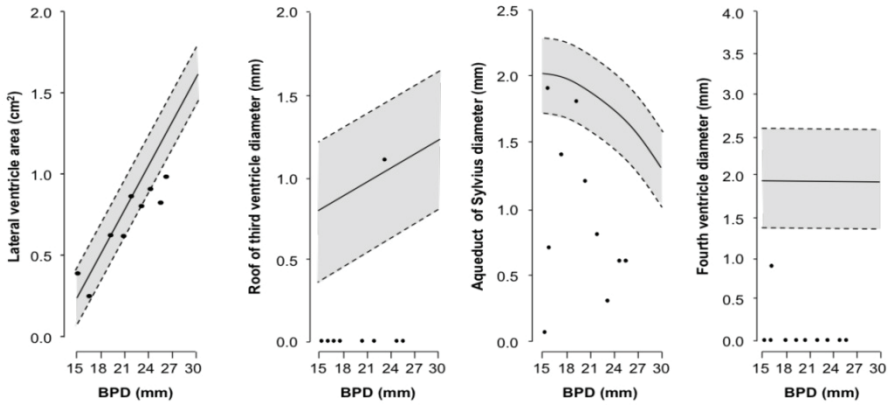


**Figure 4 –** Area of the lateral ventricles and diameters of the roof of the third ventricle,aqueduct of Sylvius and fourth ventricle in individual fetuses with open spina bifida plotted on the reference ranges (5th, 50th and 95th centiles) with fetal biparietal diameter (BPD).

**Table 4:** Median and interquartile range of measurements of the cerebral ventricular system in normal fetuses and in those with open spina bifida.

| Measurement | Normal fetuses (n=410) | Spina bifida (n=10) | P value |
|---|---|---|---|
| Area of the lateral ventricle, delta in $cm^2$ | 0.0 (-0.1 to 0.0) | -0.1 (-0.2 to 0.0) | 0.009 |
| Roof of third ventricle, delta in mm | 0.0 (-0.2 to 0.2) | -0.9 (-1.0 to -0.7) | <0.0001 |
| Aqueduct of Sylvius, delta in mm | 0.0 (-0.1 to 0.1) | -1.1 (-1.3 to -0.5) | <0.0001 |
| Fourth ventricle diameter, in multiple of the median | 1.0 (0.9 to 1.2) | 0.0 (0.0 to 0.0) | <0.0001 |

## 4.4. DISCUSSION

The findings of this study demonstrate that in fetuses with open spina bifida at 11-13 weeks' gestation the intracranial collection of CSF is substantially reduced with a decrease in the area of the lateral ventricles and diameters of the roof of the third ventricle, aqueduct of Sylvius and fourth ventricle.

In normal fetuses the area of lateral ventricles and the diameter of the roof of the third ventricle increased, the diameter of the aqueduct of Sylvius decreased and the diameter of the fourth ventricle did not change significantly with BPD. In the case of the lateral ventricles we chose to measure their area, rather than the traditional approach of measuring their diameter, because certainly within the gestational range of 11-13 weeks the diameter reflects the size of the choroid plexuses rather than the fluid within the ventricles. The third ventricle during the second trimester is visible in the transthalamic view of the brain, but at 11-13 weeks the roof of the ventricle is clearly seen at a slightly superior plane that we also used for measurement of the BPD. There are no previously reported ranges for most measurements obtained in this study, except for the diameter of the fourth ventricle. In the mid-sagittal plane at 11-13 weeks' gestation the fourth ventricle is visualized as an intracranial translucency and the diameter increases with gestation (Chaoui R et al, 2009). In our study an axial plane was used to obtain the largest diameter of this ventricle just below the cerebellum and this did not change significantly with gestational age. A recent study has also reported the measurement of the fourth ventricle in the axial plane but the transabdominal approach was used (Egle D et al, 2011).

In fetuses with open spina bifida the finding of an apparent depletion of CSF from the brain provides an insight into the pathophysiology of Chiari II malformation in the first trimester of pregnancy. In the first description of this malformation the hindbrain herniation was attributed to the presence of hydrocephalus (Stevenson LK et al, 2004). Subsequently, McLone and Knepper proposed that both the open neural tube and incomplete neurocele occlusion lead to failure of distension of the primitive cranial ventricular

system and allow CSF to leak from the entire central nervous system (McLone DG et al, 1989, 2003).

Several studies have shown that normal development of the embryonic and early fetal brain is dependent on the pressure generated by accumulation of CSF within a closed ventricular system (Desmond M et al, 2002, 2005; Levitan M et al, 2009; Gato A et al, 2009). Animal studies of induced myelomeningocele reported that failure to maintain normal intracerebral pressure results in decreased cerebral ventricles and subarachnoid space and crowding of the posterior fossa (Inagaki T et al, 1997; Danzer E et al, 2005). Our findings of decrease in the area and diameters of the entire ventricular system is compatible with the hypothesis that in this neural tube defect there is decreased intraventricular fluid pressure. Decreased fluid content in brain cavities has also been described in encephalocele and in acrania (Blaas H-G K et al, 2009).

Chiari-associated central nervous system malformations affect all the brain (McLone DG et al, 2003; Chaoui R et al, 2009; Scheier M et al, 2011; Miller E et al, 2008; Juranek J et al, 2010). Previous first trimester sonographic studies reporting on the brain abnormalities found in association with open spina bifida have focused on the posterior fossa (Chaoui R et al, 2009; Lachmann R et al, 2011; Chaoui R, Nicolaides KH, 2011). However, as shown in this study open spina bifida is also associated with sonographically detectable abnormalities in the supratentorial compartment.

In first trimester screening for open spina bifida the mid-sagittal plane obtained by transabdominal sonography is likely to be widely used because this is the same plane that is routinely examined for measurement of nuchal translucency and assessment of the nasal bone in early screening for aneuploidies (Chaoui R, Nicolaides KH, 2011). In this respect, transvaginal sonography for examination of several axial planes is unlikely to be widely accepted as a primary method of screening for spina bifida but it would rather be reserved for cases with suspicious findings in the mid-sagittal plane.

## REFERENCES

*Blaas H-G K, Eik-Nes SH.* Sonoembryology and early prenatal diagnosis of neural anomalies. Prenat Diagn 2009; 29:312-325.

*Chaoui R, Benoit B, Mitkowska-Wozniak H, Heling KS, Nicolaides KH.* Assessment of intracranial translucency (IT) in the detection of spina bifida at the 11-13-week scan. Ultrasound Obstet Gynecol 2009;34**:** 249-252.

*Chaoui R, Benoit B, Heling KS, Kagan KO, Pietzsch V, Sarut Lopez A, Tekesin I, Karl K.* Prospective detection of open spina bifida at 11-13 weeks by assessing intracranial translucency and posterior brain. Ultrasound Obstet Gynecol 2011; 38:722-26.

*Chaoui R, Nicolaides KH.* Detecting open spina bifida at the 11–13-week scan by assessing the posterior brain region: mid-sagittal or axial plane? Ultrasound Obstet Gynecol 2011; 38: 609-12.

*Danzer E, Schwarz U, Wehrli S, Radu A, Adzick NS, Flake AW.* Retinoic acid induced myelomeningocele in fetal rats: characterization by hystopathological analysis and magnetic resonance imaging. Exp Neurol 2005; 194:467-75.

*Desmond M, Leviran M, Haas A.* Internal luminal pressure during early chick embryonic brain growth: descriptive and empirical observations. Anat Rec 2005; 285: 737-47.

*Desmond M, Levitan M.* Brain expansion in the chick embryo initiated by experimentally produced occlusion of the spinal neurocoel. Anat Rec 2002; 268: 147-59.

*Egle D, Strobl I, Weiskopf-Schwendinger V, Grubinger E, Kraxner F, Mutz-Dehbalaie IS, Strasak A, Scheieir M.* Appearance of the posterior fossa at 11+3 to 13+6 gestational weeks on transabdominal ultrasound examination. Ultrasound Obstet Gynecol 2011; 38**:** 620-624.

*Gato A, Desmond M.* Why the embryo still matters: CSF and the neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis. Dev Biol 2009; 327: 263-272.

*Ghi T, Pilu G, Falco P, Segata M, Carletti A, Cocchi G, Sanini D, Bonasoni P, Tani G, Rizzo N*. Prenatal diagnosis of open and closed spina bifida. Ultrasound Obstet Gynecol 2006; 28:899-903.

*Inagaki T, Schoenwolf G, Walker M.* Experimental model: change in the posterior fossa with surgically induced spina bifida aperta in mouse. Pediatr Neurosurg 1997; 26**:**185-89.

*Juranek J.* Anomalous development of brain structure and function in spina bifida myelomeningocele. *Dev Disabil Rev 2010*; 16: 23-30.

*Lachmann, R, Chaoui, R, Moratalla, J, Picciarelli, G, Nicolaides KH.* Posterior brain in fetuses with open spina bifida at 11 to 13 weeks. Prenat Diagn 2011;31:103-106.

*Levitan M, Desmond M.* Expansion of the human embryonic brain during rapid growth: area analysis. Anat Rec 2009; 292**:** 472-80.

*McLone DG, Knepper PA.*The cause of Chiari II malformation: a unified theory. Pediatr Neurosci 1989; 15:1–12.

*McLone DG, Dias MS.* The Chiari II malformation: cause and impact. Childs Nerv Syst 2003; 19:540-50.

*Miller E, Widjaja E, Blaser S, Dennis M, Raybaud C.* The old and the new: supratentorial MR findings in Chiari II malformation. *Child Nerv Syst* 2008; 24:563-75.

*Nicolaides KH, Campbell S, Gabbe SG, Guidetti R*. Ultrasound screening for spinabifida: cranial and cerebellar signs. Lancet 1986;**2:**72-4.

