



# Fetal Syndromes - Diagnosis and Management using 3D-4D Sonography

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## INTRODUCTION

Three-dimensional ultrasound (3D US) has become a very powerful and progressively popular ultrasound technique in the last three decades (1989-2019).<sup>1</sup> Today, it is used in regular prenatal assessment in everyday clinical practice as additional method to two-dimensional ultrasound (2D US).

Over the time, 3D US equipment and technology dramatically enhanced the quality of images, shortened the time of acquisition, and at the same time improved our ability to assess and visualize the normal and to detect abnormal development of a embryo and fetus in utero.<sup>1-13</sup>

Acquired volumes can be stored, re-loaded and re-evaluated at any time. Different imaging modalities can be applied for more detailed survey. Particular region of interest such as small defects can be displayed in the ideal plane that sometimes cannot be achieved by conventional 2D US technique.<sup>3,4,9,10</sup> This increases the accuracy of the detection and diagnosis of various malformations and fetal syndromes.<sup>2</sup>

Diagnosing fetal syndromes is a major challenge both in the prenatal and postnatal period.<sup>2</sup> The syndrome is like a big puzzle whose parts need to be carefully assembled to get a whole picture.<sup>2</sup> Good multidisciplinary approach, proper communication and collaboration with parents and all physician's involved are required in the diagnostic process. All available resources and tools are needed to increase diagnostic precision in the prenatal diagnosis of fetal syndromes.<sup>4</sup>

The introduction of highly specialized software systems for three-dimensional and four dimensional ultrasound (3D/ 4D US) enable detailed assessment of the fetal anatomy and the evaluation of the dynamics of structural and functional development of the fetus in real-time.<sup>2,4</sup> Owing to this, clinical practice has also gained new functional tests such as the Kurjak's antenatal neurodevelopmental test (KANET); named in honor of its author; used for the evaluation of fetal brain function.<sup>2-4,24-31</sup> Antenatal detection and diagnosis of fetal anomalies and syndromes is shifted from the 2nd to the 1st trimester of preg-

nancy<sup>2,4,10-12</sup> partly due to technological advances and benefits of imaging quality with 3D/4D US technology, as reported by many authors.

### When to suspect a syndrome? Defining a syndrome

Common terminology used to describe fetal syndromes can sometimes be confusing. A wide variety of terms and synonyms are used. Sometimes, there is a lack of good definitions of how many major and minor criteria should be present to diagnose each syndrome. The difference in prenatal detection rates for each region or country can be partly explained by differences in screening policies and follow-up practices, as well as the possible variations in practitioners' skills and available equipment.<sup>4</sup>

Detecting one anomaly should always raise doubts about the presence of other anomalies and should therefore serve as a trigger that will encourage us to further investigate and raise awareness of the possible existence of syndromes.<sup>2,4-9</sup>

Other triggers can be: positive personal or family history of syndrome, or a child born with a syndrome, consanguinity, exposure to teratogens (drugs, radiation) and other harmful agents (e.g. infections, TORCH, Zika). Genetic syndromes in children are most commonly diagnosed on the basis of craniofacial dysmorphic features.<sup>5,8,34,35</sup>

**Clinical dysmorphology** is a branch of clinical genetics devoted to the study of abnormal human development, with emphasis on syndromes that reflect as changes in morphology of the body.<sup>32-35</sup> There are several pathophysiological mechanisms of poor fetal development (fetal maldevelopment) and these include: malformation, deformation, disruption or dysplasia.

**Malformations:** due to abnormal embryonic development, commonly defined as single, localized poor formation of tissue, which has genetic etiology. This anomaly then raises a number of other defects (e.g. anencephaly). The recurrence risk for malformations generally range from 1% to 5%.<sup>7</sup>

**Deformation** is a result of extrinsic mechanical forces on otherwise normal tissue, deforming it (e.g. abnormal faces, pulmonary hypoplasia and limb contractures that result from pro-

longed oligohydramnios or primary renal agenesis in Potter syndrome.

**Disruption** results from an extrinsic insult that destroys normal tissue, altering the formation of affected structure (e.g. amniotic band syndrome).

**Dysplasia:** when the primary defect is absence of normal organization of cells into tissue, we speak of dysplasia (e.g. achondroplasia).

