FETAL NEUROSONOGRAPHY

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1. INTRODUCTION

Central Nervous System (CNS) malformations are among the most common congenital malformations.

Neural tube defects are the most frequent and affect 0.1-0.2% of newborns. Although the incidence of intracranial anomalies is uncertain, based on data from long-term follow-up studies, it is estimated that they may occur in about 1 in 100 newborns. They are the second cause of disability in childhood and the origin of severe and permanent lesions, with no possibility of therapeutic options in most cases.

The development of the CNS has some peculiarities that differentiate it from the other organs.

The development of the different structures occurs throughout the gestation and continues after birth. For this reason, the brain is exposed to significant alterations and malformations due to the effect of accidents or different risk situations at any gestational age (infections, trauma, hypoxia, etc.). Therefore, when planning the prenatal screening for CNS anomalies, it should be taken into account that:

a. Patterns of normality change with Gestational Age (GA), and therefore the normal appearance of the CNS at different Gestational Ages must be known.
b. Lesions are progressive. It takes some time after the risk situation has arisen until the abnormality becomes evident in the image; at the same time, the image changes depending on the time that has passed since the exposure and the ultrasound evaluation.
c. A normal ultrasound in week 20-22 does not exclude a pathology that may occur later in fetal life.

The content of this protocol includes the planning of the ultrasound diagnosis of CNS anomalies, the procedure for a systematic examination, and the indications for advanced neurosonography.

2. PLANNING THE ULTRASOUND DIAGNOSIS OF CNS ANOMALIES

This is based on two levels of attention defined by the content and complexity of the scan:

- Basic ultrasound. The objective is to confirm normality and the absence of images associated with a pathology. **Its content is not limited to the 20-22 week ultrasound, but rather extends to all ultrasound scans performed**, regardless of gestational age. In the ultrasound we can find different degrees of abnormality: certain anomaly, suspected anomaly, variant of normality and alarm. Any of them justifies referral to a specialised team.

- Detailed neurosonography. Focused on the **diagnosis and characterisation of anomalies**, it is performed by specialists who study the fetal CNS as part of a multidisciplinary team. Ideally, this team should include geneticists, paediatric neurologists, neonatologists, pathologists, and specialists in different aspects of Fetal Medicine with the ability to provide the most comprehensive care of the CNS. This includes the indication for complementary tests, to provide information to parents about short- and long-term prognosis, planning of controls, the approach of the different options, and genetic counselling for future pregnancies.

3. INDICATIONS FOR ADVANCED NEUROSONOGRAPHY

1. History of CNS congenital anomaly (family or previous pregnancy affected).
2. Abnormality or suspected CNS abnormality detected in the screening ultrasound (Annex 1).
3. Persistent increased Nuchal Translucency (> 99^{th} percentile in the second trimester ultrasound).
4. Head diameter (HD) >2 SD or <2 SD.
5. Severe intrauterine growth restriction (IUGR) (< p3 o altered fetal Doppler).
6. Extracranial malformations:
    a. Facial and ocular abnormalities
    b. Congenital Heart Defect
    c. Cardiac rhabdomyomas
    d. Limb incorrectly positioned

e. Ultrasound signs associated with certain genetic syndromes

7. Potential situations of cerebral injury during pregnancy:
    a. Maternal disease (Phenylketonuria, autoimmune thrombocytopenic purpura).
    b. Suspected fetal infection by Cytomegalovirus, Toxoplasma, Rubella, Varicella or Zika by seroconversion or presence of ultrasound signs.
    c. Hypoxic-ischaemic risk situation:
        i. Severe Fetal anaemia (> 1.5 MoMs)
        ii. Fetal hydrops
        iii. Severe maternal anaemia (Hb <7)
        iv. Complications of monochorionic pregnancy: twin-to-twin transfusion syndrome, selective intrauterine growth restriction type II-III or intrauterine death of one of the foetuses
        v. High risk maternal hypoxic situations: surgery in a pregnant woman who requires general anaesthesia, severe respiratory disease
8. Exposure to teratogens that affect neurogenesis (Annex 2).
9. Variants of uncertain significance in prenatal microarrays.

4. BASIC ULTRASOUND OF THE CNS ≥ 18; SYSTEMATIC EXAMINATION

Evaluation of the CNS in a screening ultrasound is performed by means of transabdominal ultrasound. It includes the evaluation of the axial planes of the fetal head and the evaluation of the spine.

4.1 FETAL HEAD EVALUATION:

The systematic procedure includes initially performing a general scan of the axial plane of the brain followed by the acquisition of three axial images/sections with the systematic measurement of different structures, which are detailed below.
The general evaluation of the fetal head will be performed in a craniocaudal direction:

- Morphology and integrity of the skull. Degree of ossification and presence of coronal (anterior pole) and lambdoid (posterior pole) sutures, assessing the absence of early closure in these sutures (acoustic shadow).
- Checking the position of the midline and the symmetry of the intracranial structures in all planes. Many severe brain injuries are associated with deviations or distortions of the midline.

- Attention will be paid to the ventricular system, looking at the outline and periventricular area.
- The echogenicity of the brain parenchyma will be evaluated, as echogenic or cystic areas should not be present.

Subsequently, the following sections and measurements will be taken:

1. posterior horns of the lateral ventricle

1. **Transventricular section (Figure 1a).** This is the highest section in the axial plane. A good section identifies the following structures: midline, Cavum Septi Pellucidi (CSP), the lateral ventricles (LV) (anterior and posterior horns) and the parieto-occipital (P-O) sulcus. The choroid plexus is generally identified within the s (LV). Next, we will detail the peculiarities of some of these structures:
    a. CSP: in all cases between 17 and 37 weeks, the average echo at the level of the anterior third ventricle is seen to be interrupted by the CSP. Under normal conditions, the CSP acquires a rectangular morphology and corresponds to the anechogenic (fluid) space that remains between the anterior horns of the LV. The CSP is separated from the previous horns by two lines that correspond to the septi pellucidi. It will be important to always identify its presence in weeks 17-37, as well as identifying the presence of the walls of the septum and its morphology. The non-visualisation between weeks 16-37 or the alteration of its usual morphology should alert us, since it may be indicating an anomaly at the commissural level, such as agenesis or dysgenesis of the corpus callosum; while non-visualisation of the walls of the septi pellucidi could be indicating an anomaly in the holoprosencephaly (HPE) spectrum. With the aim that the absence of cavum does not go unnoticed, it is important to not confuse it with the columns of the fornix, so visualisation of the columns of the fornix is recommended (3 parallel lines), which can be identified in a section slightly lower than the transthalamic plane (**Figure 1c**).
    b. Posterior horns of the LV: in this axial section, the atrium and posterior horns (confluence of the ventricular system) that are occupied by the glomus of the choroid plexus are visualised. In this transventricular section, the measurement of the ventricular atrium will be performed. The reference of the optimal plane for taking the measurement will be at the level of the parieto-occipital fissure and the CSP in a horizontal position.

At 20 weeks, this reference coincides with the glomus of the choroid plexus. Once the reference points are located, a line perpendicular to the axis will be drawn, longitudinal of the lateral ventricles, placing the calipers on the interior of the ventricular walls (**Figure 2a**). Its value remains stable throughout the second and third trimester of gestation (6-8 mm) and is considered pathological when ≥ 10 mm, a situation known as ventriculomegaly. You should bear in mind that the size of the atrium may change during the pregnancy and that a certain asymmetry in the size of both atriums (of up to 2.5 mm) would be considered normal. An asymmetry will be deemed significant when the difference between the atriums is > 2.5 mm. Likewise, although in all cases it is recommended to evaluate the measurement of both ventricles, due to artifacts caused by the parietal bone of the proximal hemisphere, the visualisation of the hemisphere and atrium proximal to the transducer is often unclear. Although we cannot obtain a section to perform the measurement, the proximal atrium should be evaluated to check for any asymmetry at this level. To assess this, oblique axial insonation is useful. If dilatation is suspected, the examination should be completed with the advanced sections.

2. hippocampal gyrus

2. **Transthalamic section (Figure 1b, 1c).** This is a plane parallel and inferior to the transventricular plane. A good section identifies the following structures: midline, CSP, anterior or frontal horns of the LV and the . It is in this section where the measurement of brain biometrics will be performed: Biparietal Diameter (BPD), Head Circumference (HC) and Cephalic Index (CI).

a. BPD: the distance between the proximal external limit and distal internal limit of the skull.
b. HC: is the external outline of the skull. Alterations in the HC can reveal cell proliferation alterations (micro/macrocephaly). Microcephaly is considered when the HC <-3 SD and macrocephaly when the HC > 2 SD.
c. CI: BPD/occipito-frontal diameter ratio. Normal limits are between 75-85%. Below 75% is considered to be dolichocephaly, and above 85%, brachycephaly.
d. The degree of opercularisation of the Sylvian Fissure will be evaluated depending on the gestational age (**Figure 8, Annex 3.2**).

3. **Transcerebellar section (Figure 1d).** This is a slightly oblique plane, inferior to the transthalamic plane. It is obtained after discreetly tilting the transducer towards the posterior fossa. A good section identifies the following structures: CSP, the anterior or frontal horns of the LV, the thalami, the cerebellum and the cisterna magna, with a correct visualisation of the occipital bone. Next, we will provide details of the peculiarities of some of these structures:
   a. Cerebellum: in the evaluation of the cerebellum, it will be important to assess the morphology and the size. The cerebellum is shaped like a "butterfly" and is made up of the two rounded hemispheres of symmetrical morphology, dimensions and position, separated by the vermis, a more echogenic structure, centrally located between both hemispheres. The measurement of the transverse cerebellar diameter(TCD) will be taken systematically in the ultrasound of the second trimester, and in the third trimester if an anomaly is suspected.
      - TCD: It is the distance between the proximal external limit and the distal internal limit of the skull (**Figure 2b**). In millimetres, it matches the Gestational Age in weeks up to week 22-24.
   b. Cisterna magna or posterior fossa: the cisterna magna is a fluid-filled space posterior to the cerebellum. It often contains a thin septum with a symmetrical layout that correspond to normal folds of the arachnoid and are usually absent when there is a pathology. In this section, the measurement of the cisterna magna will be performed systematically in the ultrasound of the second trimester and in the third semester if an anomaly is suspected:
      - Anteroposterior diameter of the posterior fossa or cisterna magna (**Figure 2b**): the distance between the posterior vermis limit and the inner edge of the occipital bone, simulating the continuation of the middle echo. Its normal value remains stable between 2 and 10 mm during the entire pregnancy.
   c. Relationship of the IV ventricle with the cisterna magna: in the evaluation of the posterior fossa, it will also be important to rule out whether there is communication between the posterior fossa and the fourth ventricle. You can calculate the 4V index (4VI): an ultrasound marker of vermis dysgenesis/agenesis that is calculated as the ratio between the latero-lateral diameter and the anterior-posterior diameter of the fourth ventricle, which should be > 1 (**Figure 2c**).