*Scheier M, Lachmann R, Petros M, Nicolaides KH.* Three-dimensional sonographyof the posterior fossa in fetuses with open spina bífida at 11-13 week's gestation. Ultrasound Obstet Gynecol 2011; 38:625-29.

*Stevenson LK.* Chiari type II malformation: past, present and future. Neurosurg Focus 2004; 16: E5.

*Van den Hof MC, Nicolaides KH, Campbell J, Campbell S*. Evaluation of the lemon and banana signs in one hundred and thirty fetuses with open spina bifida. Am J Obstet Gynecol 1990;162:322-7.

# Chapter 5

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## DILATED FOURTH VENTRICLE IN FETUSES WITH TRISOMY 18, TRISOMY 13 AND TRIPLOIDY AT 11-13 WEEKS' GESTATION

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## ABSTRACT

**OBJECTIVE:** To determine if in fetuses with aneuploidies the diameter of the fourth cerebral ventricle at 11-13 weeks' gestation is different from euploid fetuses.

**METHODS:** The fourth ventricle at 11-13 weeks' gestation was assessed in 62 cases of trisomy 21, 32 of trisomy 18, 10 of trisomy 13 and 12 of triploidy and compared to 410 normal euploid fetuses. Transvaginal sonography was carried out and 3D brain volumes were acquired. The fetal head was assessed in an axial plane and the diameter of the fourth ventricle was measured. Values in aneuploid and euploid fetuses were compared.

**RESULTS:** The diameter of the fourth ventricle in trisomy 18, trisomy 13 and triploidy, but not in trisomy 21, was significantly higher than in euploid fetuses. In euploid fetuses the median diameter of the fourth ventricle was 1.9 mm and the 95$^{th}$ centile was 2.5 mm. The measurements were above the median and the 95$^{th}$ centile in 25 (78.1%) and 17 (53.1%) cases of trisomy 18, in 10 (100%) and 8 (80.0%) of trisomy 13 and in 10 (83.3%) and 10 (83.3%) of triploidy.

**CONCLUSIONS:** In trisomy 18, trisomy 13 and triploidy the diameter of the fourth ventricle at 11-13 weeks' gestation is increased.

## 5.1. INTRODUCTION

Ultrasound examination at 11-13 weeks' gestation was originally introduced for measurement of fetal nuchal translucency (NT) thickness and crown-rump length (CRL) in screening for aneuploidies (Nicolaides KH, 1992; Snijders RJ et al, 1998). However, it is now apparent that this scan can also lead to the diagnosis of many serious fetal defects and pregnancy complications (Syngelaki A et al, 2011; Nicolaides KH, 2011). Recently, attention has focused on the early diagnosis of open spina bifida from demonstration of a decrease in the diameter of the fourth ventricle (Chaoui R et al, 2009; Lachmann R et al, 2011; Chaoui R, Nicolaides KH, 2011; Loureiro T et al, 2012).

Ultrasound studies in the second trimester of pregnancy have reported an association between aneuploidies and the Dandy-Walker malformation (DWM) spectrum, which is characterized by complete or partial agenesis of the vermis and cystic dilation of the fourth ventricle and the posterior cranial fossa (Goetzinger RK et al, 2008; Nyberg DA et al, 1991; Ulm B et al, 1997; Ecker LJ et al, 2000).

The aim of this study is to determine if in fetuses with aneuploidies there are alterations in the diameter of the fourth ventricle at 11-13 weeks' gestation.

## 5.2. METHODS

The diameter of the fourth ventricle was measured in fetal head volumes, obtained by transvaginal 3D ultrasonography from 62 cases of trisomy 21, 32 of trisomy 18, 10 of trisomy 13 and 12 of triploidy at 11-13 weeks' gestation. Gestational age was calculated from the fetal crown-rump length (CRL) (Robinson HP et al, 1975).The study was part of a larger one in which the cerebral ventricular system was assessed in normal euploid fetuses, in aneuploid fetuses and in fetuses with open spina bifida. The operator (L.T.) that performed all measurements was not aware of the fetal condition under investigation. The values of this study on fetal aneuploidies were compared

to those of 410 normal euploid fetuses that were previously reported (Loureiro T et al, 2012). We excluded cases with open spina bifida because the results of these cases were published previously (Loureiro T et al, 2012).

The study was conducted in three university hospitals between October 2009 and December 2011. Transvaginal sonography was carried out and 3D brain volumes were acquired (transvaginal 5-9L probe, GE Voluson Expert 730, GE Voluson E8 or GE Voluson E6, GE Medical Systems, Zipf, Austria) before chorionic villous sampling for fetal karyotyping. The 3D sample box was placed in a way to contain only the fetal head and the acquisition plane was set at the level of the thalamus and mesencephalon, in a transverse view. The angle of acquisition was 40-55$^{o}$ depending on the distance between the transducer and the fetal head. Volume acquisition was during fetal quiescence and took 3 seconds to complete. The resulting 3D volume, which included the whole fetal head from crown to neck, was stored and studied *off-line*, using 4D view software (GE Medical System, version 6.0). The position of the brain was adjusted to obtain an exact mid-sagittal view in plane C. Different post-processing features to improve the quality of image were used, like candle chrome map and speckle-reduction imaging (SRI) feature. Two axial planes of the brain were obtained as previously described (Loureiro T et al, 2012). In an axial plane characterized by the butterfly image of the two chroroid plexuses with the roof of the third ventricle in the middle, we measured the biparietal diameter (BPD). The second plane was the suboccipitobregmatic one, below the cerebellum, where the largest anteroposterior diameter of the fourth ventricle was measured (Figure 1).

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination.

## STATISTICAL ANALYSIS

Continuous and categorical variables were compared using Mann-Whitney U-test and $\chi 2$-square test or Fisher's exact test, respectively.

In normal fetuses the diameter of the fourth ventricle did not change significantly with BPD and each value was expressed as a multiple of the normal median (MoM). Kruskal- Wallis test, with Bonferroni correction, was used to examine the significance of difference between each group of aneuploidies and euploid normal fetuses.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL) was used for data analyses.

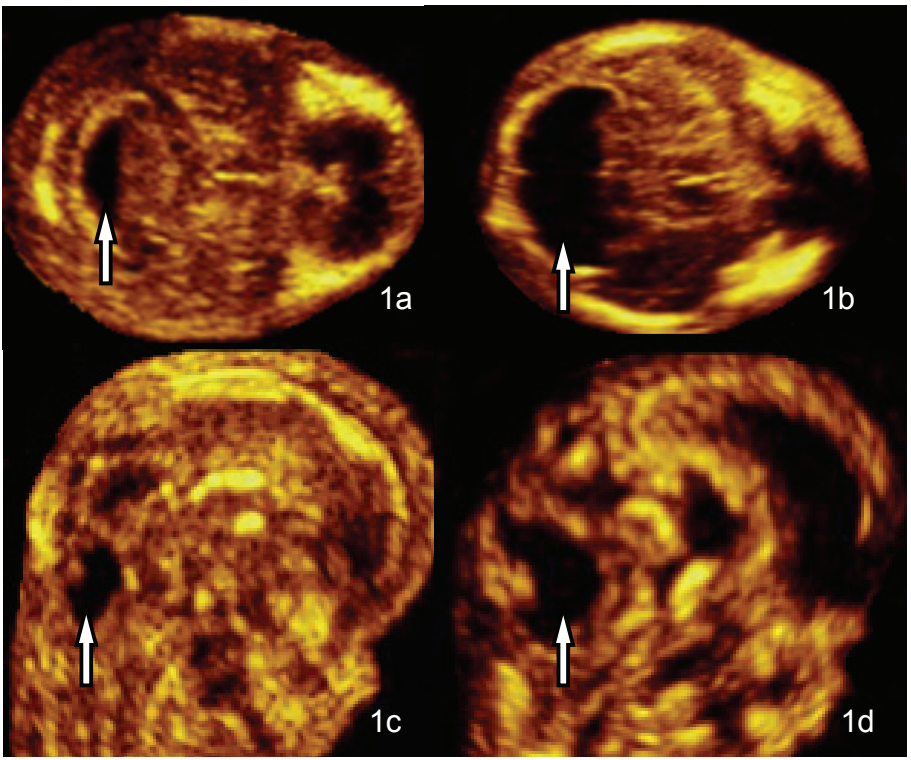


**Figure 1 –** Axial (1a and 1b) and sagittal (1c and 1d) views of the fetal brain demonstrating the fourth ventricle (arrow) in a euploid fetus (left) and one with triploidy (right).

## 5.3. RESULTS

The demographic and pregnancy characteristics of the study population are summarized in Table 1. Compared to euploid pregnancies, in trisomy 18 the BPD was lower and in trisomy 18 and triploidy the CRL was lower.

In trisomy 21 the median MoM diameter of the fourth ventricle was (1.0, interquartile range 0.9 to 1.2) was not significantly different from euploid fetuses (1.0, interquartile range 0.9 to 1.2), but the diameter was significantly increased in trisomy 18 (1.4, interquartile range 1.1 to 1.6; *P*<0.05), trisomy 13 (1.5, interquartile range 1.3 to 1.8; *P*<0.001) and triploidy (2.1, interquartile range 1.8 to 2.0; *P*<0.001).