Any of the mechanisms of fetal maldevelopment can result in altered morphology of fetal organs and systems, which can result in the formation of a fetal syndrome if many organs are involved. The term "**Syndrome**" originates from ancient Greek meaning "running together"<sup>39</sup>, representing a specific pattern of associated signs, symptoms, dysmorphic features and/or behaviors occurring together in the same person.<sup>2,6,40</sup>

We are currently aware of thousands of syndromes and their variants 4-8 and this number is rising every day. It is particularly important to learn how to identify and find the right diagnosis in severe cases. In fact, it is estimated for the human genome to have about 80,000 genes, it should probably be discovered as many rare syndromes. Over 300 syndromes are associated with some type of facial anomaly.<sup>2,4,8,9</sup> The incidence of fetal syndrome varies.<sup>8</sup> It is estimated that the real occurrence of most syndromes is likely to be much greater, but due to natural selection there is no further development.

Terminology used to describe fetal syndrome can sometimes be very confusing. Namely, different terms and their synonyms are used at the same time. In some cases, it is not well defined how important the main criteria would be, and how many of the subordinate criteria should appear for setting up a particular diagnosis. It is important to point out the difference and to distinguish between the terms: syndrome, sequences and associations.

**Syndrome:** the occurrence of anomalies, multiple malformations and / or sequences, together in a recognizable sample. They are mainly the result of one, usually genetic abnormality and have the same etiology; as for example in trisomy 21 (Down Syndrome).

**Sequence:** The sequence occurs when one developmental disadvantage results in a cascade of secondary deficiencies that cause tertiary and so on, such as: Pierre-Robin sequence: primary defect is mandibular hypoplasia, retrognathia that causes glossophtosis, secondary defect, which further disturbs normal, physiological closure of the soft palate and causes a tertiary defect: the palathoshisis of soft palate. (retrognathia-> glossophtosis-> palathoshisis). In 25% of cases, the fetus will have Stikler syndrome.<sup>41</sup> The sequence may be an isolated finding, associated with some other defect or a part of a syndrome.

**Association:** Connecting random combinations of inherited anomalies that may be the result of numerous pathogenic genetic factors (unlike syndrome). Example: VATER / VACTERL association (acronym for) (anomalies of the vertebrae - V; anal atresia- A; cardiac defects- C; trachea - oesophageal fistula - TE, kidney anomalies - R, anomalies of the limbs - L). This association is typically defined by the presence of at least three of the above-mentioned congenital anomalies.<sup>5-8</sup>

Some fetal syndromes can be detected prenatally while others cannot; some are expressed prenatally while others are not.<sup>4</sup> In many cases definitive diagnosis can be made postnatally, many years later.<sup>2</sup> It is estimated that about 1 out of 10 people, or a total of 30 million, live with rare diseases in the United States. Globally, it is about 350 million people who have one of over 7,000 known rare diseases. As already mentioned, the way to diagnosing these children is pretty long and painstaking. It takes about seven years from symptom to diagnosis, and in that period the children see at least seven doctors.<sup>35-37</sup> All this has a negative impact on the child and his family, representing emotional and financial burden on everyone, and negatively influencing and affecting the quality of life and prognosis of the child. The list of possible differential diagnoses (DD) of fetal syndromes is extensive.

Currently there are several types of online databases that can help in detecting and identifying certain patterns of anomaly in syndromes, sequences or associations. The most commonly used medical databases are: Online Mendelian Inheritance in Man (OMIM), POSSUM-web, London Dysmorphology Database (LDDDB) as part of a larger database: London Medical Database (LMD). Some of the larger databases combined their data to enrich them, allowing users a faster and more accurate search. As part

of the new FDNA (The Facial Dysmorphology Novel Analysis) database, LMD database access is integrated in combination with a brand new technology called Face2Gene.<sup>35-37</sup> Namely, with the help of the Face2Gene database, genetic search facilitates detection of dysmorphic facial features and recognizes human malformations from face photos to present a list of the most commonly used syndromes.<sup>37</sup> Patient symptoms, features and genomic data are analyzed in the network of thousands of genetic experts around the world, providing scientific insights to improve and accelerate diagnosis and therapy. Notwithstanding, all of these network databases are based mainly on the data, symptoms and features found in newborns and children, and are not directly relevant to the recognition of syndromes in the antenatal period. *Phenotype online database*<sup>38</sup> is a database for specialists dealing with prenatal detection of syndromes using ultrasonic technology so that all prenatal sonographic markers and features are involved in data analysis. Data is easily accessed, ultrasonic markers and symptoms are combined, anomaly list that may still appear within the diagnosis is obtained, and some features that are identified in the parents can even be included.<sup>38</sup>