4.2. SPINE EVALUATION

The examination is performed in three planes at the level of the vertebral column: sagittal, axial and coronal, by means of a morphology scan (**Figure 3**). The entire vertebral column will be evaluated, with special attention to the lumbosacral region.

a. Sagittal plane: in this plane, the ossification centres of the vertebral bodies and the posterior arches must be identified (2 lines), evaluating the position, ossification and integrity of the bodies and the lateral processes of all the vertebrae. The regular position of the ossification centres must be confirmed, as well as the integrity of the skin covering the entire spine, which is one of the more evident signs of normality.
b. Coronal plane: evaluation of the spine in the coronal plane is a dynamic process throughout the entire column. Depending on the orientation of the ultrasound from ventral to dorsal, the following will be evaluated:
    - integrity of the vertebral bodies (1 line),
    - integrity of the vertebral bodies and posterior arches (3 lines) and
    - integrity of the posterior arches alone (2 lines).
c. Transverse or axial plane: it is also a dynamic process throughout the entire spine. The 3 ossification centres will be evaluated (vertebral bodies, posterior arches and lateral processes) and the skin covering each vertebra. The morphology of the thoracic and lumbar vertebrae is triangular, while the cervical vertebrae are quadrangular, and the sacral ones are flat.

If the evaluation is not conclusive, because it is a breech presentation, a transvaginal ultrasound should be performed or a new ultrasound should be scheduled in order to evaluate the most caudal portion of the spine.

5. PROCESS OF ADVANCED NEUROSONOGRAPHY OF THE CNS ≥ 18 w

This consists of the multiplane evaluation of the CNS. To perform this, initially we will evaluate the same axial sections described above in the basic screening ultrasound (transabdominal), although a more detailed evaluation of the different structures will be performed. After the axial sections, the coronal and sagittal sections will be acquired and evaluated, preferably obtained by transvaginal ultrasound (TVUS), reserving the transabdominal route for cases where fetal static or other reasons prevent the performance of the TVUS. In any case, it is very important to keep in mind that the highest resolution and quality of the different structures is obtained if the degree of flexion of the fetal head facilitates insonation through the acoustic window provided by the fontanelles and sutures, so that the sections obtained have a quality and resolution similar to neonatal intracranial ultrasound. Obtaining them will often require a gentle manipulation of the foetus with the free hand, i.e. the one not holding the transducer.

calcarine sulci

The procedure includes the evaluation of the morphology, position, and echogenicity of the different intracranial structures, cerebral gyri and fissures. The evaluation of the gyri should be carried out throughout the entire neurosonographic examination in the different planes, mainly evaluating the Sylvian fissure and the cingulate, parieto-occipital (P-O) and . It is very important to keep in mind that both its appearance and morphological characteristics correlate precisely with gestational age.

In order to quantify cortical maturation, we will use the degrees described by Pistorius (**Annex 3.6**).

Finally, the size of certain intracranial structures will be evaluated. The normal ranges are considered to be those measurements that are between the 5th centile and the 95th centile, following the normality curves described according to gestational age (**Annex 3**).

5.1. AXIAL PLANES:

In the advanced examination, we will add the following evaluations:

a. **Transventricular section**: assessment of the parieto-occipital sulcus: degree of maturation (**Figure 8, Annex 3.3**).
b. **Transthalamic section**: to the screening ultrasound procedure (measurement of BPD, HC, cephalic index), we will incorporate:
    i. Third ventricle (**Figure 6**): measurement of its maximum diameter by placing the calipers on the inner edge of the ventricular walls.

    i. optic chiasma

    ii. Assessment of the : in a section slightly lower than the transthalamic plane and with a tilting towards the orbits, we can evaluate the optic chiasma. It is identified by its hyperechoic appearance, characteristic X-shape and its location in the middle of the Willis polygon.
c. **Transcerebellar section**. Detailed evaluation of the morphology of the fourth ventricle with the posterior fossa.

5.2 CORONAL PLANES:

We will always begin the examination by performing a coronal scan in the anteroposterior direction, assessing the integrity of the skull, the presence of the normal-looking sagittal suture (absence of acoustic shadow and/or ridge) and the subarachnoid space. Subsequently, we will obtain the following planes (**Figure 4**):

a) **Transfrontal section**: this is the most anterior section, through the anterior fontanelle, at the level of the orbits and sphenoid bones. It is a plane in front of the corpus callosum, so we must confirm that the interhemispheric fissure is not interrupted and identify the most anterior portion of the anterior horns of the lateral ventricles in front of the genu of the corpus callosum. In this section, the presence and degree of maturation of the olfactory grooves will also be assessed from 28 weeks of gestation (**Figure 8**).

b) **Transcaudate section**: this is one of the most important planes in the fetal neurosonography. It is obtained by tilting or sliding the transducer towards the most posterior part of the anterior fontanelle. From top to bottom, the following will be evaluated:

    i. Subarachnoid space (it can be measured if it is subjectively enlarged). Measurement of craniocortical and sinocortical distances (**Figure 6**). The normal values of the sinocortical distance remain stable throughout gestation (3.0 ± 0.9 mm).

    ii. Superior longitudinal sinus.

    iii. Interhemispheric fissure interrupted by the genu of the corpus callosum (it is visualised as a hypoechoic band that crosses the two hemispheres).

    iv. Anterior horns of the lateral ventricles separated from the CSP by the septum. Measurement of the height of the anterior horns (craniocaudal), placing the calipers on the inner edge of the ventricular walls, so they are perpendicular to the long axis of the horns (**Figure 6**). < 3.5 mm is considered normal.

    v. CSP that, when normal, acquires a triangular or trapezoid shape below the corpus callosum.

    vi. Caudate nuclei. the germinal matrix, starting from the 2nd trimester, is located preferably at the level of the caudate nucleus, a place where frequent haemorrhaging occurs, due to the fragility of its vessels. Thus, we will assess echogenicity, as well as the absence of cystic images at this level.

    vii. Degree of maturation of the cingulate sulcus (**Figure 8, Annex 3.3**).

c) **Transthalamic section**: obtained through the anterior fontanelle after tilting the transducer or through the open sagittal suture. Just like the transcaudate plane, in this section we will identify the sagittal sinus, the anterior horns, and the CSP, although in this posterior section the caudates are no longer identified and now new structures appear, such as:
   i. Thalamus
   ii. Third ventricle, located in the midline, between the thalami
   iii. Optic chiasma: bell-shaped, moderately echogenic, in the midline near the base of the skull.
      - Measurement of the optic chiasma in the coronal plane: the lateral and superior limits of the chiasma will be defined through the use of the colour Doppler, in which we will visualise the supracavernous segment of the internal carotid artery just below the limits of the chiasma and the anterior cerebral artery above it (**Figure 6**).
   iv. Sylvian fissure of both hemispheres, where the morphology can be evaluated (**Figure 8, Annex 3**)

d) **Transcerebellar section**: this is the only coronal section that is obtained through the posterior fontanelle, facilitating the visualisation of more posterior structures, such as:
   i. Occipital horns of the lateral ventricles. In this section you can perform the measurement of the proximal atrium. The measurement will be carried out at the level of P-O sulcus, just before the bifurcation with the calcarine sulcus (**Figure 6**)
   ii. Interhemispheric fissure
   iii. Cerebellum, with the identification of the two cerebellar hemispheres and the vermis
   iv. Tentorium position
   v. Degree of maturation of calcarine sulcus (**Figure 8, Annex 3.3**)

5.3 SAGITTAL PLANES

We will always begin the examination with a sagittal scan. From the centre, the transducer will move from right to left, paying attention to the position of the lateral ventricles and echogenicity of the periventricular tissue, as well as the identification of midline structures. Next we will obtain the following planes (**Figure 5**):

a) **Midsagittal section**: this facilitates the direct visualisation of the structures that are in the midline. Depending on the evaluation of the supra or infratentorial structures, this plane must be obtained through the anterior (for the assessment of the anterior midsagittal structures) or posterior fontanelle (for infratentorial structures) or even through the non-ossified sagittal suture. Systematically, the following structures will be assessed:

i. Corpus Callosum: this can begin to be fully visualised between 18-20 weeks. In the evaluation of CC, the quantitative measurement of the length of the corpus callosum will be as important as its qualitative evaluation. Other aspects should be carefully evaluated, such as the presence of the different parts of the CC (from front to back: beak, rostrum, body and splenium), thickness and echogenicity
    - Length of the corpus callosum (**Figure 6**): the measurement will be made with the calipers in the “out-to-out” position, from the most anterior part of the corpus callosum to the most posterior part of the splenium.
    - Thickness of the corpus callosum (**Figure 6**): if a subjective evaluation results in a thickening or thinning of the corpus callosum, it should be measured by placing the calipers on the internal edge of the two hyperechogenic lines that limit it (callosum sulcus on the upper part and the interface with the CSP and cavum vergae at the bottom) at the level of the genu, body and splenium. Normal values are between 2 and 7 mm.

ii. CSP: anechogenic structure just below the CC. As the gestation advances, the CSP collapses from the posterior part to the front part. A delay of the collapse in the advanced stages of gestation is considered to be a variant of normality (cavum vergae).

iii. Fornix, structures that delimit the CSP below.

iv. Third ventricle that continues with the Sylvian aqueduct and at the same time with the fourth ventricle. In the sagittal section, the fourth ventricle acquires a triangular shape, and from 18 weeks it must be completely covered and separated from the great cistern.

v. Vermis: the vermis presents an ultrasound structure different from the cerebellar hemispheres. The vermis lobes are echogenic and oriented in a “radial” arrangement towards the fastigium. In general, the primary and secondary fissure can be distinguished. The height of the vermis will be systematically measured, while the anteroposterior diameter will only be measured if it is subjectively reduced (**Figure 6**). The ratio between height and anteroposterior diameter is approximately 1:1.

- Height: the measurement is made out-to-out from the most cranial to most caudal portion, parallel to the axis of the brainstem.
- Anteroposterior diameter of the vermis: it will be measured out-to-out following a horizontal line of the vermis at the level of the fastigium.

vi. Brainstem with the pons: under normal conditions the pons will not be measured. It will only be measured in cases of suspected pathology in the fossa posterior (cerebellum) or when it specifically seems to be reduced (**Figure 6**).

- A-P pons measurement: measured from the anterior edge of the pons (excluding the basilar artery, which is identified as a pulsatile element) to the most anterior border of the fourth ventricle, perpendicular to the axis of the brainstem.

vii. Posterior fossa or cisterna magna,

viii. Insertion and orientation of the tentorium

The application of colour Doppler in the sagittal plane facilitates the identification of the anterior cerebral artery, the pericallosal artery and its branches, and the vein of Galen, which can be helpful in some cases of pathology of the corpus callosum or midline vascular malformations.

b) **Parasagittal planes**: these are oblique to the midsagittal plane and symmetrical on each side. They are obtained through the sagittal suture or the posterior fontanelle, and make it possible to evaluate the morphology, outline and content of the lateral ventricles in the section of the 3 horns (frontal, temporal and occipital horns). We can evaluate in detail the choroid plexuses, the periventricular area and the brain parenchyma.
They also make it possible to assess the cortical maturation of the external surface and sometimes the Sylvian fissure.