**Table 1:** Demographic and pregnancy characteristics of the study population.

| Measurement | Euploid fetuses (n=410) | Trisomy 21 (n=62) | Trisomy 18 (n=35) | Trisomy 13 (n=10) | Triploidy (n=12) |
|---|---|---|---|---|---|
| **Maternal age in years, median (IQR)** | 31.5 (28.0-35.0) | 37.0 (34.0-40.0)** | 37.0 (30.0-40.5)** | 32.0 (24.0-36.5) | 30.5 (25.8-35.8) |
| **Gestational age in days, median (IQR)** | 87.6 (84.8-90.4) | 88.8 (85.5-93.0) | 84.1 (81.3-88.7)* | 86.0 (83.5-87.6) | 83.2 (80.3-86.4)* |
| **Fetal biparietal diameter in mm, median (IQR)** | 21.0 (19.1-23.4) | 21.1 (18.9-23.2) | 18.8 (17.6-21.1)** | 19.3 (17.8-20.3) | 19.5 (16.8-24.2) |
| **Fetal crown rump length in mm, median (IQR)** | 60.8 (55.5-66.3) | 62.9 (56.7-71.3) | 54.2 (49.3-62.7)* | 57.7 (53.1-60.7) | 52.7 (47.6-58.5)* |

***IQR** = Interquartile range*
*Comparisons by Kruskal-Wallis test, with Bonferroni correction for multiple comparisons. * P<0.05 **P<0.001.*

In the euploid fetuses the median diameter of the fourth ventricle was 1.9 mm and the 95$^{th}$ centile was 2.5 mm. The diameter of the fourth ventricle was above the median and the 95$^{th}$ centile in 25 (78.1%) and 17 (53.1%) of the 32 cases of trisomy 18, in 10 (100%) and 8 (80.0%) of the 10 cases of trisomy 13 and in 10 (83.3%) and 10 (83.3%) of the 12 cases of triploidy (Figure 2).

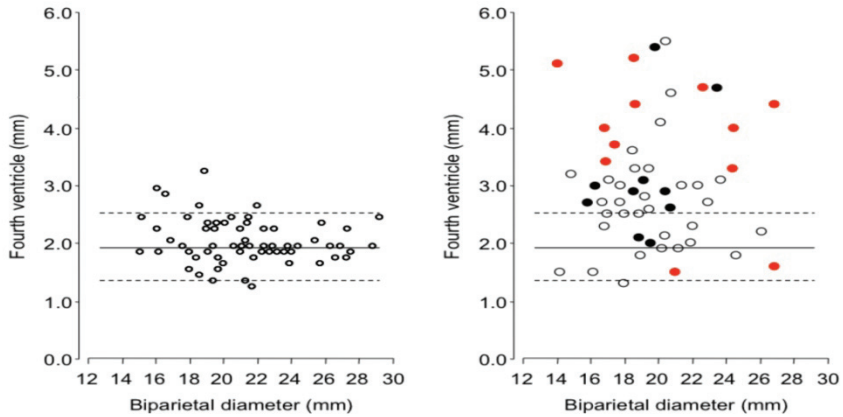


**Figure 2–** Diameter of the fourth ventricle in relation to BPD in fetuses with trisomy 21 (left) and trisomy 18 (right, open circles), trisomy 13 (right, black circles) and triploidy (right, red circles) plotted on the reference range of euploid fetuses (5$^{th}$, 50$^{th}$ and 95$^{th}$centiles).

## 5.4. DISCUSSION

The findings of this study demonstrate that at 11-13 weeks' gestation the anteroposterior diameter of the fourth ventricle in fetuses with trisomy 18, trisomy 13 and triploidy, but not in trisomy 21, is higher than in euploid fetuses. We measured the fourth ventricle in a suboccipitobregmatic plane below the cerebellum, using 3D brain volumes which had been acquired by transvaginal ultrasound. This approach combined the advantages of good resolution and correct alignment of the small brain structures in three orthogonal planes.

In the second trimester the Dandy-Walker Malformation (DWM) is commonly associated with aneuploidies, mainly trisomy 18, trisomy 13 and triploidy, but not trisomy 21 (Goetzinger RK et al, 2008; Nyberg DA et al, 1991; Ulm B et al, 1997; Ecker LJ et al, 2000). A previous study investigated the possible association between aneuploidies and the diameter of the fourth ventricle measured in the mid-sagittal view of the fetal profile at 11-14 weeks' gestation (Papastefanou I et al, 2011). In 17 aneuploid fetuses, including nine with trisomy 21, three with trisomy 18, three with trisomy 13, one with trisomy 20 and one with triploidy, the mean diameter of the fourth ventricle, corrected for crown-rump length, was significantly increased. In our study an axial plane was used to obtain the largest diameter of this ventricle just below the cerebellum (Loureiro T et al, 2012). In trisomy 21 the diameter of the fourth ventricle was not significantly different from euploid fetuses but in trisomy 18, trisomy 13 and triploidy the diameter was increased.

At 8-9 weeks' gestation the roof of the fourth ventricle contains two areas lined by flattened ependymal cells: the anterior or rostral and posterior or caudal membranous areas separated by the plica choroidea, which subsequently develops into the choroid plexus (O'Rahilly R et al, 1990). Cystic malformations in the posterior fossa have been classified on the basis of their embryological origin into those of the rostral area with abnormal development of the cerebellum as in DWM and those of the caudal area with inadequate opening of the foramina of Magendie and Luschka, which are often transient and of no pathological significance (Kollias SS et al, 1993; Blake JA, 1898; Wilson JT, 1906). Consequently, possible explanations for the dilated fourth ventricle in trisomies 18 and 13 and triploidy are delayed development of the posterior fossa or, in some cases, an underlying DWM. Sonographic studies have reported that a cyst in the posterior fossa in early pregnancy can be a transient finding in normal fetuses (Bronshtein M et al, 1998; Goldstein I et al, 2002). Bronshtein et al described 21 fetuses with isolated enlargement of the fourth ventricle at 14-16 weeks' gestation which became normal on follow-up scans at 22-23 weeks (Bronshtein M et al, 1998). However, a posterior fossa cyst detected in the first trimester scan has also been described in association with subsequently diagnosed DWM (Achiron R et al, 1993; Nizard J et al, 2005). The effectiveness of ultrasound

examination at 11-13 weeks' gestation and measurement of fetal NT thickness in early screening for aneuploidies are well established (Nicolaides KH et al, 2011). During this scan systematic examination of the fetal anatomy can lead to the diagnosis of many serious fetal defects (Syngelaki A et al, 2011). The finding of a small fourth ventricle can alert the ultrasonographer to the presence of open spina bifida (Chaoui R et al, 2009; Lachmann R et al, 2011; Chaoui R, Nicolaides KH, 2011; Loureiro T et al, 2012). As demonstrated in this study a large fourth ventricle is commonly found in fetuses with those aneuploidies which are associated with other brain defects, including the DWM. Trisomy 18, trisomy 13 and triploidy are associated with low serum free ß-hCG and PAPP-A and several easily detectable sonographic features, including increased NT, early onset growth restriction, tachycardia, holoprosencephaly, cardiac defects, exomphalos and megacystis. Assessment of the fourth ventricle may improve further the already high performance of first trimester biochemical and sonographic screening for these aneuploidies (Nicolaides KH, 2011). Further investigations are needed to determine the effectiveness of assessment of the fourth ventricle in first trimester screening for DWM in euploid fetuses.

## REFERENCES

*Achiron R, Achiron A, Yagel S.* First trimester transvaginal sonographic diagnosis of Dandy-Walker malformation. J Clin Ultrasound 1993;21:62-64.

*Blake JA.* The roof and lateral recesses of the fourth ventricle, considered morphologically and embryologically. J Comp Neur 1898; 10:79-106.

*Bronshtein M, Zimmer EZ, Blazer S.* Isolated large fourth ventricle in early pregnancy – a possible benign transient phenomenon. Prenat Diagn 1998;18:997-1000.

*Chaoui R, Benoit B, Mitkowska-Wozniak H, Heling KS, Nicolaides KH.* Assessment of intracranial translucency (IT) in the detection of spina bifida at the 11-13-week scan. Ultrasound Obstet Gynecol 2009; 34: 249-252.

*Chaoui R, Nicolaides KH.* Detecting open spina bifida at the 11–13-week scan by assessing the posterior brain region: mid-sagittal or axial plane? Ultrasound Obstet Gynecol 2011; 38: 609-612.

*Ecker LJ, Shipp TD, Bromley B, Benacerraf B.* The sonographic diagnosis of Dandy-Walker and Dandy-Walker variant: associated findings and outcomes. Prenat Diagn 2000;20:328-332.

*Goetzinger RK, Stamilio DM, Dicke JM, Macones GA, Odibo AO.* Evaluating the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common central nervous system malformations. Am J Obstet Gynecol 2008;199:285.e1-285.e6.

*Goldstein I, Makhoul IR, Tamir A, Rajamim BS, Nisman D.* Ultrasonographic nomograms of the fetal fourth ventricle. J Ultrasound Med 2002;21:849-856.

*Kollias SS, Ball WS Jr, Prenger EC.* Cystic malformations of the posterior fossa: differential diagnosis clarified through embryologic analysis. Radiographics 1993;13:1211-1231.

*Lachmann R, Chaoui, R, Moratalla, J, Picciarelli, G, Nicolaides KH*. Posterior brain in fetuses with open spina bifida at 11 to 13 weeks. Prenat Diagn 2011;31:103-106.

*Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH.*Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation.Ultrasound Obstet Gynecol 2012; 39: 620-4.

*Nicolaides KH, Azar GB, Byrne D, Mansur CA, Marks K*. Nuchal translucency: ultrasound screening for chromosomal defects in the first trimester of pregnancy. BMJ 1992;304:867-869.

*Nicolaides KH.* Turning the pyramid of prenatal care. Fetal Diagn Ther 2011;29:183-196.

*Nicolaides KH.* Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7-1.

*Nyberg DA, Mahony BS, Hegge FN, Hickok D, Luthy DA, Kapur R*. Enlarged cisterna magna and the Dandy-Walker malformation: factors associated with chromosome abnormalities. Obstet Gynecol 1991;77: 436 442.

*Nizard J, Bernard JP, Ville Y.* Fetal cystic malformations of the posterior fossa in the first trimester of pregnancy. Fetal Diagn Ther 2005;20:146-151.