#### Clinical application of 3D ultrasound in the antenatal detection of fetal syndromes.

The systematic approach (according to guidelines) to fetal assessment by two-dimensional (2D) ultrasound is still a gold standard and should always be followed to avoid mistakes. When evaluating structures such as the face or brain, the advanced 3D / 4D US modalities gives a whole range of additional information that cannot be obtained by 2D technique.<sup>3,16</sup>

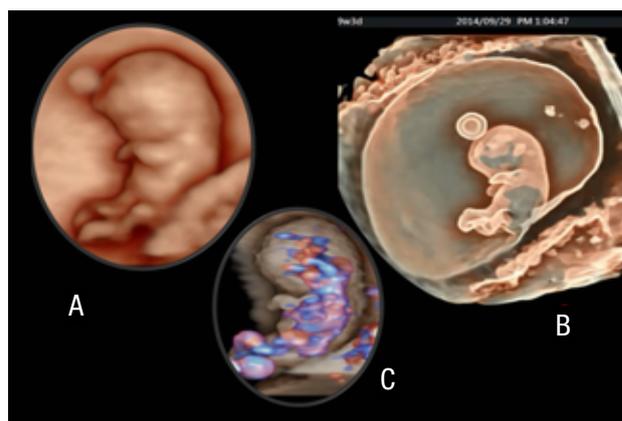
Systematic review of 525 articles on 3D / 4D ultrasound by Goncalves et al.<sup>42</sup>, found that 3D US provides additional diagnostic information for diagnosing facial anomalies, particularly facial clefts, neural tube defects and skeletal malformation. Merz and Welter<sup>43</sup> examined a large group of 3472 fetuses with 2D and 3D ultrasound intended to detect fetal anomalies. The total number of detected defects was 1012. Comparing the 2D and 3D techniques, 3D US showed superiority with 60.8% of detected anomalies, which concerned more favorable visualization of target areas in different views (e.g. Multiplanar, Surface View).<sup>43,44</sup>

Only in recent years high-frequency probes and high-resolution displays called High-Definition Live (HDlive) technology have revolutionized the quality of sonographic imaging. 3D HDlive mode of display uses the advantages of 'shadowing effects' to enhance the visualization of the desired details in the image.<sup>44</sup> Unlike the conventional 3D surface display that uses a fixed virtual light source and reflects light from the surface of the skin, HDlive modality calculates the spread of light through the skin and tissue.<sup>10,12,16,31,44</sup> Shadows are created where the light was moved through the dense tissue. The virtual light source can be easily changed and directed from any angle and manipulated in this way to improve segmentation of the tissue structure, define precise contours and highlight important clinical details.<sup>10,44</sup> This is suitable for observing the surfaces, especially in the face area.<sup>46-50</sup> Any suspicious surface or malformation can be shown and investigated much better than conventional 2D ultrasound.

Changing the virtual light angle, it can be perfectly adjusted to highlight something and thus gain a depth perception in the visualization of an area of interest that may be anomalous. Transparent (translucent) effect is obtained if the light source is located behind the object.<sup>44,49</sup> Improved smoothing performance is obtained by applying volume-speckle reduction imaging (V-SRI) on high-quality multi-planar 3D / 4D images using volume (voxel) compared to traditional single-slice imaging (pixels).

The 3D HDlive mode can be used successfully throughout the entire pregnancy.<sup>16</sup> In the first trimester, normal and abnormal development of embryos and fetuses can be monitored and evaluated to the finest details. Only few years ago, new applications in 3D ultrasound called HDlive Silhouette (Flow) and Flow (Flow) were launched. HDlive Silhouette revealed the clinical significance of simultaneous imaging of internal morphology through the outer surface in a transparent manner (Figure 1,2).

This helps in determining the exact localization and volume of internal structures that may be hyperechoic as bone, or hypoechoic structures (Figure 2 A-D) such as cysts.<sup>49,50</sup> HDlive Flow technology adds more spatial resolution to conventional angiogram. Combining both techniques at the same time (HDlive Silhouette and



**Figure 1. A-C**

1A, B: 3D HDlive Silhouette fetal presentation at 9 + 3 gestational weeks. With the different intensity of Silhouette mode, we get more or less transparent picture in "see through fashion".

1A: The surface details.

1B: See differences in the details of the previous picture (A), see the details of the internal organs filled with fluid, such as a ventricular system in the brain, an egg yolk, etc.