5.4 SPINE EVALUATION

In the advanced examination, the level of the conus medullaris must be assessed (**Figure 7**), which is the terminal part of the spinal cord and is identified sonographically as a triangular structure with two surrounding echogenic lines at the end of the spinal cord. The filum terminale begins at the tip of the conus medullaris and extends caudally as a thin and fibrous filament to insert into the first coccygeal vertebra. The conus medullaris will have its final location in L1-L2 from 24 weeks of gestation. Previously, we would find it lower depending on the weeks of gestation (L4 or lower between 13-18 weeks; L2- L3 between 18-24 weeks).

5.5. ARTERIAL AND VENOUS VASCULARISATION

In selected cases, the use of colour Doppler can improve the diagnostic capacity of neurosonography, which is why it would be indicated in cystic images, suspicion of aneurysms or arteriovenous anomalies, and in heterogeneous intra- or extraparenchymal images.

5.6 THREE-DIMENSIONAL ULTRASOUND

Can be used to obtain head volumes for subsequent delayed analysis by the same team or sent to experts. We recommend a routine neurosonographic examination using multiplanar images, due to the greater quality and detail of the images obtained, reserving the 3D study for certain cases.

6. EARLY ULTRASOUND 11-17 WEEKS

Although most CNS malformations cannot be diagnosed until after 20 weeks, early assessment of intracranial structures enables the diagnosis of certain pathologies (in many cases lethal), such as the acrania-exencephaly-anencephaly sequence, holoprosencephaly, encephalocele or open neural tube defect and the suspicion of others that will require follow-up until we can confirm their diagnosis (such as posterior fossa pathology).

It is important to know the brain's appearance from an early age, because the ultrasound images at 11-13 weeks will differ from the appearance at 14-17 weeks, as a consequence of rapid changes in the development of the CNS.

For the early assessment of intracranial structures, the use of transvaginal ultrasound is recommended, although in patients with BMI 25 Kg/m2, and if the aim is not to assess the posterior fossa, a transabdominal assessment can be performed with the high-frequency transducers.

<u>6.1 EARLY BASIC ULTRASOUND OF THE CNS</u>

Although the diagnostic capacity is limited in the early stages (<18 weeks), every time we perform a fetal anatomical assessment, the intracranial structures must be included in the assessment. Generally, the first anatomical assessment of the foetus will be performed in the first trimester screening ultrasound (11-13 weeks). The planes/structures to be evaluated at each moment are as follows:

11-13 weeks (Figure 9)

a) Axial plane scan: assessment of the cranial shell from 11 weeks in axial section (and, if possible, in coronal section) to rule out the presence of bone defects or distortion zones. The correct visualisation of fetal skull ossification can rule out ossification anomalies such as acrania-exencephaly-anencephaly or encephalocele.

b) Transventricular section: assessment of the integrity of the midline (interhemispheric fissure and falx) and of the lateral ventricles that are occupied in their posterior 2/3 by the choroid plexus: the hemispheres must be symmetrical and separated by a complete and uninterrupted midline. In this section is where we should suspect alterations in the formation of the prosencephalon, such as lobar or alobar holoprosencephaly. In > 50% of foetuses from 13 weeks onwards, we can identity an ovoid anechogenic image at the level of the interhemispheric fissure, which corresponds to the cavum veli. Given the dominance of the LV and plexus at this gestational age, in the transventricular section the ventricles and plexus acquire the "butterfly wings" sign. The anterior horns look rounded and full of CSF. Although the brain parenchyma is very thin, on the lateral surface of the hemispheres, the insula (future Sylvian fissure) is detected as a small depression (< 2 mm).

c) Transthalamic section: this is a more caudal section than the previous one. In this section, the most anterior part of the choroid plexus, separated by the interhemispheric line, is identified. At a posterior level, the interhemispheric line is interrupted by the third ventricle with the two thalami that, at this gestational age, acquire an ovoid morphology. In a lower transthalamic section, posterior to the third ventricle, an anechogenic space with rectangular morphology is identified that corresponds to the Sylvian aqueduct, prominent at this gestational age. Around the Sylvian aqueduct you can see the Tectum. This is the preferred section for measuring the biparietal diameter, although its measurement is also accepted in the transventricular section.

   - BPD measurement: in our centre, the measurement will be carried out from "out-to-in", and this measurement will be applied for estimating fetal growth in the 2nd trimester.

d) Spine: assessment in 2 planes (sagittal and axial) to evaluate correct vertebral integrity and alignment. The skin should be seen intact from the cervical vertebrae to the sacrum, as well as their correct alignment (absence of scoliosis).

The vertebral bodies show ossification, but the neural arches, which are still cartilaginous, are isoechoic or hypoechoic. Direct systematic examination of the fetal spine and the skin that covers it allows for a precise early diagnosis of 50% of the open defects.

14-17 weeks (Figure 10):

a) Transventricular section: the brain parenchyma can be better assessed. In the anterior part, an irregularity may start to be hinted at, which will be the CSP, visible > 17 weeks, and behind the cavum veli, although from week 14-15, it will collapse and no longer be visible under normal conditions.

b) Transthalamic section: the third ventricle and the thalami will be increasingly evident. However, the Sylvian aqueduct will be collapsing during these weeks of gestation, almost disappearing from 17 weeks of pregnancy.

c) Transcerebellar section: at the beginning of the second trimester, it will be possible to start evaluating the developing vermis and the brainstem, whilst remembering that the cerebellum has a very different appearance than what we can see from 18-20 weeks. In these weeks of gestation, the cerebellum is butterfly-shaped and the upper vermis is already present and isoechoic. As the weeks of gestation progress, the vermis will develop craniocaudally, a process that will end at 18 weeks. Before this process is completed, it will be normal to see communication between the fourth ventricle and the cisterna magna.

6.2. EARLY ADVANCED NEUROSONOGRAPHY OF THE CNS

An early NSG will be indicated in the following situations:

1) Suspected CNS malformation on early ultrasound

2) Clinical history (of parents or previous pregnancies) of:

a) Neural tube defect

b) Midline abnormalities (holoprosencephaly spectrum)

c) History of severe vermis pathology

3) Patients undergoing antiepileptic treatment who have not performed prophylaxis correctly with folic acid at high doses.

This ultrasound will be performed from 12+3 weeks (ideally > 13 weeks).

Ultrasound markers have been described for early detection or suspicion of Neural Tube Defect (NTD) and anomalies of the posterior fossa. Based on the available literature and our centre's own previous experience, the following markers will be recommended:

AXIAL PLANE (Figure 11)

The same sections reported in the basic CNS examination will be made: transventricular and transthalamic, although markers of NTD and posterior fossa pathology will be integrated. In addition, the evaluation of the transcerebellar section in the axial plane will be added.

a) Transthalamic section: apart from the BPD, the head circumference will also be measured.

   - Head circumference: Measurement around widest part of the head. There are tables of normality available after 12 weeks.

   In this section, certain early open NTD markers will be evaluated:

   - Distance between the Sylvian aqueduct and the occipital bone: If < 2.5 mm (Finn M, et al. UOG 2011) this will be suggestive of open NTD.
   - Presence of the "crash sign" (Ushakov F et al., Ultrasound Obstet Gynecol 2019). This sign is due to the posterior displacement of the midbrain and Sylvian aqueduct along with a moulding of the occipital bone as a consequence of the impact of the cerebellum to the bone.

b) Transcerebellar section: it will be important to remember that the cerebellar hemispheres are already developed at 11 weeks of gestation, while the vermis presents a progressive cranio-caudal formation during the first trimester of gestation, starting from week 11 to week 18 of gestation. For this reason, before 18 weeks, a physiological communication between the fourth ventricle and the cisterna magna will be identified in the most caudal projection. In a higher transcerebellar section, this communication is interrupted by the presence of the plexus of the fourth ventricle.

   The absence of the plexus of the fourth ventricle has been shown to be a marker with a high PPV for cystic anomalies at the level of the posterior fossa (DWM) and chromosomal anomalies (Volpe P, UOG 2016). As a NTD marker in this section, the Cisterna Magna (CM) will be measured:

   - CM measurement in the axial plane: placement of the calipers perpendicular to the greater axis between the limit of the plexus of the fourth ventricle and the occipital bone in the Transcerebellar high section. Be careful not to confuse the occipital bone with the meninges. A measurement of CM < p5 will be suggestive of open NTD, while CM > p95 will indicate probable cystic pathology of the posterior fossa (García-Posada et al. UOG 2013).

SAGITTAL PLANE (Figure 12):

In the sagittal plane between 11-13.6 weeks, apart from the measurement of the nasal bone and nuchal translucency, a posterior fossa evaluation will be performed, using the same section described and used for the measurement of nuchal translucency (NT) according to the Fetal Medicine Foundation (https://fetalmedicine.org/fmf-certification-2/nuchal-translucency-scan), considering an optimal section to be when the thalami, brainstem or midbrain, the choroid plexus of the fourth ventricle, the fourth ventricle or intracranial translucency (IT), the cisterna magna and the occipital bone are visible. Several markers have been described to evaluate the posterior fossa in this view. For ease and reproducibility, the measurement of the Cisterna Magna (CM) will be proposed.

- CM measurement in the sagittal plane: placement of the calipers perpendicular to the major axis between the limit of the plexus of the fourth ventricle and the occipital bone. Be careful not to confuse the occipital bone with the presence of meninges. A CM measurement < p5 will be suggestive of open NTD, while CM > p95 will indicate probable fossa cystic pathology later on (García-Posada et al. UOG 2013).

7. INDICATIONS FOR MAGNETIC RESONANCE (MRI)

Magnetic Resonance Imaging is a complementary diagnostic tool of NSG that in some situations provides information of great clinical value. In general terms, the MRI will always be performed after an NSG has been performed by an expert examiner (it is not considered a screening technique) and not before 18 weeks of gestation.