*O'Rahilly R, Müller F.* Ventricular system and choroid plexuses of the human brain during the embryonic period proper. Am J Anat 1990; 189: 285-302.

*Papastefanou I, Souka AP, Pilalis A, Panagopoulos P, Kassanos D.* Fetal intracranial translucency and cisterna magna at 11 to 14 weeks: reference ranges and correlation with chromosomal abnormalities. Prenat Diagn 2011;31:1189-1192.

*Robinson HP, Fleming JE.* A critical evaluation of sonar crown rump length measurements. Br J Obstet Gynaecol 1975;182:702-710.

*Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH.* UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;352:343-346.

*Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH.* Challenges in the diagnosis of fetal non chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011; 31: 90-102.

*Ulm B, Ulm R, Deutinger J, Bernaschek G.* Dandy-Walker malformation diagnosed before 21 weeks of gestation: associated malformations and chromosomal abnormalities. Ultrasound Obstet Gynecol 1997;10:167-170.

*Wilson JT.* On the nature and mode of origin of the foramen of Magendie. J Anat 1906;71:423-428.

# Chapter 6

Ultrasound Obstet Gynecol 2012; 40: 282-7.

## LATERAL VENTRICLES IN FETUSES WITH ANEUPLOIDIES AT 11-13 WEEKS' GESTATION

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## ABSTRACT

**OBJECTIVE:** To examine the possible association between aneuploidies and fetal lateral cerebral ventriculomegaly in the first trimester of pregnancy.

**METHODS:** Three dimensional brain volumes were acquired by transvaginal ultrasound at 11-13 weeks' gestation in 410 euploid fetuses and in 63 fetuses with trisomy 21, 34 with trisomy 18 and seven with trisomy 13. Lateral ventricles were assessed in a transverse view, just above the roof of the third ventricle and measurements of the areas of the lateral ventricles and choroid plexuses were obtained. The ratio between choroid plexus and lateral ventricle areas (CLR) was calculated. Measurements in aneuploid fetuses were compared to those in euploid fetuses.

**RESULTS:** In euploid fetuses the area of the lateral ventricles and choroid plexuses areas increased, whereas the CLR decreased with fetal biparietal diameter. In fetuses with trisomy 21 lateral ventricle area and choroid plexus area were smaller but CLR was not significantly different from that in euploid fetuses. In trisomy 18 and 13, CLR was significantly smaller than in euploid fetuses. The CLR was below the 5$^{th}$ centile of normal range in 11 (32.4%) fetuses with trisomy 18 and in six (85.7%) with trisomy 13.

**CONCLUSIONS:** There is evidence of ventriculomegaly at 11-13 weeks' gestation in most fetuses with trisomy 13 and one third of fetuses with trisomy 18.

## 6.1. INTRODUCTION

Fetal cerebral ventriculomegaly may result from chromosomal and genetic defects, brain abnormalities, hemorrhage or infection, but in many cases no clear etiology is identified. In 14 published series in the 1980s and early 1990s concerning a combined total of 690 fetuses with ventriculomegaly during the second and third trimesters of pregnancy the mean incidence of aneuploidies was 13% and the commonest aneuploidies were trisomies 21, 18 and 13 (Nicolaides KH et al, 1990). It was noted also that the incidence of aneuploidies was inversely related to the severity of ventriculomegaly; in a series of 420 fetuses with ventriculomegaly the incidence of aneuploidies was 22% in those with mild to moderate ventriculomegaly and 6% in those with severe disease (Snijders RJM et al, 1995).

The aim of this study was to examine the possible association between ventriculomegaly and aneuploidies in the first trimester of pregnancy.

## 6.2. METHODS

The study was part of a project investigating the fetal cerebral ventricular system at 11-13 weeks' gestation; the results concerning the fourth ventricle in fetuses with aneuploidies and all ventricles in fetuses with open spina bifida were published previously (Loureiro T et al, 2012, 2012). The study was conducted in three university hospitals between October 2009 and December 2011. The control group consisted of 410 normal fetuses and the study group included 63 cases of trisomy 21, 34 of trisomy 18 and 11 of trisomy 13 (four cases of trisomy 13 were subsequently excluded because of holoprosencephaly). Gestational age was calculated from the fetal crown–rump length (CRL) (Robinson HP et al, 1975).The operator (L.T.) who performed all measurements was not aware of the fetal condition under investigation.

All patients underwent transvaginal sonography (transvaginal 5-9L probe, GE Voluson Expert 730, GE Voluson E8 or GE Voluson E6, GE Medical Systems, Zipf, Austria) and three-dimensional (3D) ultrasound brain volumes were

acquired before chorionic villus sampling for fetal karyotyping. As a reference view, we used a transverse plane of the fetal head at the level of the thalamus and mesencephalon and the image was magnified to occupy the entire screen. The 3D sample box was placed in a way to contain only the fetal head and the acquisition angle was 40-55$^{o}$. To minimize movement artifacts, volume acquisition was done during fetal quiescence and took 3 seconds to complete. The resulting 3D volume was stored and processed offline, using 4D view software (GE Medical System, version 6.0). Using the multiplanar mode, the position of the brain was adjusted to obtain an exact vertical mid-sagittal view in Plane C. The landmark used for anteroposterior tilting was alignment of the roof of the third ventricle with the anterior border of the first cervical vertebra which are seen as echogenic dots at the middle of the fetal neck. The candle chrome map and speckle-reduction imaging (SRI) features were used to improve the image. The reference views for the measurements were identified in Plane A. The fetal biparietal diameter (BPD) was measured as previously described (Loureiro T et al, 2012). The lateral ventricles and the choroid plexus were assessed in a transverse view, just above the roof of the third ventricle and the caudate nucleus, which is a modified plane as compared to previously published data (Loureiro T et al, 2012) since, in this plane, the eminence created by the caudate nucleus is avoided (Figure 1). The area of each lateral ventricle and each choroid plexus was measured by tracing the border of these structures, and the average of two measurements was considered. The ratio of choroid plexus to lateral ventricle areas was then calculated.

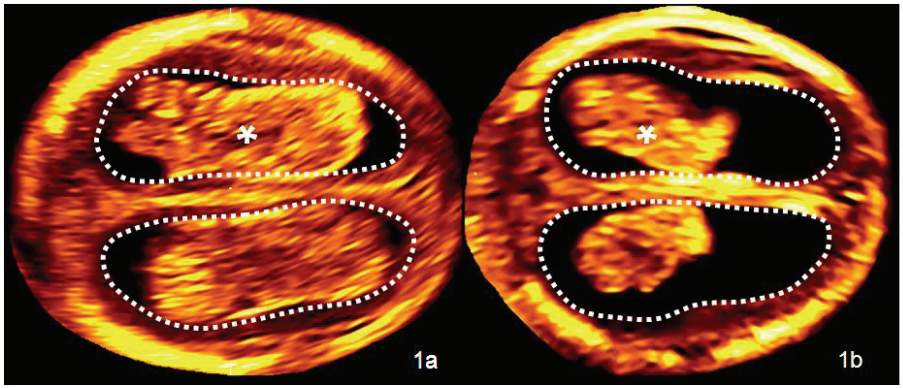



**Figure 1 –** Axial view of fetal brain demonstrating lateral ventricles (dotted line) and choroid plexuses (*) in a euploid fetus (left) and in a fetus with trisomy 13 (right). In both fetuses the biparietal diameter was 24 mm.

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination.

## STATISTICAL ANALYSIS

Continuous variables were compared using Kruskal-Wallis test and each of the aneuploid groups was compared to the euploid group by Mann-Whitney *U*-test with Bonferroni correction for multiple comparisons. In the euploid group regression analysis was used to examine whether each measurement changed with the BPD and to determine whether the relationship was linear or non-linear. A reference range ($5^{th}$, $50^{th}$ and $95^{th}$ centiles) for each measurement was constructed, based on the relation with BPD. Measurements in the euploid fetuses and in those with a chromosomal abnormality were expressed as a difference from expected normal mean for BPD (delta value). Kruskal-Wallis test was used to examine the significance of differences within the groups and, subsequently, each group of aneuploidy was compared to the euploid group using the Mann-Whitney *U*-test with Bonferroni correction for multiple comparisons. The statistical

software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.

## 6.3. RESULTS

The demographic and pregnancy characteristics of the study population are summarized in Table 1. Compared to euploid fetuses, the BPD and CRL values were smaller in fetuses with trisomy 18.

**Table 1.** Demographic and pregnancy characteristics of the study population according to ploidy status.

| Characteristic | Euploid (n=410) | Trisomy 21 (n=63) | Trisomy 18 (n=34) | Trisomy 13 (n=7) |
|---|---|---|---|---|
| Maternal age (years) | 31.5 (28.0-35.0) | 37.0 (34.0-40.0)** | 37.0 (31.0-40.3)** | 31.5 (24.0-36.3) |
| Gestational age (days) | 87.6 (84.0-90.0) | 88.0 (85.0-93.0)* | 85.5 (83.0-90.5) | 87.0 (84.0-91.8) |
| Fetal biparietal diameter (mm) | 21.1 (19.1-23.4) | 21.2 (18.9-23.1) | 18.7 (17.2-20.9)** | 19.3 (17.8-20.3) |
| Fetal crown rump length (mm) | 60.8 (55.5-66.3) | 63.1 (56.6-71.4) | 53.4 (49.5-62.7)* | 57.4 (51.3-60.4) |

*Data are given as median (interquartile range). Comparisons were made using the Kruskal–Wallis test, with post–hoc Bonferroni correction for multiple comparisons. * P<0.05; **P<0.001*

In euploid fetuses the lateral ventricle and choroid plexus areas increased, whereas the ratio of choroid plexus area to lateral ventricle area decreased with fetal BPD (Figure 2 and 3).