1C: 3D HDlive Silhouette and Flow showing the same fetus as above. Facing the details of early fetal circulation in the brain, heart and umbilical cord.<sup>16</sup>

Flow) can display the exact location of the vascular structure within the body and organs and accurately determine the direction of the vascular flow (3D HDlive bidirectional power Doppler) (Figure 1C). These two new applications allow visualization of blood circulation within the fetus, the various parts of the fetal brain and lung flow.<sup>49,50</sup> Using different color shades of the skin by the HDlive application, brought a great news as it gives the impression of a living fetus with even more realistic and impressive imaging illustrations.<sup>16,49-51</sup> Many of the above-mentioned innovations in 3D / 4D ultrasound applications are particularly useful in prenatal detection and visualization of fetal anomalies and discrete details.<sup>52-55</sup>

Together with this sonographic diagnostic tools, we should also mention something new that will greatly assist in accelerating postnatal recognition of rare syndromes. The fascinating combination of science, research and new technologies is jointly implemented in a new research program titled 'Give Face Syndrome'. Facial Dysmorphology Novel Analysis (FDNA) is a new technology that facilitates the detection of dysmorphic features and recognizable patterns of human malformations in newborns, children and adults, to provide comprehensive and updated neurogenetic references available to everyone online.<sup>36,37,55</sup>



Figure 2 A-D

A-D: The same fetus at 9 weeks of gestation. Note the difference in imaging details.

A: VCI mode- sagittal image of the fetus- increased nuchal translucency (NT)

B-D: 3D HDlive Silhouette images with the increasing silhouette mode from B to D. Ending up with the transparent view. Notice the increased accumulation of the fluid in the neck area and the cystic structures within: Cystic hygroma coli.<sup>16</sup>

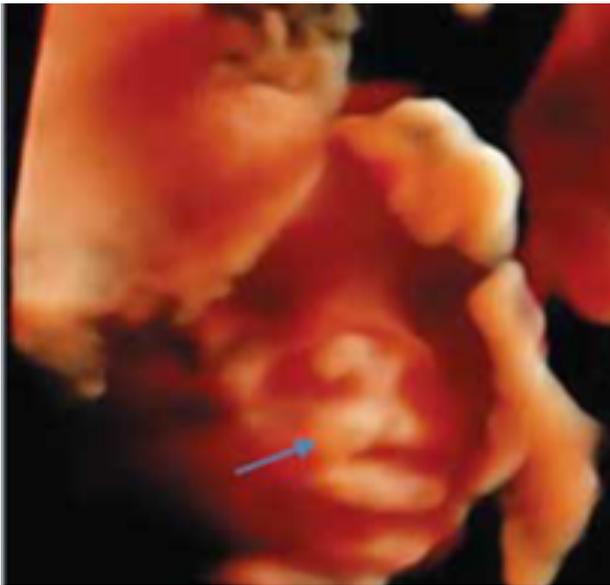
### Syndromes featuring primarily craniofacial anomalies

In this section, it will be described various syndromes featuring primarily craniofacial anomalies and their associated defects that can be detected by standard 2D ultrasound techniques, and demonstrate how the recognition is enhanced by advanced 3D / 4D techniques in order to increase the accuracy of prenatal diagnosis.

As mentioned earlier, we know more than 300 syndromes associated with some type of facial anomaly: most commonly: with cleft lips and / or palate, micrognathia and hypoplasia of the maxilla and the face (Goldenhar syndrome, Treacher-Collins, Pierre-Robin sequences as part of syndrome, Apert syndrome, van der Woude syndrome)<sup>2,4,8,9</sup> These syndromes can be divided by various features, such as orofacial cleft, craniosynostosis, pharyngeal arch abnormalities and simply facial dysmorphism. From embryologic point of view, the face is made up of five facial prominences that surround the future mouth and all meet in one point called the philtrum, a small recess above the upper lip.<sup>5,6,56,57</sup>

### How to distinguish what is normal and what is not?

Although we all have the same basic features, we also have our own recognizable features. There is evidence that the human brain (fusiform gyrus) has a specialized mental module devoted to processing facial recognition features. Researchers in various parts of the world work on understanding how fusiform gyrus mechanisms follow information: how we recognize faces and interpret their various facial expressions. Fetuses with dysmorphic characteristics are diagnosed, as noted earlier, according to the criteria to be met, which are also used in postnatal assessment. These include: head shape and closure of the skull bones, facial asymmetry, hyper / hypotelorism of the eyes, inclined eyes (mongoloid, antimongoloid position (Figure 3), low set or abnormal ears, micro or retrograde jaw confirmed by measuring Jaw-Index,<sup>58</sup> cleft lips and / or palate.<sup>5</sup> In the process of face evaluation, we must also consider ethnic variations and normal differences. For example, epicanthal fold may be normal for Asiatic Asian and non-Asiatic people, but can be considered as a dysmorphic feature of syndromes such as Down, Turner, Noonan,