There are innumerable indications for MRI of the fetal CNS, the most common being: ventriculomegaly, absence of cavum septi pellucidi, posterior fossa malformation, injury or destruction of the parenchyma, suspicion of craniosynostosis and neural tube defects. The indication and time of realisation should be agreed by a multidisciplinary team. However, some of the following are common situations in which we perform a fetal MRI to complete the neurosonographic assessment:

- Severe and/or progressive ventriculomegaly
- Suspected alteration of cortical development
- Microcephaly (-3 SD) and macrocephaly (+2 SD)
- Intracranial haemorrhage (grade II/III or intraparenchymal)

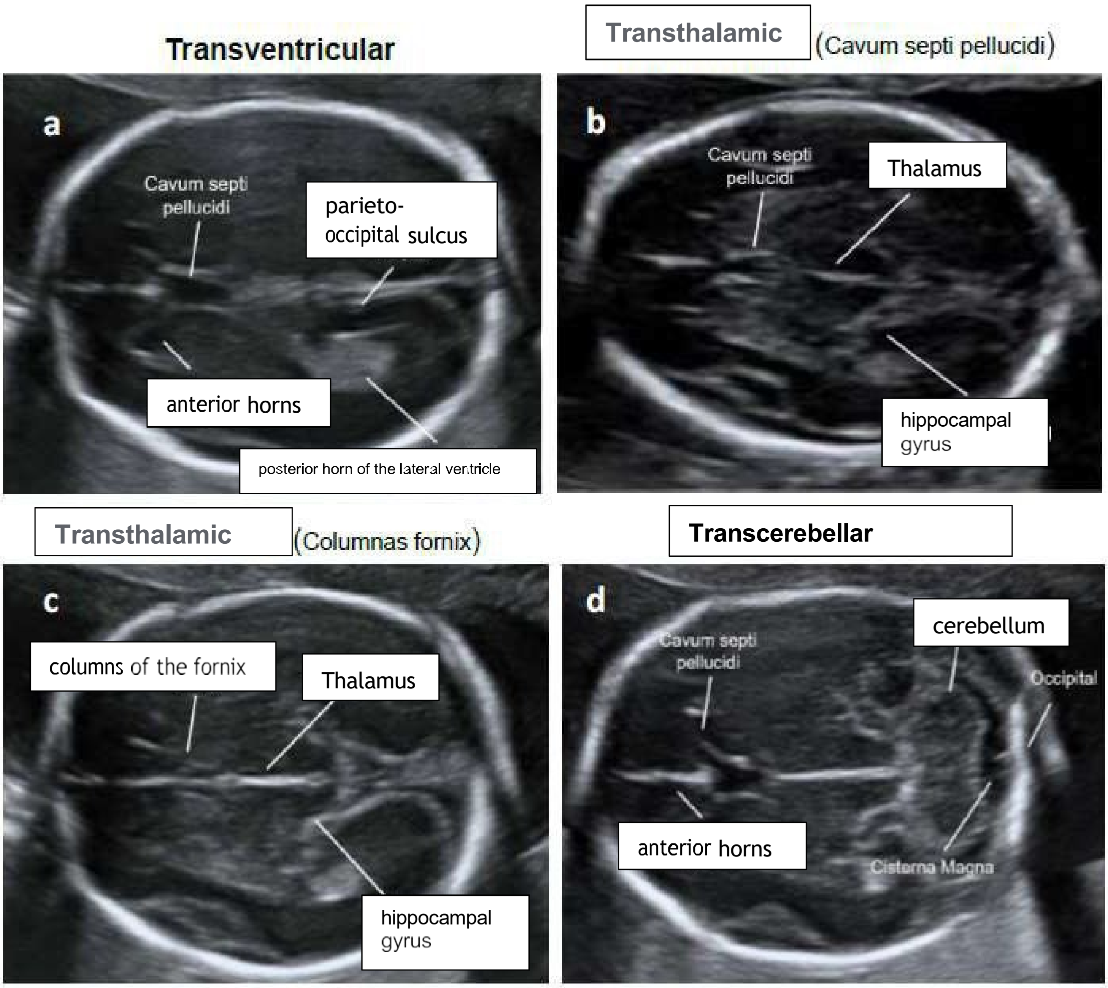
- Posterior fossa anomalies
- Fetal infection (CMV, Zika)
- Vascular anomalies
- Hypoxic episodes
- Intracranial tumours
- Rhabdomyomas
- History of complex CNS malformation
- Unsatisfactory NSG scan

Cases in which neurosonography is inconclusive are considered optional indications to rule out anomalies. Among others:

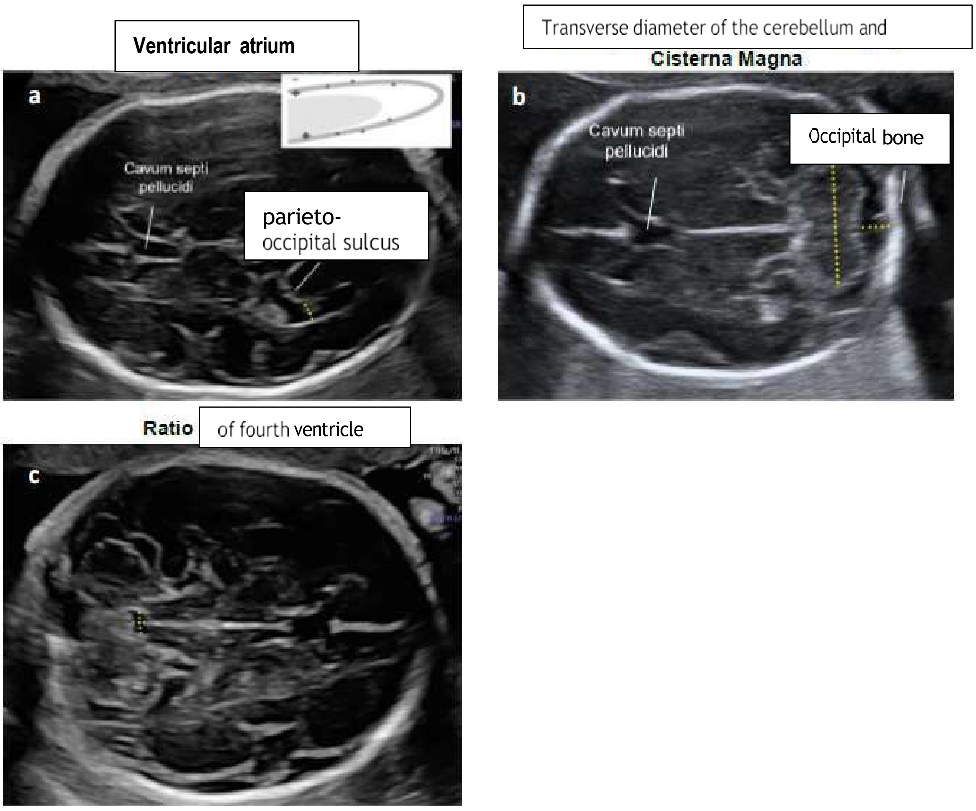
- Severe oligoanhydramnios
- Persistent breech presentation
- Maternal obesity (BMI > 40)

7. FIGURES

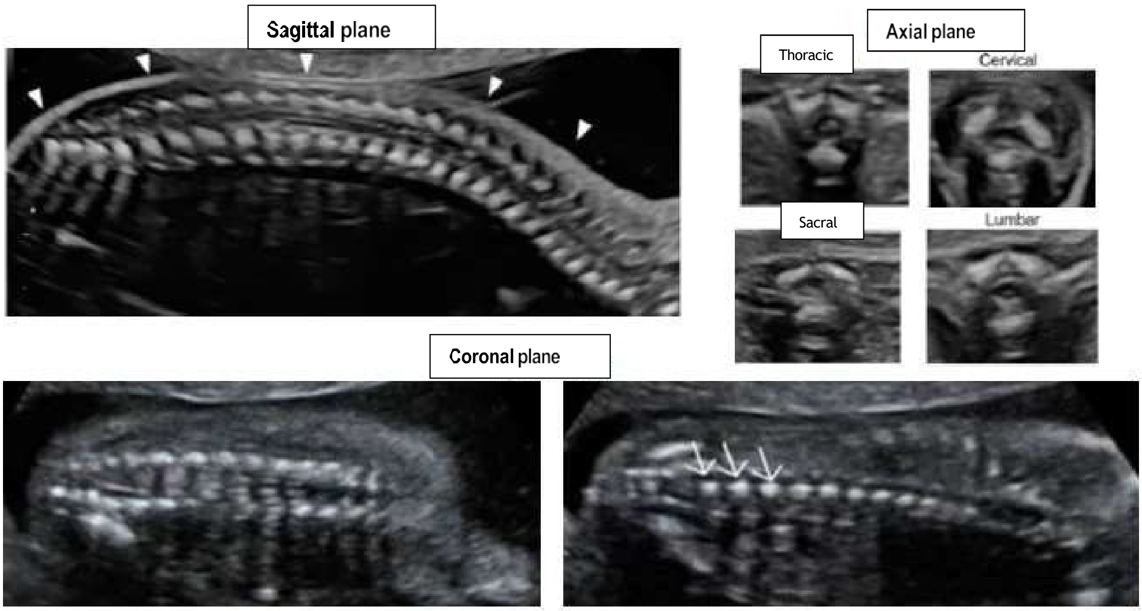
1. Basic intracranial sections in neurosonography