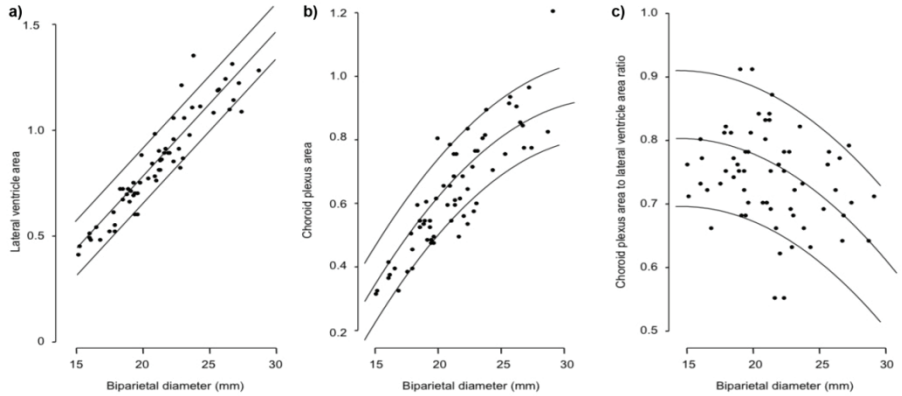



**Figure 2 –** Lateral ventricle area (measured in $cm^2$) (a), choroid plexus area (measured in $cm^2$) (b) and ratio of choroid plexus area to lateral ventricle area (c) in relation to biparietal diameter in trisomy 21 fetuses at 11-13 weeks' gestation, plotted on reference range of euploid fetuses ($5^{th}$, $50^{th}$ and $95^{th}$ centiles).

*Expected lateral ventricle area = 0.578 + 0.068 x BPD; adjusted $R^2$ = 0.870, SD = 0.08; P<0.001.*

*Expected choroid plexus area = –1.004 + 0.116 x BPD –0.002 x $BPD^2$; adjusted $R^2$=0.740; SD = 0.07; P<0.001,*

*Expected choroid plexus lateral to ventricle ratio = 0.636 + 0.023 x BPD – 0.001 x $BPD^2$; adjusted $R^2$ = 0.206; SD = 0.06; P<0.001.*

In fetuses with trisomy 21, the lateral ventricle and choroid plexus areas were significantly smaller than those in euploid fetuses (Figure 2, Table 2). In trisomy 18 fetuses, the ratio of choroid plexus area to lateral ventricle area was significantly smaller than that in euploid fetuses. In trisomy 13 fetuses, the choroid plexus and the ratio of choroid plexus area to lateral ventricle area were significantly smaller than those in euploid fetuses (Figure 3). The ratio of choroid plexus area to lateral ventricle area ratio was below the $5^{th}$ centile of the euploid fetuses in 11 (32.4%) of the 34 fetuses with trisomy 18 and in six (85.7%) of the seven fetuses with trisomy 13.

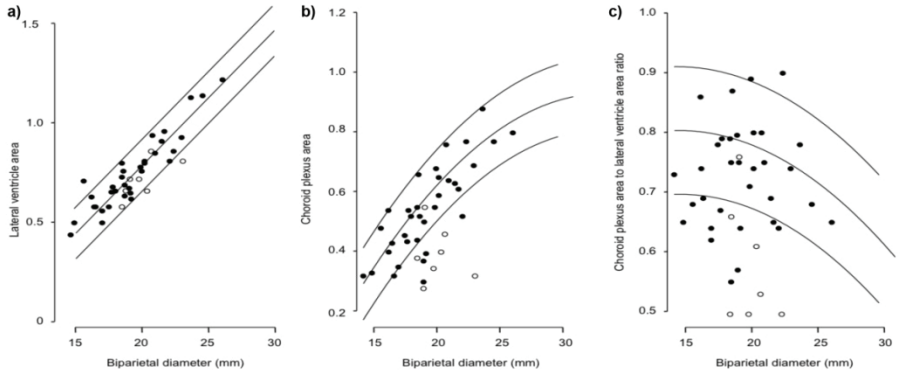



**Figure 3 –** Lateral ventricle area (measured in $cm^2$) (a), choroid plexus area (measured in $cm^2$) (b) and ratio of choroid plexus to lateral ventricle area (c) in relation to biparietal diameter in fetuses with trisomy 18 (closed circles) and trisomy 13 (open circles) at 11-13 weeks' gestation, plotted on reference range of euploid fetuses ($5^{th}$, $50^{th}$ and $95^{th}$centiles).

**Table 2:** Area of lateral ventricles and choroid plexuses in euploid and aneuploid fetuses at 11-13 weeks' gestation.

| Measurement | Euploid fetuses (n=410) | Trisomy 21 (n=63) | Trisomy 18 (n=34) | Trisomy 13 (n=7) |
|---|---|---|---|---|
| Lateral ventricle (delta in $cm^2$) | 0.000 (-0.049 to 0.045) | -0.028 (-0.073 to 0.008)* | 0.022 (-0.030 to 0.061) | -0.049 (-0.145 to 0.004) |
| Choroid plexus area (delta in $cm^2$) | -0.007 (-0.046 to 0.044) | -0.032 (-0.087 to 0.020)* | -0.023 (-0.060 to 0.041) | -0.229 (-0.272 to -0.156)** |
| Ratio of choroid plexus area to lateral ventricle area (delta) | 0.002 (-0.039 to 0.042) | -0.020 (-0.071 to 0.029) | -0.048 (-0.123 to -0.002)** | -0.244 (-0.357 to -0.132)** |

*Data are given as median (interquartile range). Comparisonswere made by Kruskal–Wallis test, with post–hoc Bonferroni correction for multiple comparisons. * P<0.05 **P<0.001.*

## 6.4. DISCUSSION

The findings of this study demonstrate that in normal fetuses at 11-13 weeks' gestation the areas of the lateral ventricles and choroid plexuses increase, whereas the ratio of choroid plexus area to lateral ventricle ratio decreases with fetal BPD. The area of both the lateral ventricles and the choroid plexuses was smaller in trisomy 21 fetuses than in euploid fetuses and the ratio of choroid plexus to lateral ventricle was not significantly altered. In trisomies 18 and 13, compared to euploid fetuses, the ratio of choroid plexus area to lateral ventricle area was smaller.

In the second and third trimesters of pregnancy, diagnosis of ventriculomegaly is based on demonstration of an increase in width of the lateral ventricular atrium in relation to the hemisphere (Nicolaides KH et al, 1990; Chervenak FA et al, 1984). In the first trimester the cortex is very thin and it was therefore anticipated than, in fetuses with ventriculomegaly, the width of the ventricle may not be increased. Consequently, we measured the area of the ventricle to capture any possible distortion in shape. Additionally, we determined the area of the choroid plexuses relative to that of the ventricles, because an early sign of ventriculomegaly during the second trimester of pregnancy is apparent shrinkage of the choroid plexuses. Previous studies reported that at 14-21 weeks' gestation the choroid plexus normally fills the lateral cerebral ventricle from side to side (Chinn DH et al, 1983) and that, with development of ventricular dilatation, the choroid plexus separates from the medial wall of the lateral ventricle (Cardoza JD et al, 1988; Hertzberg BS et al, 1994). A previous study at 14 weeks of gestation onwards established a normal range of the ratio between occipital horn height to choroid plexus thickness and reported that it was above the 95$^{th}$ centile in 31 of 32 cases of confirmed ventriculomegaly (Monteagudo A et al, 1994).

The observation that in trisomy 21 fetuses the areas of both the lateral ventricles and choroid plexuses in relation to BPD were reduced could be the consequence of smaller brain volume and/or relative brachycephaly. There is supportive evidence for both findings from previous postnatal,

prenatal and animal studies. A magnetic resonance imaging study in 16 patients with trisomy 21 reported that their brain volume was reduced (Pinter J et al, 2001). An anthropometric study in 199 patients with trisomy 21 between 6 months and 61 years of age reported the finding of brachycephaly due to shorter head length than head width (Allanson JE et al, 1993). In two postmortem studies with a combined total of 415 fetuses with trisomy 21, aborted at 15-40 weeks' gestation, the ratio of the BPD to occipitofrontal diameter (OFD) or head circumference was significantly higher than that in normal fetuses (Stempfle N et al, 1999; Guihard-Costa AM et al, 2006). A 3D ultrasound study of 72 fetuses with trisomy 21 at 11-13 weeks' gestation reported that the fetal head volume was smaller than that in chromosomally normal fetuses (Falcon O et al, 2005). Another 3D ultrasound study at 11-13 weeks' gestation reported that in 100 trisomy 21 fetuses, compared to 300 euploid fetuses, both BPD and OFD were smaller but the BPD to OFD ratio was higher (Borenstein M et al, 2006). In trisomy 16 mice, an animal model of trisomy 21, the brain is significantly smaller than in euploid mice during embryonic days 10-17, which corresponds to gestational weeks 7-18 in humans (Haydar TF et al, 1996).

The ratio of choroid plexus area to lateral ventricle area was below the 5$^{th}$ centile of normal range in 32% of the fetuses with trisomy 18 and in 86% of those with trisomy 13. The pathophysiological significance of this finding is uncertain. In all of our cases with aneuploidies the parents elected to terminate pregnancy, and it was therefore not possible to determine whether a low ratio of choroid plexus area to lateral ventricle area during the first trimester would have evolved into lateral ventriculomegaly during the second trimester. Previous studies during the second and third trimester reported an association between ventriculomegaly and aneuploidies but the prevalence of ventriculomegaly in fetuses with trisomies 18 and 13 was only 4-15% and 3-39%, respectively (Snijders RJM et al, 1995; Goetzinger RK et al, 2008; Papp C et al, 2006, 2007). It could be postulated that a low ratio of choroid plexus area to lateral ventricle area at 11-13 weeks is a transient finding in some aneuploid fetuses and possibly is related to abnormal development of the ventricular system and choroid plexus, perturbed cellular function and decreased neurogenesis (Zhang J et al, 2006; Owen-Lynch PJ et al, 2003).