**Figure 4**  
3D HDlive surface rendering, US imaging of the fetus at 14 weeks of gestation. Notice: subtle and small paramedian cleft lip only.<sup>16</sup>

Williams and Fetal Alcohol Syndrome etc. Different nose forms and shapes is another example (Mediterranean, African, Asian, Latin and Caucasian).

The paramedian cleft lip (CL), cleft palate (CP) or combination of the two-cleft lip and palate (CLP) are one of the most common fetal facial anomalies and one of the most common anomalies of the fetus at all.

This can be an isolated finding (Figure 4) in less than 50% of the cases or in combination with associated anomalies as part of diverse syndromes. If this is an isolated finding, it can be elegantly repaired and reconstructed with a professional cranio-maxillofacial surgeon engagement.

It can be distinguished: a bilateral complete lip and palate cleft that can easily be identified even in the first trimester (Figure 5,10), and on the other hand, a unilateral complete cleft of the lips and palate, or an incomplete cleft of the lip that has only subtle indications and can easily be overlooked (Figure 9). The median facial clefts are the most severe anomalies, regularly part of some serious and complex sequences or syndromes, such as holoprosencephaly, Patau (trisomy 13) (Figure 6,7,12), Edwards syndrome

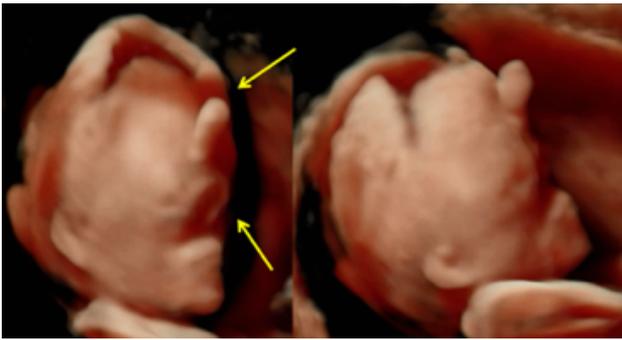


**Figure 5**  
3D HDlive surface rendering of the fetal face at 23+3 weeks of gestation. Notice: bilateral cleft lip and palate (CLP). Because of the protrusion of inner maxillary segment under the nose, anomaly is easily detected.<sup>16</sup>

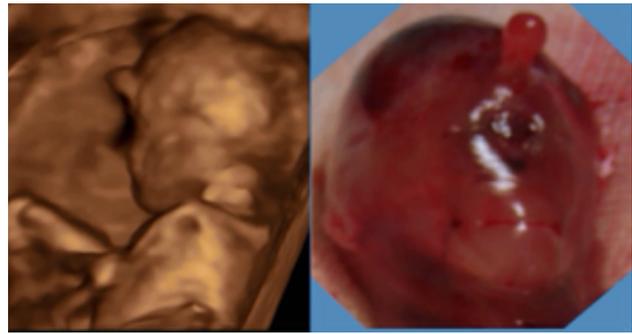
(trisomy 18) and Aicardi syndrome.<sup>60</sup> Trisomy<sup>13</sup> is the commonest chromosomal abnormality associated with alobar holoprosencephaly (fetal face: cyclopia, cebocephaly, flat nose, facial hypoplasia and lip clefts) (Figure 6,7,12). Nevertheless, it is to be remembered that 75% of fetuses with holoprosencephaly have a normal karyotype! (Figure 8,10) Unfortunately, most of the fetuses with CL, CP or CLP have a high incidence of chromosomal abnormalities and other related syndromic anomalies.<sup>61</sup>

**Van der Woude Syndrome (VdW)** carriers have up to 50% of the cases facial clefting. VdW syndrome is autosomal dominant mode of inheritance which accounts for about 2% of all cases with CL and CP.<sup>16</sup> An partial one-sided small defect CL can easily be overlooked by the usual 2D US (Figure 4)<sup>16</sup>.