2. Included measurements in basic neurosonography

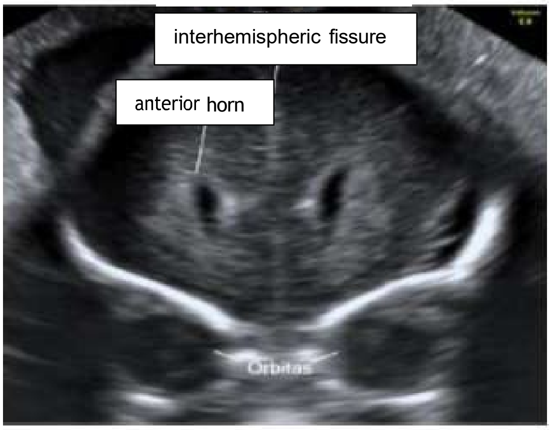


3. Spine examination in basic neurosonography

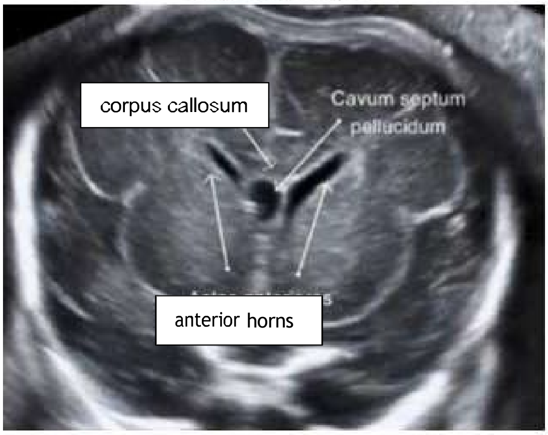


4. Advanced sections in neurosonography: coronal planes

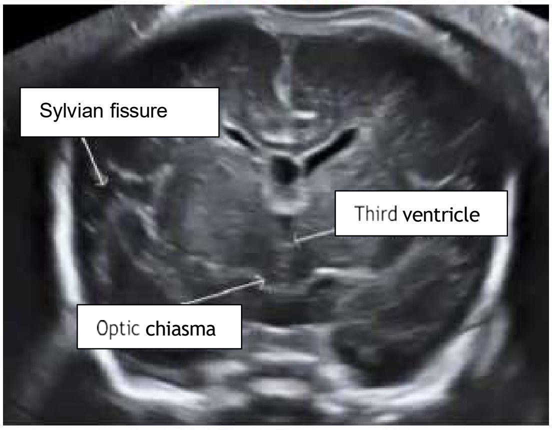
Transfrontal section



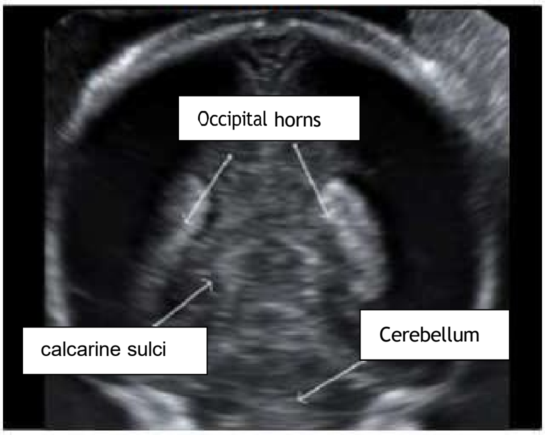
Transcaudal section



Transthalamic section



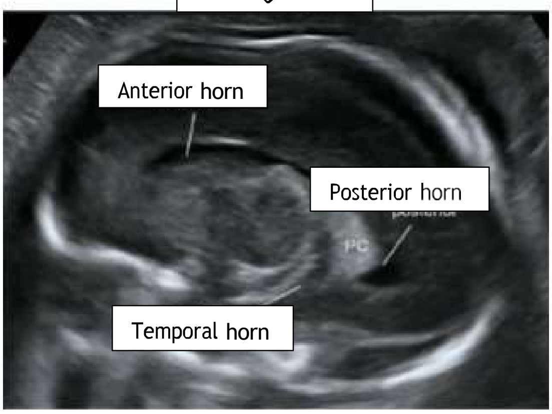
Transcerebellar section



5. Advanced sections in neurosonography: sagittal planes

Mid-sagittal

Parasagittal



6. Measurements included in advanced neurosonography

Third ventricle

Ratio of fourth ventricle

Length of corpus callosum

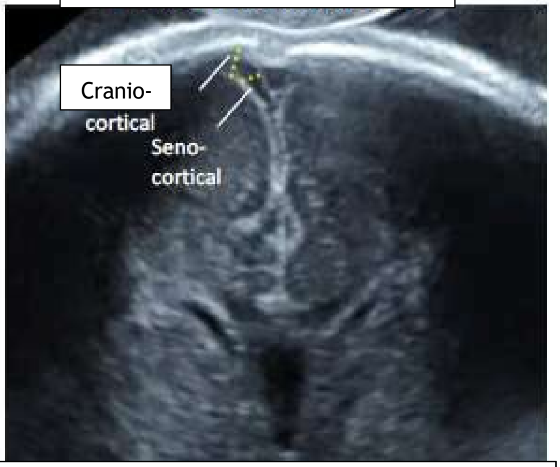
Thickness of corpus callosum

Height and AP diameter of vermis

AP diameter of bulge

Anterior horns

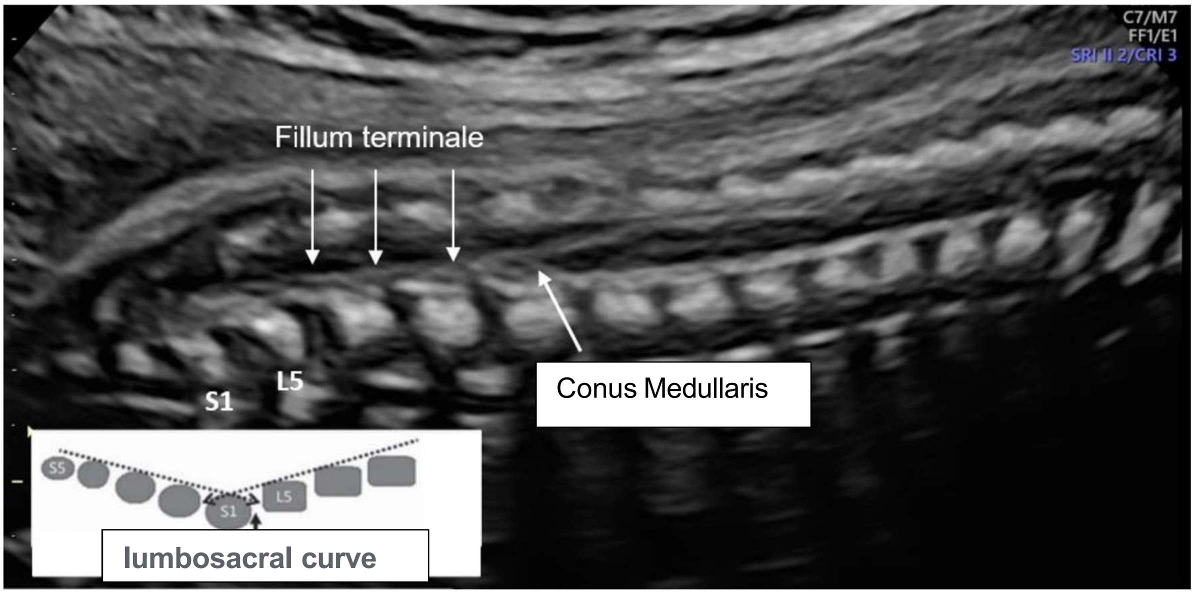
Subarachnoid space



Optic chiasma

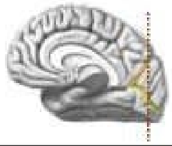
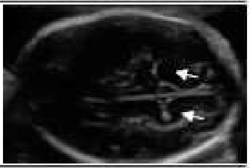
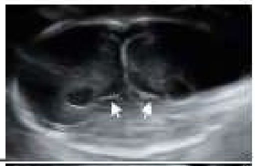
Measurement of ventricular atrium coronal section

7. Evaluation of the Conus Medullaris



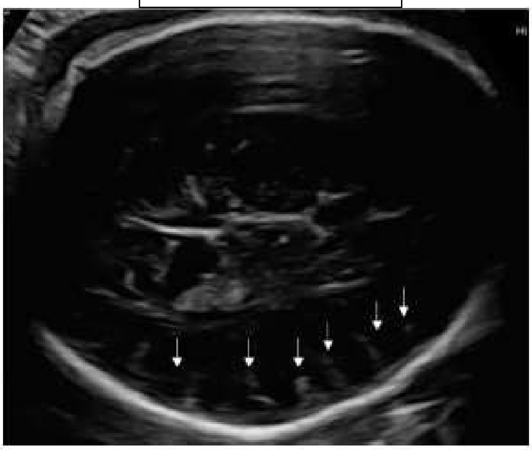
8. Cortical maturation

Toi et al. Ultrasound Obstet Gynecol 2004; 24: 706–15

Primary fissures and sulci	Appears	What you should see		
Sylvian	18	20		
Parieto-occipital	18	20		
Calcarine	20	22		
Cingulate	23	24		

Olfactory sulci

Circumvolutions

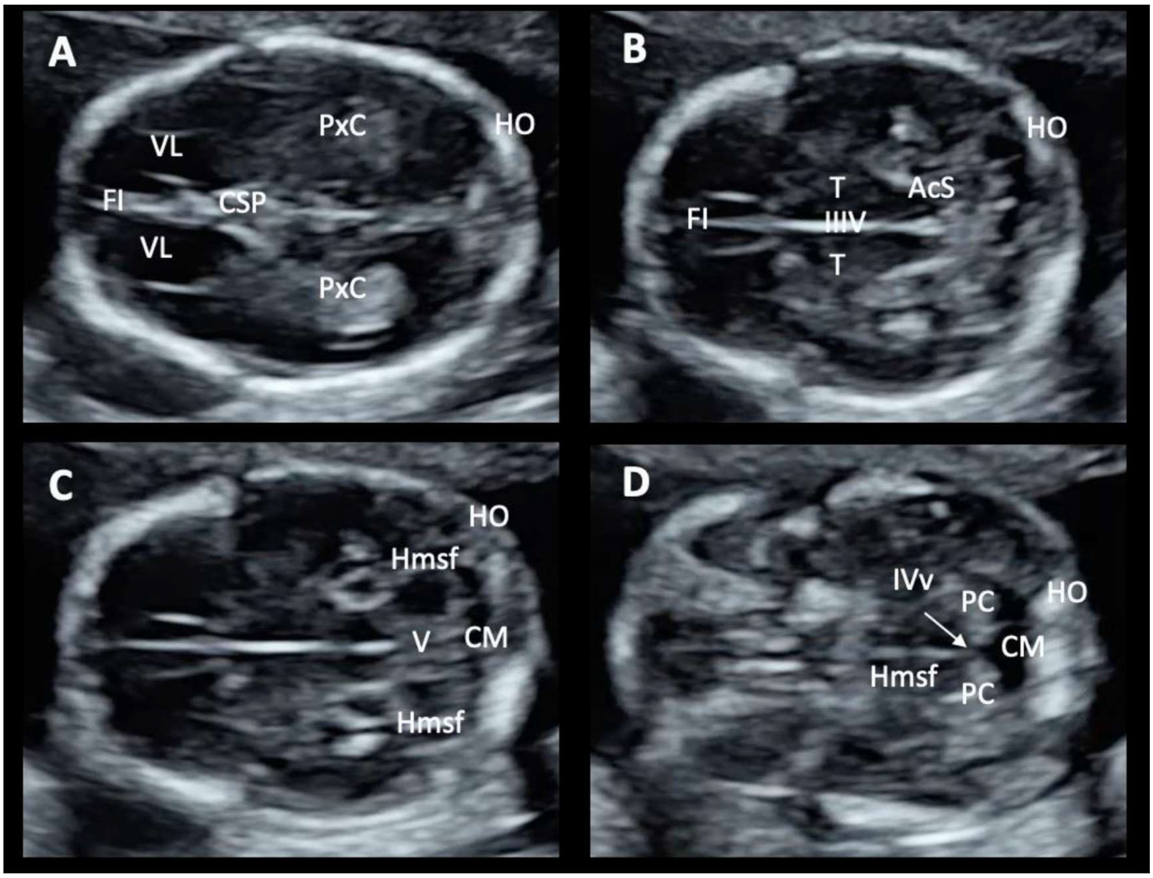


CNS evaluation in the axial plane in a 12 weeks foetus: Transabdominal acquisition. **A)** Transventricular section identifying interhemispheric fissure (IF); Cavum veli interpositi (Cvi); choroid plexus (PxC); Lateral ventricles (VL); future SylvianFissure (FS); Cranial bone (HO). **B)** Transthalamic section identifying choroid plexuses (PxC); Interhemispheric fissure (IF); Third ventricle (IIIV), Thalamus (T); Silvian Aqueduct (AcS); Occipital bone (HO).