Cerebrospinal fluid (CSF) is primarily produced by choroid plexus (Oresković D et al, 2010). There is evidence that the immature choroid plexus selectively transfers proteins from blood to CSF; thus, in the early brain, CSF has high concentrations of proteins including albumin, alpha-fetoprotein and proteoglycans (Saunders NR et al, 1999; Zappaterra MD et al, 2007; Gato A et al, 2009). At 11-13 weeks' gestation the immature choroid plexus of the lateral ventricle is large and has abundant glycogen which is thought to have a nutritive and anabolic role (Sturrock RR, 1979; Dziegielewska KM et al, 2001). In the rat hydrocephalic model, changes in CSF content, including reduced proteoglycan, are detectable prior to morphological brain defects (Pourghasem M et al, 2001) and it is therefore possible that disturbed CSF regulation may be associated with abnormalities in brain ventricle structure. Aneuploidies are associated with altered maternal serum concentrations of various proteins produced by the fetal–placental unit (Nicolaides KH, 2011) and it is likely that aneuploidies are associated with alterations concerning other proteins which could result in increased production of CSF and transient ventriculomegaly.

Ultrasound examination at 11-13 weeks' gestation is widely used in first trimester combined screening for aneuploidies but also in early diagnosis of major defects (Syngelaki A et al, 2011). Examination of the axial BPD plane of the fetal head, characterized by the butterfly image of the two choroid plexuses, is an integral part of the first-trimester scan and is useful in the detection of acrania and alobar holoprosencephaly. The finding of a contracted choroid plexus should raise the suspicion of ventricular dilatation and motivate the search for other defects associated with trisomies 18 and 13.

## REFERENCES

*Allanson JE, O'Hara P, Farkas LG, Nair RC.* Anthropometric craniofacial pattern profiles in Down syndrome. Am J Med Genet 1993;47:748-52.

*Borenstein M, Dagklis T, Csapo B, Sotiriadis A, Nicolaides KH*. Brachycephaly and frontal lobe hypoplasia in fetuses with trisomy 21 at 11 + 0 to 13 + 6 weeks. Ultrasound Obstet Gynecol 2006;28:870-5.

*Cardoza JD, Roy A, Podrasky AE.* The dangling choroid plexus: a sonographic observation of valuein excluding ventriculomegaly. AJR 1988: 151:767-770.

*Chervenak FA, Berkowitz RL, Tortora M, Chitkara U, Hobbins JC*. Diagnosis of ventriculomegaly before fetal viability. Obstet Gynecol 1984;64:652-6.

*Chinn DH, Callen PW, Filly RA*. The lateral cerebral ventricle in early second trimester. Radiology 1983; 148: 529-31.

*Dziegielewska KM, Ek J, Habgood MD, Saunders NR*. Development of the choroid plexus. Microsc Res Tech 2001;52:5-20.

*Falcon O, Cavoretto P, Peralta C, Csapo B, Nicolaides KH.*Fetal head to trunk volume ratio in chromosomally abnormal fetuses at 11 + 0 to 13 + 6 weeks of gestation. Ultrasound Obstet Gynecol 2005;26:755–60.

*Gato A, Desmond ME*. Why the embryo still matters: CSF and neuroepithelium as interdependent regulators of embryonic brain growth, morphogenesis and histiogenesis. Dev Biol 2009;327:263-72.

*Goetzinger RK, Stamilio DM, Dicke JM, Macones GA, Odibo AO*. Evaluating the incidence and likelihood ratios for chromosomal abnormalities in fetuses with common central nervous system malformations. Am J Obstet Gynecol 2008;199:285 e1-285 e6.

*Guihard-Costa AM, Khung S, Delbecque K, Menez F, Delezoide AL.* Biometry of face and brain in fetuses with trisomy 21. Pediatr Res 2006;59:33-8.

*Haydar TF, Blue ME, Molliver ME, Krueger BK, Yarowsky PJ.* Consequences of trisomy 16 for mouse brain development: corticogenesis in a model of Down syndrome. The Journal of Neuroscience 1996;16:6175–82.

*Hertzberg BS, Lile R, Foosaner D, Kliewer M, Paine S, Paulson EK, Carroll BA, Bowie J.* Choroid plexus-ventricular wall separation in fetuses with normal-sized cerebral ventricles at sonography: postnatal outcome. AJR 1994;163:405-10

*Loureiro T, Ferreira AFA, Ushakov F, Montenegro N, Nicolaides KH*. Dilated fourth ventricle in fetuses with trisomy 18, trisomy 13 and triploidy at 11-13 weeks' gestation. Fetal Diagn Ther 2012, Jul 26 [Epub ahead of print].

*Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH*. Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation. Ultrasound Obstet Gynecol 2012; 39: 620-4.

*Monteagudo A, Timor-Tritsch IE, Moomjy M*. In utero detection of ventriculomegaly during the second and third trimesters by transvaginal sonography. Obstet Gynecol 1994;4:193-8.

*Nicolaides K, Berry SM, Snijders R, Thorpe-Beeston JG, Gosden C.* Fetal lateral cerebral ventriculomegaly: Associated malformations and chromosomal defects. Fetal Diagn Ther 1990;5:5-14.

*Nicolaides KH.* Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7-15.

*Oresković D, Klarica M.* The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res Rev 2010;64:241-62.

*Owen-Lynch PJ, Draper CE, Mashayekhi F, Bannister CM, Miyan JA*. Defective cell cycle control underlies abnormal cortical development in the hydrocephalic Texas rat. Brain 2003;126:623-31.

*Papp C, Beke A, Ban Z, Szigeti Z, Toth-Pal E, Papp Z.* Prenatal diagnosis of trisomy 13: analysis of 28 cases. J Ultrasound Med 2006;25:429-35.

*Papp C, Ban Z, Szigeti Z, Csaba A, Beke A, Papp Z.* Role of second trimester sonography in detecting trisomy 18: a review of 70 cases. J Clin Ultrasound 2007;35:68-72.

*Pinter J, Eliez S, Schmitt E, Capone G, Reiss A.* Neuroanatomy of Down's syndrome: a high-resolution MRI study. Am J Psychiatry 2001; 158: 1659–1665.

*Pourghasem M, Mashayekhi F, Bannister CM, Miyan J.* Changes in the CSF fluid pathways in the developing rat fetus with early onset hydrocephalus. Eur J Pediatr Surg 2001;11:S10-S13.

*Robinson HP, Fleming JE*. A critical evaluation of sonar crown rump length measurements. Br J Obstet Gynaecol 1975;182:702-710.

*Saunders NR, Habgood MD, Dziegielewska KM.* Barrier mechanisms in the brain, II. Immature brain. Clin Exp Pharmacol Physiol 1999;26:85-91.

*Snijders RJM, Farrias M, von Kaisenberg C, Nicolaides KH.* Chromosomal defects in fetal abnormalities. In Snijders RJ, Nicolaides KH (eds): Ultrasound Screening for Chromosomal Defects. Parthenon Publishing Group 1995: pp7-9.

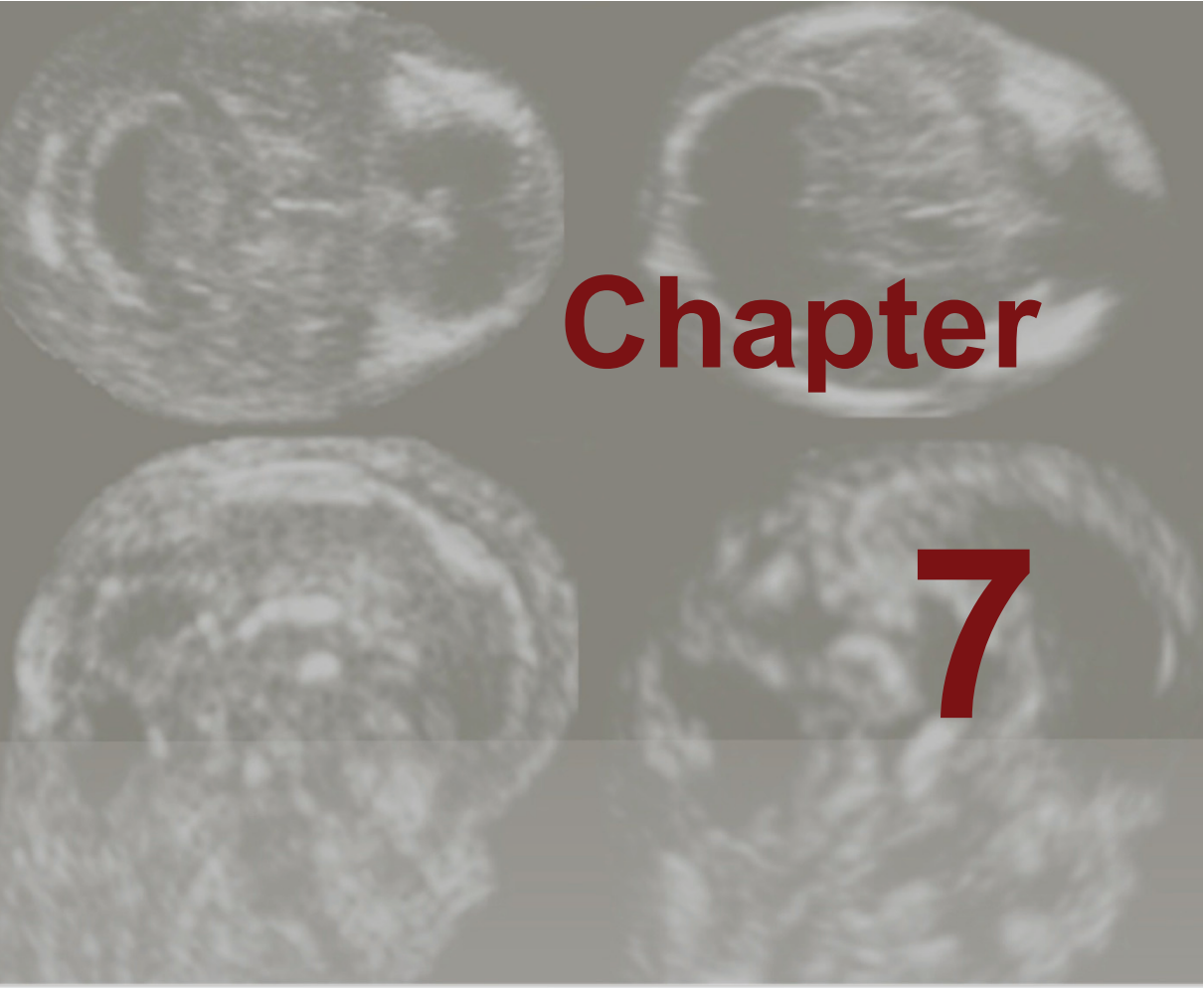
*Stempfle N, Huten Y, Fredouille C, Brisse H, Nessmann C.* Skeletal abnormalities in fetuses with Down's syndrome: a radiographic post-mortem study. Pediatric Radiol 1999;29:682-8.