*Three dimensional multiplanar display and HDlive surface view* is a better method for detecting all forms of clefts and facial malformation. Bilateral CL may sometimes be missed because it does not change the face symmetry. The two-sided complete CL and CP, on the other hand, will most likely be detected due to the protrusion of the inner maxillary segment below the nose (Figure 5,10), which is an obvious and unusual mass when looking at the face profile.<sup>5</sup>



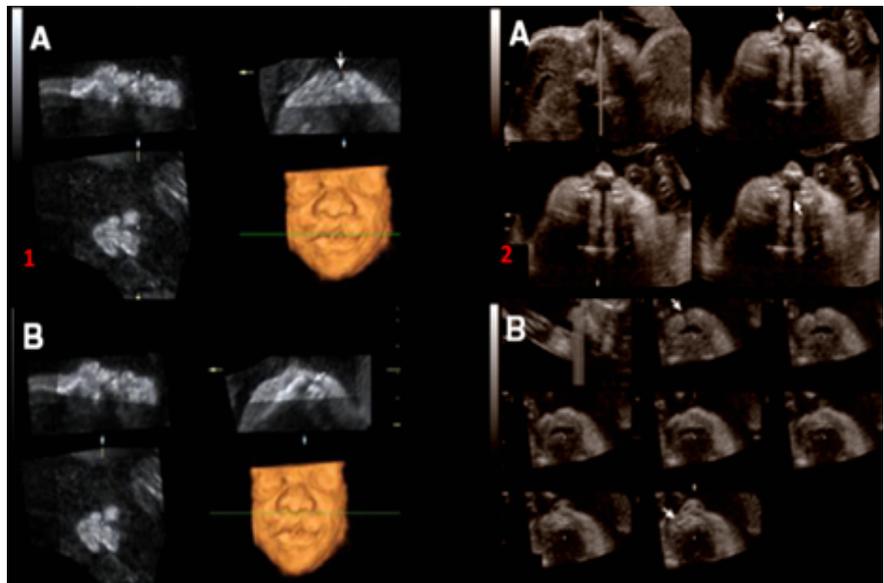
**Figure 6**  
3D HDlive surface rendering, the same fetus from the front and profile. Notice. Abnormal shape of the head, wide open sutures of the skull, anomalies of the midline of the fetal face: cyclopia (lower arrow), proboscis (upper arrow) and holoprosencephaly and low set ears are seen. All features of trisomy<sup>13</sup>- Patau syndrom.<sup>16</sup>



**Figure 7**  
Image on the left: 3Dsurface rendering, profile: notice: proboscis!  
Image on the right: the same fetus after miscarriage:notice similarities with the prenatal imaging (proboscis, cyclopia)



**Figure 8:** 3D HDlive surface rendering of the fetus at 13 weeks of gestation. Notice abnormal shape of the head, holoprosencephaly, bilateral CLP, microphthalmia, low set ears. But NORMAL karyotype!



**Figure 9:** 1A: 3D Multiplanar imaging, small, only subtle paramedian cleft lip seen on this image but, 1B: movement of the green dot across the mandible, small alveolar defect becomes evident.  
2A, 2B: A: Bilateral CLP; detected by tomographic ultrasonic imaging technique (TUI)  
B: Isolated cleft lip Izolirani – integrity of the fetal palate can be obtained by TUI imaging method

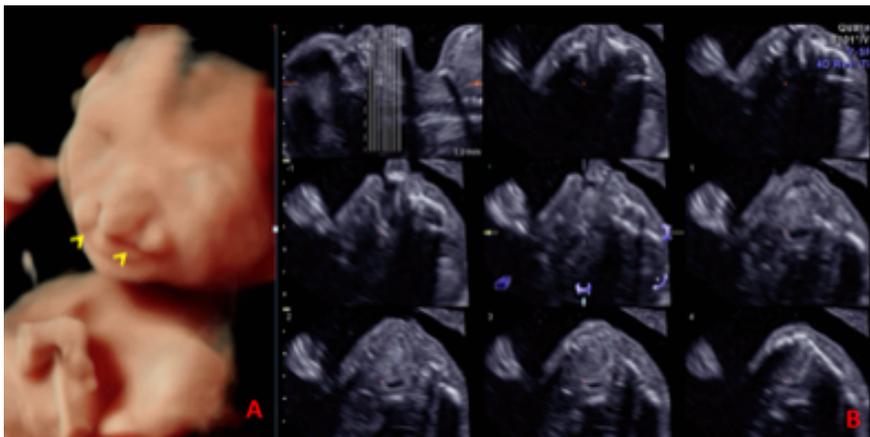
When checking for cleft of the lip, 3D HDlive techniques are very useful. For detection of cleft palate, the use of tomographic ultrasonic imaging (TUI) (Figure 9,10) allows better determination of the extent of the cleft in relation to other structures.<sup>16</sup>