9. Basic sonographic examination of the fetal brain at 11-13 weeks' gestation

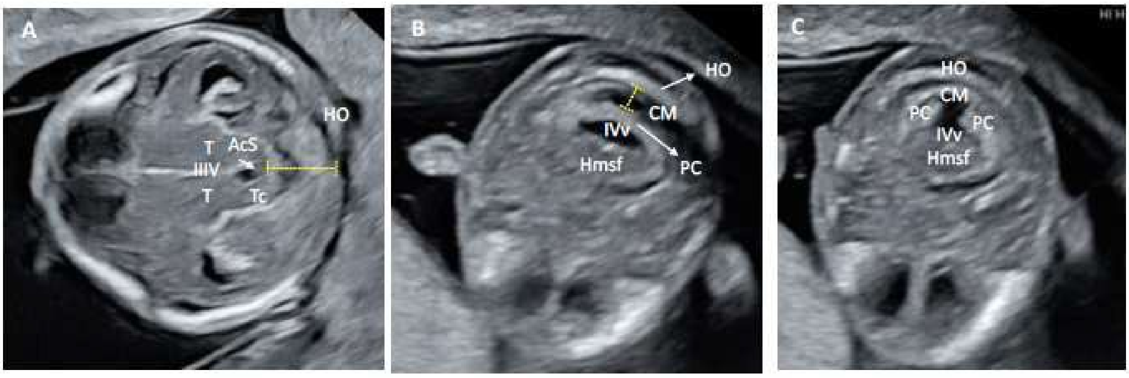
Evaluation of the CNS in an axial section of a 12 weeks foetus: Transabdominal acquisition. **A)** Transventricular plane identifying the interhemispheric fissure (FI); Cavum veli interpositi (Cvi); choroid plexus (PxC); Lateral Ventricles (VL), future Sylvian fissure (FS), cranial bone (HO). **B)** Transthalamic section identifying choroid plexus (PxC); interhemispheric fissure (FI); third ventricle (III V), Thalamus, Sylvian aqueduct (AcS); occipital bone (HO).

10. Basic sonographic examination of the fetal brain at 14-17 weeks' gestation



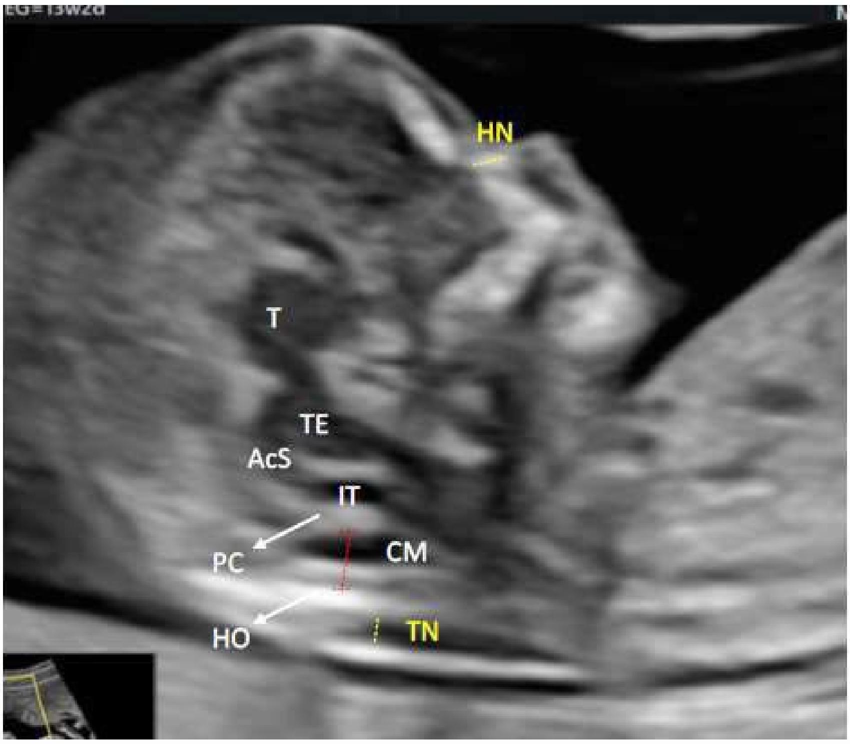
Evaluation of the CNS in an axial section of a 16 weeks foetus. A) Transventricular section identifying interhemispheric fissure (FI); choroid plexus (PxC); lateral ventricles (VL); future CSP (FS); occipital bone (HO). **B)** Transthalamic section identifying the interhemispheric fissure (FI); third ventricle (IIIV), thalamus (T), Sylvian aqueduct (AcS); occipital bone. **C)** Transcerebellar high section identifying both hemispheres of the cerebellum (Hmsf); superior vermis (V); Cisterna Magna (CM); occipital bone (HO). **D)** Transcerebellar low section identifying the communication between the cisterna magna (CM) with the fourth ventricle (IVv);both hemispheres of the cerebellum (Hmsf); fourth ventricle plexus (PC); cisterna magna (CM); occipital bone (HO).

11. Advanced sonographic examination of the fetal brain at first trimester – axial planes



Transthalamic section in the axial plane in a normal 13 weeks foetus: Transvaginal acquisition. **A)** Low transthalamic section identifying Third ventricle (IIIV); Silvian Aqueduct (AcS); Thalamus (T); Tectum (Tc); Occipital bone (HO). Yellow line demonstrates AcS – HO measurement. **B)** High transcerebellar section with the cerebellar hemispheres (Hmsf) adopting a dumbbell shape; Plexus of the fourth ventricle (PC); Occipital bone (HO); IV ventricle (IV V); Cisterna Magna (CM). The yellow line illustrates the CM measurement in the axial plane**. C)** Low transcerebellar section identifying normal communication <18 weeks between fourth V and CM.

12. Advanced neurosonographic examination of the fetal brain at first trimester – sagittal section



Normal sagittal section in a 13 weeks foetus: Transabdominal acquisition. The measurement of the nasal bone (HN) and nuchal translucency (TN) are identified. From top to bottom, the Thalamus (T) is identified; Brainstem (TE); Silvian Aqueduct (AcS); Intranuchal translucency or fourth ventricle (IT); Cisterna magna (CM); Plexus of the fourth ventricle (PC); Occipital bone (HO). Red line demonstrates the measurement of CM in the sagittal plane.

ANNEX 1. NORMALITY TABLES

1. Head circumference (mm)

Kurmanavicius et al. Br J Obstet Gynaecol, 1999; 106(2):126-35

Gestational age (weeks)		5th percentile	50th percentile	95th percentile	SD
12	59.7	72.1	84.5	7.6	
13	73.3	86.1	98.9	7.8	
14	86.7	99.9	113.1	8.0	
15	99.9	113.5	127.0	8.2	
16	112.9	126.8	140.7	8.5	
17	125.6	139.9	154.2	8.7	
18	138.1	152.7	167.4	8.9	
19	150.2	165.2	180.3	9.1	
20	162.1	177.5	192.9	9.4	
21	173.6	189.4	205.2	9.6	
22	184.9	201.0	217.1	9.8	
23	195.7	212.2	228.7	10.0	
24	206.2	223.1	240.0	10.3	
25	216.4	233.6	250.9	10.5	
26	226.1	243.7	261.3	10.7	
27	235.5	253.4	271.4	10.9	
28	244.4	262.7	281.1	11.2	
29	252.9	271.6	290.3	11.4	
30	260.9	280.0	299.1	11.6	
31	268.4	287.9	307.3	11.8	
32	275.5	295.3	315.1	12.1	
33	282.1	302.2	322.4	12.3	
34	288.1	308.7	329.2	12.5	
35	293.6	314.5	335.5	12.7	
36	298.6	319.9	341.2	13.0	
37	303.0	324.6	346.3	13.2	
38	306.8	328.8	350.9	13.4	
39	310.0	332.4	354.8	13.6	
40	312.6	335.4	358.2	13.9	
41	314.6	337.7	360.9	14.1	
42	315.9	339.4	363.0	14.3	

2. Transverse cerebellar diameter (mm)

Sherer et al. Ultrasound Obstet Gynecol. 2007; 29:32-37.

Gestational age (weeks)	5th percentile	50th percentile	95th percentile	SD
14	1.32	1.40	148	0.05
15	1.39	1.48	1.57	0.05
16	1.47	1.57	1.67	0.06
17	1.56	1.67	1.78	0.07
18	1.66	1.78	1.91	0.08
19	1.77	1.91	2.04	0.08
20	1.89	2.04	2.19	0.09
21	2.02	2.18	2.34	0.10
22	2.16	2.33	2.50	0.10
23	2.30	2.49	2.67	0.11
24	2.46	2.65	2.84	0.12
25	2.61	2.82	3.02	0.12
26	2.77	2.99	3.21	0.13
27	2.94	3.17	3.40	0.14
28	3.11	3.35	3.59	0.15
29	3.29	3.54	3.79	0.15
30	3.46	3.73	3.99	0.16
31	3.64	3.91	4.19	0.17
32	3.82	4.10	4.39	0.17
33	4.00	4.30	4.59	0.18
34	4.18	4.49	4.79	0.19
35	4.35	4.67	4.99	0.19
36	4.53	4.86	5.19	0.20
37	4.70	5.05	5.39	0.21
38	4.87	5.23	5.58	0.22
39	5.04	5.41	5.77	0.22
40	5.20	5.58	5.96	0.23
41	5.36	5.75	6.14	0.24

3. Third ventricle (mm)
Sari et al. Acta Radiol. 2005; 46(6):631-635

Gestational age (weeks)	3rd percentile	10th percentile	50th percentile	90th percentile	97th percentile
12	1.0	1.0	1.0	1.0	1.0
13	1.0	1.0	1.0	1.1	1.1
14	1.0	1.0	1.1	1.2	1.2
15	1.0	1.0	1.1	1.2	1.2
16	1.0	1.0	1.2	1.3	1.3
17	1.0	1.1	1.2	1.3	1.4
18	1.2	1.2	1.3	1.4	1.5
19	1.2	1.3	1.4	1.5	1.6
20	1.2	1.2	1.4	1.5	1.6
21	1.3	1.3	1.4	1.5	1.6
22	1.3	1.3	1.5	1.6	1.7
23	1.3	1.3	1.5	1.7	1.7
24	1.3	1.3	1.5	1.6	1.7
25	1.4	1.4	1.5	1.8	1.9
26	1.4	1.4	1.5	1.8	2.0
27	1.5	1.5	1.7	1.9	2.0
28	1.4	1.5	1.6	2.0	2.1
29	1.6	1.6	1.8	2.2	2.3
30	1.7	1.7	1.9	2.2	2.3
31	1.8	1.8	2.0	2.4	2.5
32	2.0	2.0	2.0	2.5	2.5
33	2.0	2.0	2.5	2.5	2.6
34	2.3	2.3	2.5	2.7	2.7
35	2.4	2.4	2.5	2.6	2.7
36	2.5	2.5	2.6	2.7	2.7
37	2.5	2.5	2.7	2.8	2.8
38	2.9	2.9	3.0	3.2	3.2
39	3.0	3.0	3.2	3.4	3.5
40	3.0	3.1	3.4	3.6	3.6

4. Corpus callosum length (mm)

Cignini et al. J Ultrasound Med. 2014; 33(6):1065-1078

Gestational age (weeks)	Inferior 95% CI	Mean	Superior 95% CI	SD
19	17.45	18.78	20.10	1.33
20	19.59	21.02	22.46	1.43
21	21.66	23.20	24.74	1.54
22	23.65	25.30	26.94	1.65
23	25.56	27.31	29.07	1.76
24	27.38	29.24	31.10	1.86
25	29.10	31.07	33.04	1.97
26	30.73	32.81	34.89	2.08
27	32.26	34.45	36.63	2.18
28	33.68	35.97	38.26	2.29
29	34.98	37.38	39.78	2.40
30	36.17	36.68	41.18	2.51
31	37.23	39.85	42.46	2.61
32	38.17	40.89	43.61	2.72
33	38.97	41.80	44.62	2.83
34	39.63	42.56	45.50	2.94
35	40.14	43.19	46.23	3.04
36	40.51	43.66	46.81	3.15
37	40.72	43.98	47.24	3.26