*Sturrock RR.* A morphological study of the development of the mouse choroid plexus. J Anat 1979;129:777-93.

*Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH*. Challenges in the diagnosis of fetal non chromosomal abnormalities at 11-13 weeks. Prenat Diagn 2011;31:90-102.

*Zappaterra MD, Lisgo SN, Lindsay S, Gygi SP, Walsh CA, Ballif BA*. A comparative proteomic analysis of human and rat embryonic cerebrospinal fluid. J Proteome Res 2007;6:3537-48.

*Zhang J, Williams MA, Rigamonti D.* Genetics of human hydrocephalus. J Neur 2006;253:1255-66.



# Chapter 7

## General discussion
## Main conclusions

## 7.1. GENERAL DISCUSSION

Several studies suggest that it is possible to perform a detailed anatomical evaluation by ultrasound during first trimester. Nevertheless, the results obtained cannot be generally extrapolated to routine settings. The higher scores are usually the result from highly skilled motivated experts and do not reflect the reality. Brain assessment has been addressed cautiously. This is in part because the brain is the only structure in the fetus that carries on modifying its anatomy during normal development, but also results from the limited data available in the literature concerning early normal brain development and its ultrasonographic appearance.

There are obvious major fetal brain abnormalities that can always be detected at the 11 to 13 weeks' scan like anencephaly and alobar holoprosencephaly. Some other central nervous system defects, like open spina bifida, have been considered to be nearly undetectable at 11-13 weeks' gestation until recent years, because cranial sonographic signs were thought to mainly manifest during the second trimester. Nevertheless, the recently described markers for early screening and diagnosis of open spina bifida like the fourth ventricle will soon change the present clinical scenario. Some other brain abnormalities, such as agenesis/hypoplasia of the vermis, still show prenatal diagnostic inconsistencies until 24 weeks' gestation and hydrocephalus is rarely depicted during the first trimester. But this reflects more the lack of knowledge rather than validated information.

A new model of prenatal care which will be based on the results of a comprehensive assessment at 11-13 weeks and early identification of high-risk groups will certainly adopt a more thorough anatomical survey of the fetus and of brain development in particular, during the first trimester of pregnancy. Selection of cases of increased risk of deviant development of brain structures will promote further research that will define the best protocol for their follow up. It may be possible that some markers can be more informative at 11-13 week's gestation than later on, as it happens with nuchal translucency.

To describe the significant developmental events of the normal early fetal brain is required to understand and describe early pathology. The main objective of this thesis was evaluation of the cerebral ventricular system at 11-13 weeks' gestation both in normal fetuses and in those affected by aneuploidy or open spina bifida. The ultimate goal was to find brain markers in frequent conditions associated with serious handicap like trisomy 21, trisomy 18, trisomy 13 and open spina bifida that may be useful in screening. In order to accomplish this aim, the following research plan phases were established:

1. Describe the normal ventricular system in euploid fetuses and test the reproducibility of measurements.
2. Determine if in fetuses with open spina bifida at 11-13 weeks' gestation there are alterations in the cerebral ventricular system.
3. Determine if in fetuses with aneuploidies the diameter of the fourth cerebral ventricle at 11-13 weeks' gestation is different from euploid fetuses.
4. Examine the possible association between aneuploidies and lateral cerebral ventriculomegaly at 11-13 weeks' gestation.

### 7.1.1. DESCRIBE THE NORMAL VENTRICULAR SYSTEM IN EUPLOID FETUSES AND TEST THE REPRODUCIBILITY OF MEASUREMENTS

In normal fetuses the area of the lateral ventricles and choroid plexuses increased, whereas the choroid plexus to lateral ventricle ratio decreased with fetal biparietal diameter (BPD). The third ventricle diameter increased with BPD, the fourth ventricle diameter did not change significantly with BPD and aqueduct diameter decreased with gestational age. The evaluation of lateral ventricles and choroid plexus is highly reproducible concerning both intraobserver and interobserver variability. Smaller size structures, like third and fourth ventricles and aqueduct of Sylvius, present higher deviations concerning interobserver assessment.

### 7.1.2. TO DETERMINE IF IN FETUSES WITH OPEN SPINA BIFIDA AT 11-13 WEEKS' GESTATION THERE ARE ALTERATIONS IN THE CEREBRAL VENTRICULAR SYSTEM

In open spina bifida, compared to the normal fetuses, the measurement of lateral ventricle area, the diameters of the roof of the third ventricle, aqueduct of Sylvius and fourth ventricle were significantly decreased ($P<0.0001$).

### 7.1.3. TO DETERMINE IF IN FETUSES WITH ANEUPLOIDIES THE DIAMETER OF THE FOURTH CEREBRAL VENTRICLE AT 11-13 WEEKS' GESTATION IS DIFFERENT FROM EUPLOID FETUSES

The diameter of the fourth ventricle in trisomy 18, trisomy 13 and triploidy, but not in trisomy 21, was significantly higher than in euploid fetuses. In the euploid fetuses the median diameter of the fourth ventricle was 1.9 mm and the 95$^{th}$ centile was 2.5 mm. The measurements were above the median and the 95$^{th}$ centile in 25 (78.1%) and 17 (53.1%) cases of trisomy 18, in 10 (100%) and 8 (80.0%) of trisomy 13 and in 10 (83.3%) and 10 (83.3%) of triploidy.

### 7.1.4. TO EXAMINE THE POSSIBLE ASSOCIATION BETWEEN ANEUPLOIDIES AND LATERAL CEREBRAL VENTRICULOMEGALY IN THE FIRST TRIMESTER OF PREGNANCY

In trisomy 18 and 13 the ratio between the choroid plexuses area and the lateral ventricles area (CLR) was significantly smaller than in euploid fetuses. The CLR was below the 5$^{th}$ centile of normal range in 11 (32.4%) fetuses with trisomy 18 and in six (85.7%) with trisomy 13.

## 7.2. STRENGTHS AND LIMITATIONS

The large number of cases that were evaluated, the application of strict criteria for the measurements, the use of high frequency ultrasound probes to acquire the volumes and of 3D multiplanar mode for a correct alignment of the brain structures in three orthogonal planes give strength to these results. Nevertheless, higher deviations concerning interobserver assessment in small structures like the third and fourth ventricles and aqueduct were observed. The clinical significance of this variation is probably not relevant concerning the fourh ventricle, where the 95$^{th}$ centile corresponds to 2.5 mm. The answer would be to improve training as this is a new approach that surely has a learning curve or by the development of software to identify and quantify hypoechoic regions within a 3D dataset, providing an automatic estimation of their absolute dimensions.

In all of the cases of aneuploid fetuses the parents elected to have pregnancy termination and it was therefore not possible to determine how the brain markers that were significant in these abnormal fetuses, like dilatation of the fourth ventricle and reduced choroid plexus area to lateral ventricle area, would have evolved during the second trimester. Further investigations are needed to determine the effectiveness of assessment of the fourth ventricle in first trimester screening for DWM in euploid fetuses.