**Mandibular anomalies** (agnathia, micrognathia, retrognathia) (Figures 11-15) have been described in more than 100 different syndromes.<sup>62</sup> It appears to be very common as isolated anomaly, but also commonly as part of heterogeneous syndromes. By 2D US abnormal profile (Figure 11, 12) is first to be detected. Abnormal fetal pro-

file is noted! With the application of different 3D US modalities, it is possible to investigate in detail and to get a complete impression on the fetal appearance and possible existence of other orofacial anomalies.

**Pierre-Robin Sequence (PRS)**

It is characterized by a triad of orofacial anomalies consisting of retrognathia, glossoptosis and median cleft of the soft palate. Mandibular hypoplasia (Figure 11) is a primary deficiency that occurs early in pregnancy between the 7th and 11th week of pregnancy. The tongue maintains high in the oral cavity, which subsequently pre-



**Figure 10:**

3D HDlive surface imaging: bilateral CLP; fetus with multiple anomalies but: NORMAL KARYOTYPE!

**B:** TUI display –notice: the extent of the cleft !<sup>16</sup>

vents the normal tongue placement and prevents the soft palate from fusing.<sup>16, 63</sup> Prenatal diagnosis of micrognathia in PRS can be set very early in the first trimester of pregnancy, by using 3D ultrasound and its applications.<sup>64,65</sup> Micrognathia can be quantified by "Jaw-index" = the ratio of the AP mandible and BPD diameter. If the ratio is  $<0.23$ , a micrognathia diagnosis<sup>58</sup> can be set.

More than 40 PRS syndromes have been described, most common of which are Stickler's syndrome (SS) and 22q11.2 deletion syndrome (Di George Syndrome)<sup>18-23</sup>.

Pooh and Kurjak published the sequence of images of mandibular hypoplasia and slow development of jaw in case of PRS during pregnancy. 16 Serial 3D imaging can be used to clearly document the mandibular growth over a few weeks. 16 Accelerated compensatory growth of the lower jaw is expected during the 1st year of life, and adjustment of the child's profile can be expected from the 3rd-6th year of life.<sup>54, 66-69</sup>

Isolated PRS (without any other associated malformation) occurs in about 50% of cases, however, in the second half of the cases PRS is part of the syndrome. The clinical manifestations of syndrome depend on the persistence and severity of related anomalies.<sup>5</sup> The nature of these anomalies is diverse, most commonly anomaly of the 1st pharyngeal arch, various chromosomal disorders (DiGeorge syndrome), collagenopathy or syndromes associated with the use of toxic substances in pregnancy, such as alcohol (fetal syndrome alcohol FAS), etc. In the study of 115 cases of PRS patients, as expected, 54% had an isolated isolate finding. (5%), facial and hemifacial microsomy (3%), other defined (3.5%) and undefined. Other syndromes:

Stickler (18%), Velocardiofacial syndrome (7%), Treacher-Collins syndrome (9%)<sup>22</sup>.

Facial dysmorphism usually derives from a combination of migration disorders and inadequate formation of facial mesenchym (especially when it is associated with disorders of the 1st and the 2nd pharyngeal arch).<sup>61, 67</sup>

#### Goldenhar syndrome (GS)

Synonyms are: Hemifacial microsomy or oculo-auriculo-vertebral syndrome (OAV). This is a combination of migration disorder and inadequate formation of facial mesenchym (disorders of the first and second throat arch).<sup>61, 67</sup> GS is characterized by a wide range of main and as-