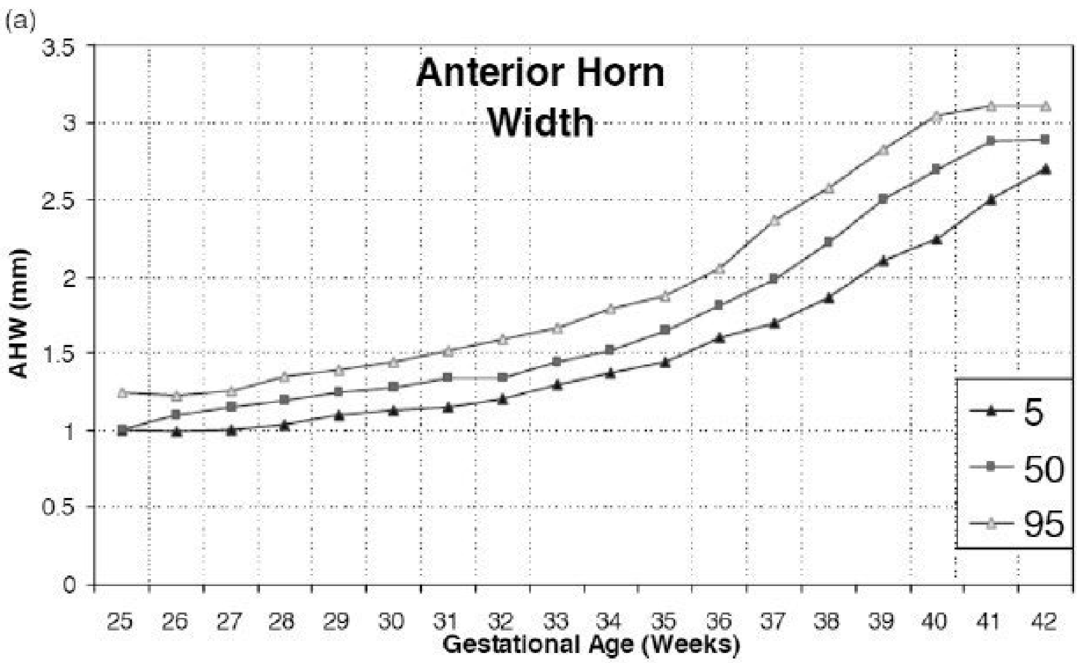
5. Cerebellar vermis height (mm)

Cignini et al. Plos One. 2016; 11(1): e0147528

Gestational age (weeks)	Inferior 95% CI	Mean	Superior 95% CI	SD
20	10.69	11.27	11.85	0.58
21	11.29	11.96	12.63	0.67
22	11.95	12.71	13.47	0.76
23	12.65	13.50	14.35	0.85
24	13.38	14.32	15.26	0.94
25	14.13	15.16	16.19	1.03
26	14.89	16.01	17.13	1.12
27	15.64	16.85	18.06	1.21
28	16.37	17.67	18.97	1.30
29	17.08	18.47	19.86	1.39
30	17.74	19.22	20.71	1.48
31	18.34	19.91	21.48	1.57
32	18.88	20.54	22.20	1.66
33	19.34	21.09	22.84	1.75
34	19.70	21.54	23.38	1.84
35	19.97	21.90	23.82	1.93

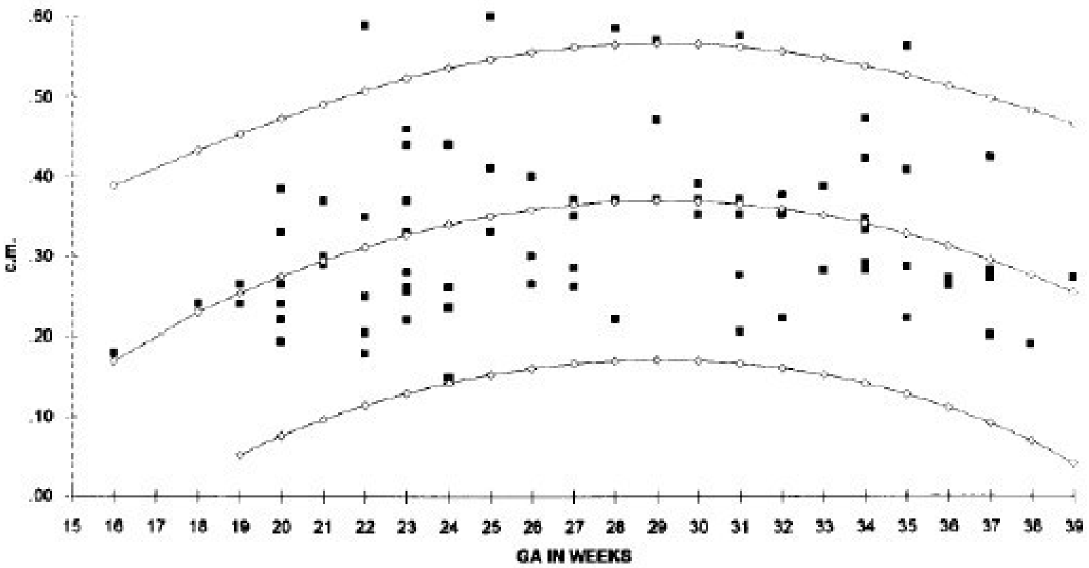
6. Anterior horn of the lateral ventricles

Sondhi et al. Acta Paediatr. 2008; 97(6):738-744



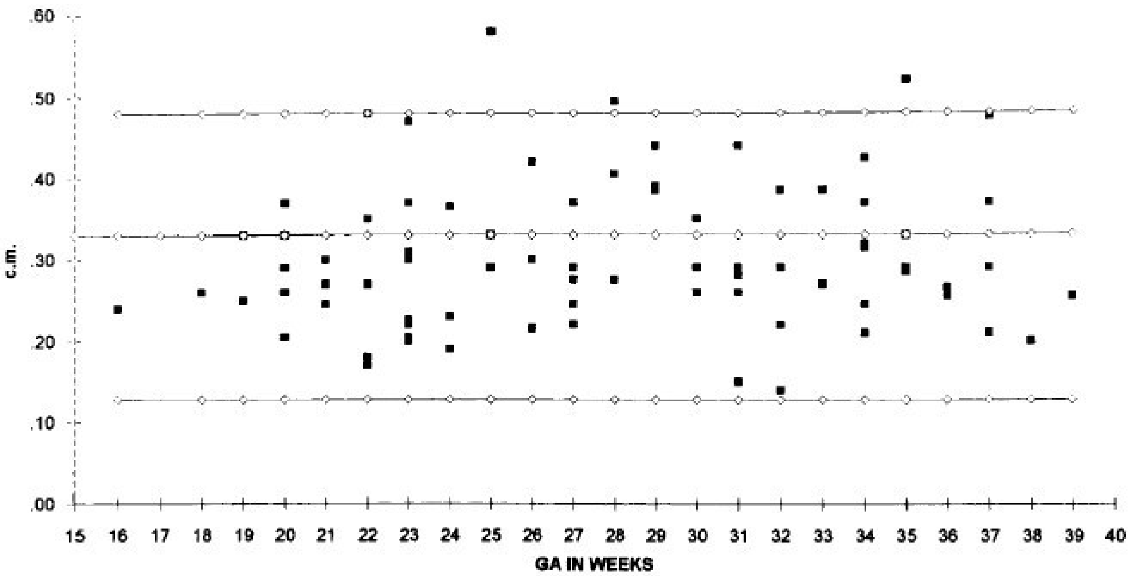
7. Cranio-cortical space

Malinger et al. Prenat Diagn. 2000; 20:890-893



8. Sinus-cortical space

Malinger et al. Prenat Diagn. 2000; 20:890-893



9. Transverse diameter of the cavum septi pellucidi (mm)

Falco P et al. Ultrasound Obstet Gynecol, 2000.16(6):549-53

Gestational age (weeks)	- 2 SD	Mean	+ 2 SD
15	1.8	2.8	3.7
16	2.1	3.2	4.2
17	2.4	3.5	4.6
18	2.7	3.9	5.0
19	2.9	4.2	5.5
20	3.2	4.5	5.8
21	3.4	4.8	6.2
22	3.6	5.1	6.6
23	3.7	5.3	6.9
24	3.9	5.5	7.2
25	4.0	5.8	7.5
26	4.1	5.9	7.8
27	4.2	6.1	8.0
28	4.3	6.3	8.3
29	4.3	6.4	8.5
30	4.3	6.5	8.7
31	4.4	6.6	8.8
32	4.3	6.7	9.0
33	4.3	6.7	9.1
34	4.3	6.7	9.2
35	4.2	6.7	9.3
36	4.1	6.7	9.4
37	4.0	6.7	9.4
38	3.8	6.6	9.4
39	3.7	6.6	9.5
40	3.5	6.5	9.4
41	3.3	6.4	9.4

10. Ratio (AP diameter/longitudinal) of the cavum septi pellucidi (mm)
Karl Ultrasound Obstet Gynecol 2017; 50: 336–341

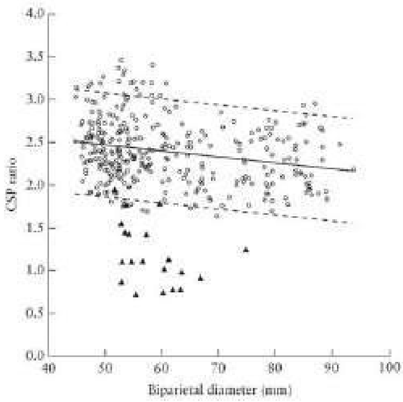
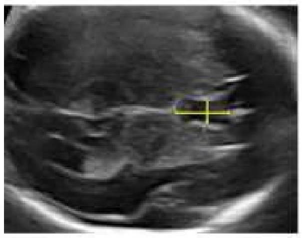


Figure 5 Individual measurements of length-to-width ratio of cavum septi pellucidi (CSP) in normal fetuses (○), with reference range (median and 5th and 95th centiles), and in 20 fetuses with partial agenesis of the corpus callosum (▲), in relation to biparietal diameter.

$$\text{CSP ratio} = \frac{\text{CC length}}{\text{CC width}}$$

95% P-ACC → CSP ratio < p5
80% p-ACC → CSP ratio < 1.5
40% p-ACC → CSP ratio < 1



Cavum Normal

Partial agenesis of corpus callosum (p-ACC)