## 7.3. MAIN CONCLUSIONS

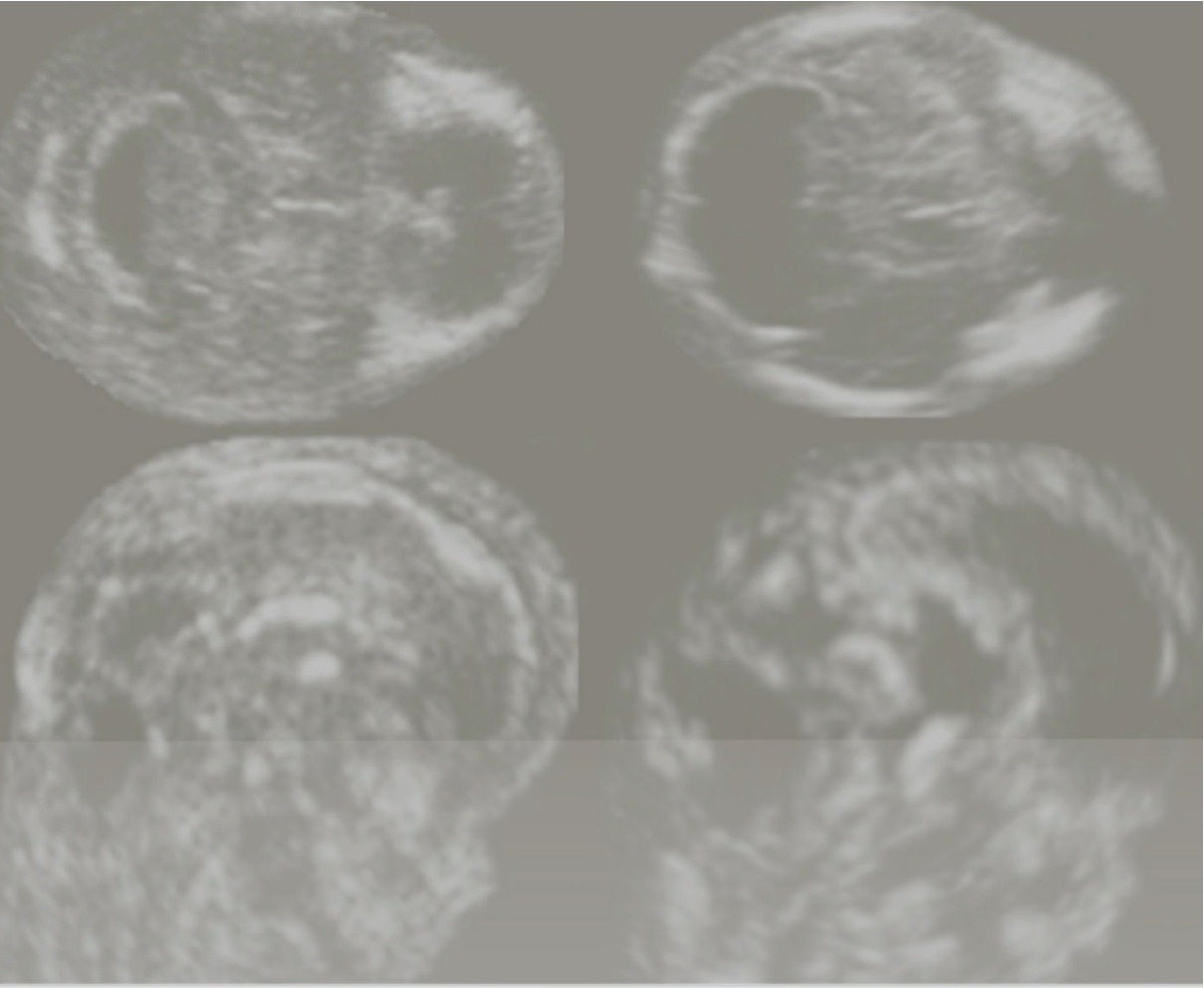
- Measurement of the cavities of the cerebral ventricular system, like the lateral ventricle area and the diameters of the third ventricle, aqueduct and fourth ventricle, is feasible at 11-13 weeks' gestation.

- There are statistically significant differences in size of cerebral ventricular system cavities between fetuses with open spina bifida and normal ones and between aneuploid and euploid fetuses at 11-13 weeks' gestation.

- In fetuses with open spina the intracranial collection of cerebrospinal fluid is substantially reduced at 11-13 weeks' gestation.

- In trisomy 18, trisomy 13 and triploidy the diameter of the fourth ventricle is increased at 11-13 weeks' gestation.

- Most fetuses with trisomy 13 and one third of fetuses of trisomy 18 have evidence of ventriculomegaly at 11-13 weeks' gestation.



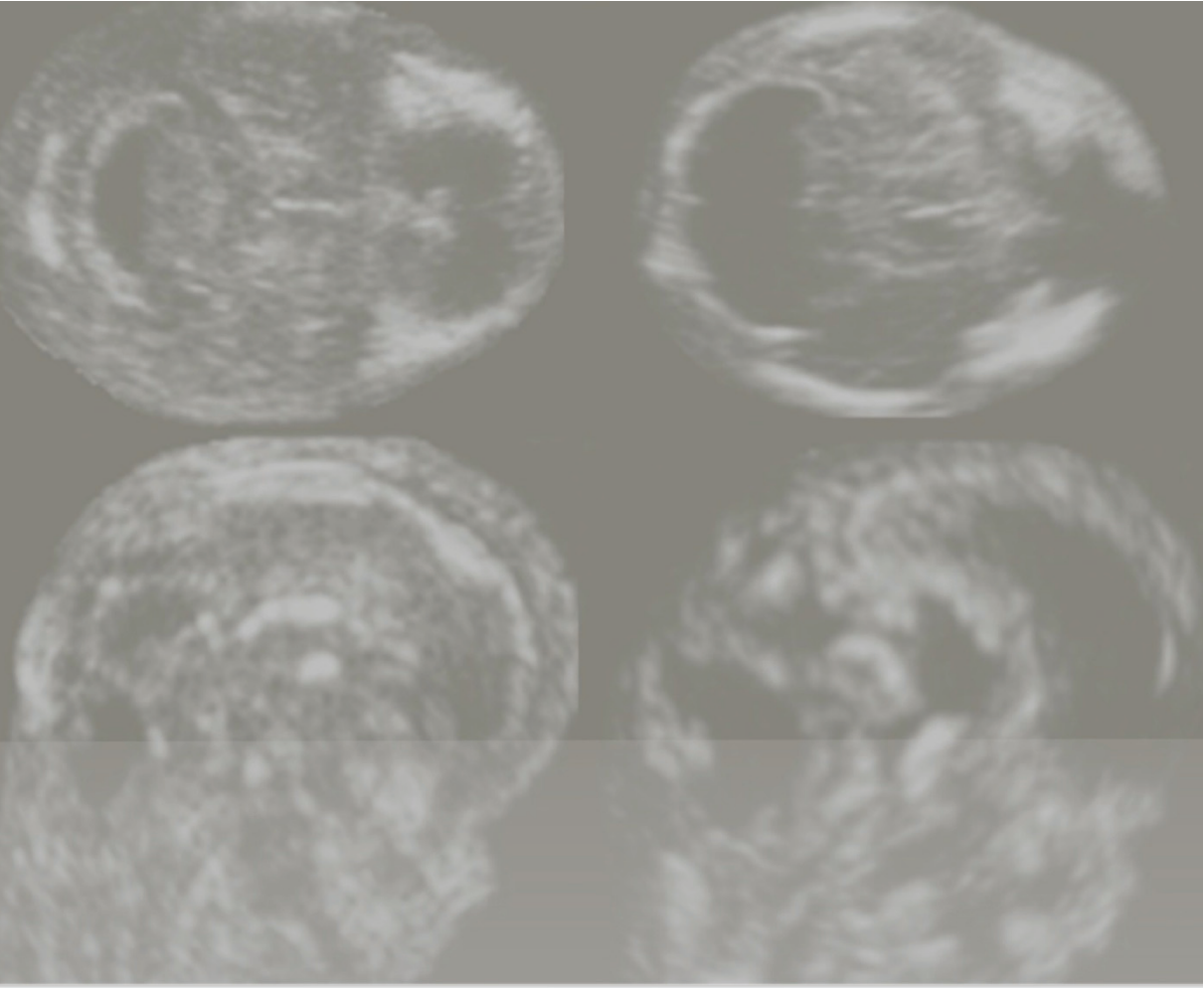
# LIST OF PUBLICATIONS

This PhD thesis is based on the following scientific publications:

- *Loureiro T, Ushakov F, Montenegro N, Gielchinsky Y, Nicolaides KH.* Cerebral ventricular system in fetuses with open spina bifida at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol 2012; 39: 620-4.*

- *Loureiro T, Ferreira AFA, Ushakov F, Montenegro N, Nicolaides KH.* Dilated fourth ventricle in fetuses with trisomy 18, trisomy 13 and triploidy at 11-13 weeks' gestation. *Fetal Diagn Ther* Jul 26 [Epub ahead of print].

- *Loureiro T, Ushakov F, Nerea M, Montenegro N, Nicolaides KH.* Lateral ventricles in aneuploidies at 11-13 weeks' gestation. *Ultrasound Obstet Gynecol* 2012; 40: 282-7.



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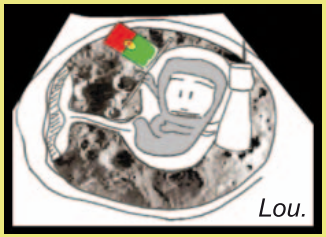
*'I have always wanted to peek inside the head and see what the brain is doing.'*

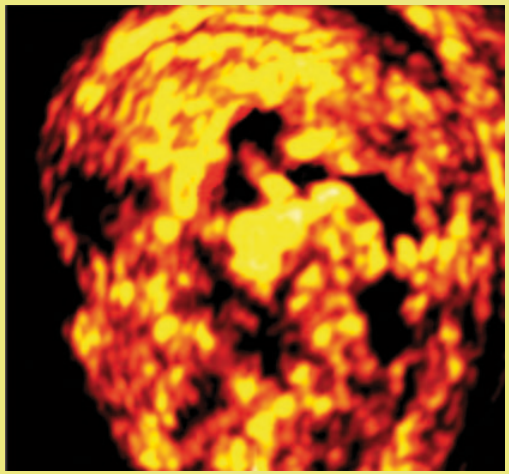
[Teresa Loureiro]




Loureiro interest in Medicine dates to her childhood, but she has also become fascinated by Literature and Art from an early age. By the age of 17, she had regularly published short stories, and poems in the portuguese Journal *Diário de Notícias (DN) Jovem*, a literary supplement for young authors.

Her hobbies include drawing and oil-painting and some collections of her ultrasound cartoons have been published in *Ultrasound Journal of Obstetrics and Gynecology*.

She has always been a great fan of dancing, sports and *siesta*.



' If the human brain were so simple that we could understand it, we would be so simple that we couldn't. '

Lyall Watson

Dissertação de candidatura ao grau de Doutor em Medicina, na área de Obstetrícia, submetida à Faculdade de Medicina da Universidade do Porto

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