11. Anteroposterior diameter of the pons (mm)

Mirlesse V et al. Prenat Diagn, 2010 30(8):739-45

Gestational age (weeks)	5th centile	10th centile	25th centile	Mean	75th centile	90th centile	95th centile
21	6.7	7.0	7.4	7.9	8.4	8.9	9.1
22	6.9	7.3	7.8	8.4	9.0	9.5	9.8
23	7.2	7.6	8.2	8.8	9.5	10.1	10.4
24	7.6	8.0	8.6	9.3	10.0	10.6	11.0
25	8.1	8.5	9.2	9.9	10.6	11.2	11.6
26	8.5	9.0	9.6	10.4	11.1	11.7	12.1
27	8.9	9.4	10.1	10.8	11.6	12.2	12.6
28	9.4	9.8	10.5	11.3	12.3	12.7	13.1
29	9.8	10.2	10.9	11.7	12.4	13.2	13.5
30	10.2	10.6	11.3	12.1	12.9	13.6	14.0
31	10.5	11.0	11.7	12.5	13.3	14.0	14.4
32	10.8	11.3	12.0	12.9	13.7	14.5	14.9
33	11.0	11.5	12.3	13.3	14.2	15.1	15.6
34	11.2	11.8	12.7	13.7	14.7	15.7	16.3
35	11.4	12.0	13.0	14.1	15.3	16.3	16.9
36	11.6	12.3	13.3	14.5	15.8	16.9	17.6

12. Optic chiasma

Viñals et al. Ultrasound Obstet Gynecol. 2016; 48:733-738

Gestational age (weeks)	3rd centile	5th centile	50th centile	95th centile	97th centile
21	5.6	5.6	6.0	6.3	6.3
22	5.6	5.6	6.0	7.5	7.5
23	5.4	5.48	6.4	7.74	7.9
24	5.7	5.74	6.5	7.96	8.0
25	5.5	5.56	6.8	9.0	10.0
26	5.7	5.7	7.0	8.1	8.1
27	6.8	6.8	7.0	8.1	8.1
28	7.1	7.1	7.85	8.6	8.6
29	6.9	6.9	8.2	8.7	8.7

13. A) Measure of cisterna magna between 11.0-14.1 weeks of pregnancy

Pauta M et al., Fetal Diagn Ther 2019

CRL (mm)	5th centile	10th centile	50th centile	90th centile	95th centile
45	0.94	1.2	1.66	2.28	2.44
46	0.96	1.2	1.67	2.30	2.48
47	0.98	1.21	1.69	2.32	2.53
48	1.00	1.22	1.70	2.34	2.57
49	1.03	1.23	1.72	2.37	2.61
50	1.05	1.25	1.74	2.39	2.65
51	1.07	1.26	1.76	2.42	2.70
52	1.10	1.28	1.78	2.45	2.74
53	1.13	1.30	1.81	2.49	2.80
54	1.15	1.32	1.84	2.54	2.85
55	1.18	1.34	1.87	2.58	2.91
56	1.21	1.37	1.91	2.63	2.97
57	1.24	1.40	1.94	2.68	3.02
58	1.27	1.42	1.98	2.73	3.08
59	1.29	1.44	2.01	2.77	3.14
60	1.32	1.47	2.04	2.81	3.19
61	1.34	1.49	2.06	2.86	3.25
62	1.36	1.51	2.10	2.90	3.30
63	1.39	1.54	2.13	2.95	3.36
64	1.41	1.57	2.16	3.00	3.42
65	1.44	1.59	2.20	3.05	3.48
66	1.46	1.62	2.23	3.10	3.55
67	1.49	1.64	2.26	3.15	3.61
68	1.51	1.66	2.29	3.20	3.67
69	1.53	1.68	2.32	3.25	3.74
70	1.55	1.70	2.34	3.29	3.80
71	1.57	1.72	2.36	3.33	3.86
72	1.59	1.73	2.39	3.37	3.92
73	1.60	1.75	2.41	3.40	3.97
74	1.62	1.76	2.43	3.44	4.02
75	1.63	1.78	2.46	3.48	4.07
76	1.65	1.80	2.48	3.52	4.12
77	1.66	1.81	2.51	3.56	4.17
78	1.67	1.84	2.54	3.6	4.22
79	1.69	1.86	2.57	3.65	4.28
80	1.70	1.88	2.61	3.7	4.33
81	1.72	1.91	2.64	3.75	4.40
82	1.74	1.93	2.68	3.81	4.46

13. B) Measure of cisterna magna between 15-32 weeks of pregnancy

Napolitano M et al., UOG 2020.

Gestational age (weeks)	3rd centile	5th centile	50th centile	95th centile	97th centile
15+0	1.71	1.82	2.82	4.36	4.64
16+0	1.96	2.08	3.2	4.92	5.24
17+0	2.19	2.33	3,56	5.44	5.79
18+0	2.41	2.56	3.59	5.92	6.29
19+0	2.61	2.77	4.2	6.36	6.75
20+0	2.8	2.97	4.48	6.67	7.17
21+0	2.97	3.15	4.73	7.12	7.55
22+0	3.12	3.31	4.97	7.45	7.90
23+0	3.26	3.46	5.18	7.75	8.21
24+0	3.39	3.6	5.37	8.02	8.50
25+0	3.51	3.72	5.55	8.27	8.76
26+0	3.62	3.83	5.71	8.50	8.99
27+0	3.72	3.94	5.85	8.70	9.21
28+0	3.81	4.03	5.99	8.89	9.41
29+0	3.9	4.12	6.11	9.06	9.59
30+0	3.97	4.20	6.22	9.22	9.75
31+9	4.04	4.27	6.33	9.36	9.90
32+0	4.11	4.34	6.42	9.49	10.04
33+0	4.17	4.40	6.51	9.62	10.17
34+0	4.22	4.46	6.59	9.73	10.28
35+0	4.27	4.51	6.66	9.83	10.39
36+0	4.32	4.56	6.73	9.92	10.49

ANNEX 1. Ultrasound markers of CNS pathology

- Atrium =/> 10 mm
- Cisterna magna =/> 10 mm.
- Cisterna magna < 2 mm.
- Morphologic alterations or absence of the cavum septi pellucidi
- Cranial morphology alterations
- Alteration in cephalic biometrics (standard deviation below or above 2)
- Intracranial cystic structures
- Morphological alteration of the anterior horn of the lateral ventricles
- Morphological or biometric alteration of the cerebellum
- Echogenic alteration of the cerebral parenchyma
- Circumvolution alteration

ANNEX 2: teratogens that affect neurogenesis

- Alcohol (doses greater than 2 units, twice a week).
- Drugs of abuse: cocaine, opioids and design drugs.
- Drugs:
    a. Anticoagulants: Acenocoumarol, Warfarin.
    b. Antiepileptics: valproic acid (NTD), Carbamazepine (NTD), phenobarbital (malf. CNS). Topiramate, lamotrigine, levetiracetam and gabapentin are safe drugs.
    c. Antimetabolites and Cytostatic agents.
    d. Retinoids.
- Exposure to high doses of mercury (> 20 µg/l in urine): intake greater than 50 g per week of fish with high content (swordfish, red tuna and pike).
- High dose radiation exposure (=> 5 Rads): abdominal, pelvic or lumbosacral spine CT or with barium enema, radiotherapy for oncological treatment, etc.

ANNEX 3: Complementary tables

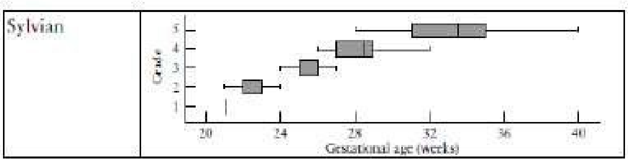
1. Anteroposterior vermis diameter

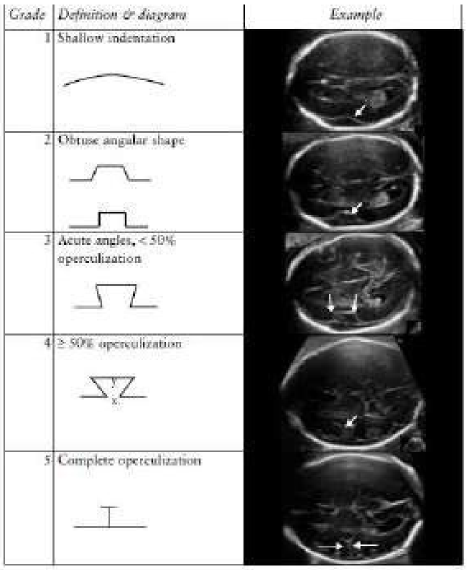
Leibovitz Ultrasound Obstet Gynecol 2014; 44:575-580

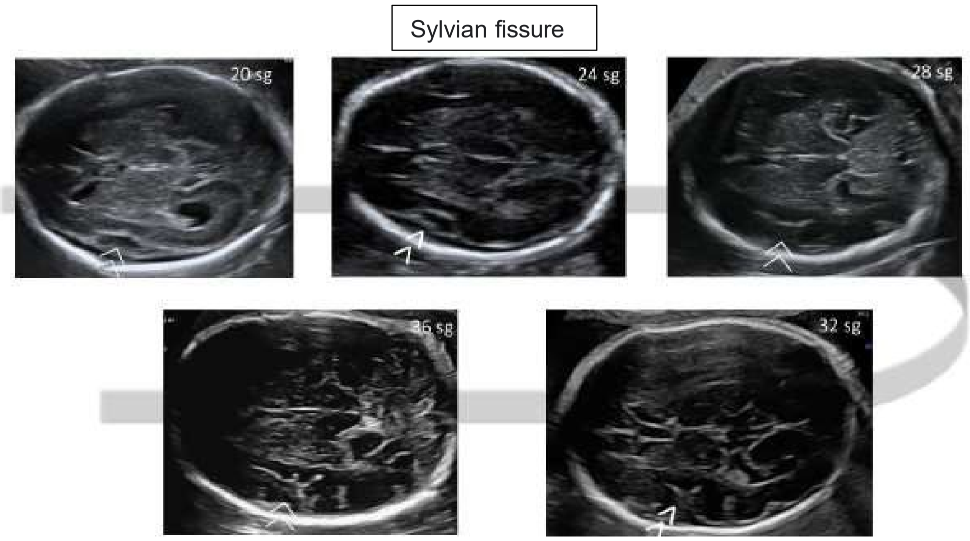
Gestational Week	Number of fetuses	Anteroposterior Vermian Diameter (cm)		
		Mean	Mean-2SD*	Mean+2SD*
15w+1d – 16w+0d	13	0.42	0.34	0.50
16w+1d – 18w+0d	10	0.43	0.29	0.57
18w+1d – 20w+0d	11	0.58	0.37	0.80
20w+1d – 22w+0d	10	0.76	0.57	0.95
22w+1d – 23w+0d	14	0.82	0.59	1.06
23w+1d – 24w+0d	55	0.87	0.63	1.10
24w+1d – 25w+0d	19	0.90	0.70	1.10
25w+1d – 27w+0d	11	0.98	0.72	1.24
27w+1d – 29w+0d	12	1.08	0.75	1.40
29w+1d – 31w+0d	15	1.19	0.81	1.56
31w+1d – 33w+0d	33	1.26	0.82	1.69
33w+1d – 35w+0d	15	1.33	0.93	1.73

2. Estimated evolution of the Sylvian fissure

Pistorius et al. Ultrasound Obstet Gynecol 2010; 36:700-8







3. Estimated evolution of the Parieto-occipital, Calcarine and Cingulate sulci

Pistorius et al. Ultrasound Obstet Gynecol 2010; 36:700-8