**Table 1:** Main features of Goldenhar syndrome

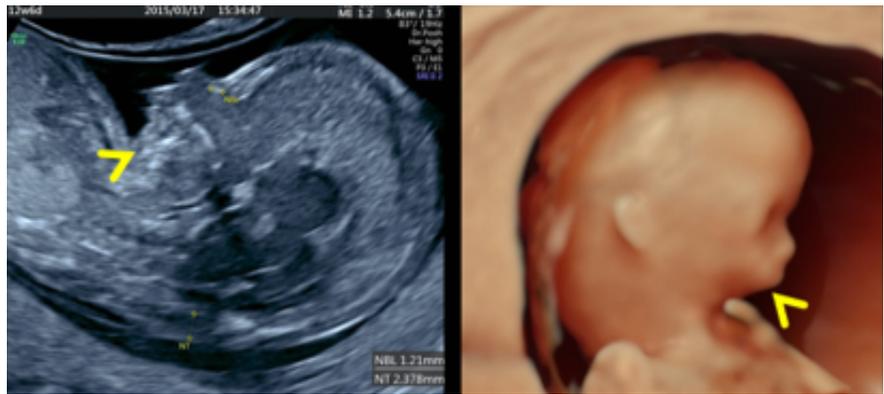
Goldenhar syndrom	GS
Asymetric (unilateral) hypoplasia of the face	70-85%
Mandibular hypoplasia- PRS	
"Skin tags" around the ear	
Hypoplasia of the ear	
Malformation/ microphthalmia of the eye	
Unilateral cleft lip/ palate	
Anomaly of vertebrae (hemivertebrae)	
CHD	
CNS anomalies- Corpus Callosum Lipoma	
Malformations of gastrointestinal system	

sociated features that can differ in the severity from one to the other case (Table 1).

The classic feature of GS is asymmetric (mostly unilateral) facial hypoplasia. (Figure 13.14) Fetuses with GS have major anomalies such as unilateral mandibular hypoplasia involving the temporomandibular joint, multiple pre-auricular skin tags (Figure 13,14) around ear, ear

**Figure 11**

A: left image: 2D midsagittal view of the fetus, notice: abnormal fetal profile and shape of the head, increased NT and prominent micrognathia (arrow).  
 B: right image: the same fetus as in the left image, 3D HDlive surface rendering modality. uz notice: all mention above and detail of the low set ears,<sup>16</sup>

**Figure 12**

3D HDlive surface rendering: notice: cleft lip and palate (CLP) and hypoplasia of the mandible. Case of Trisomy 13 (Patau syndrome at 21 gestational weeks)



hypoplasia/ aplasia and/ or eye malformations (anophthalmia and microphthalmia) and vertebral anomalies (Typically, these malformations are one-sided (70-85%) and give an asymmetric facial appearance. There have been various theories about the emergence of this syndrome. Some authors assumed that the problem could be a one-way blood flow disorder (ischemia) to the 1st and 2nd pharyngeal arch that could occur during the time period between the 4th and

8th week of pregnancy.<sup>70</sup>Wang et al.<sup>71</sup> analyzed data from a large register of congenital anomalies in Spain and established the association between diabetic mothers and the increased risk of their children born with GS syndrome. It is assumed that poorly controlled mother's diabetes interfere with the migration of cells into the cephalic portion of the neural ridge causing this syndrome.<sup>71</sup>

### PRENATAL DIAGNOSIS

The first sonographic indicator to detect this syndrome may be the discovery of facial asymmetry due to hemifacial microsomia (Table 2, 3) or a small detail such as a very typical periauricular skin pendant (tag) (Figure 13, 14). This is most commonly diagnosed in the second and third trimesters of pregnancy. With 3D surface rendering, more details can be visualized. Unilateral craniofacial anomaly, (cerebral hemisphere hypoplasia), eye (micro / anophthalmia), low set ears with various malformations, face (soft tissue asymmetry), kidney (hydronephro-

sis) etc. Using 3D HDLive imaging technology, even small facial details or other parts of the body can be visualized in a very detailed way, which can be of great help in counseling the parents. Differential diagnosis should be based on Treacher's syndrome (TCS), Hellerman-Streiff syndrome, Delleman's syndrome, Nager's syndrome, Townes-Brocks syndrome).

Genetic counseling: GS occurs rarely and sporadically, but for the first-degree relatives the possibility of repetition (RR) is estimated at

2%. Due to the complexity of the clinical picture, multidisciplinary approach to problem is important.<sup>2,4</sup> The combination of micrognathia along with low set ears is a common occurrence in many syndromes.

### Treacher-Collins syndrome (TRC)

The discovery of bilateral symmetrical facial hypoplasia, periauricular tags in combination with micrognathia, may be part of Treacher-Collins (TCS) or some other syndrome such as Nager or Miller syndrome.

TCS is the disorder of the first and second pharyngeal arch. This is a congenital disturbance of craniofacial development induced by mutations in the long arm of chromosome 5 (5q32) of TCOF1 gene.<sup>72</sup> It is depicted by bilateral symmetric oto-mandibular dysplasia (Figure 15). Furthermore, there is downward slanting of a palpebral fissure, lower eyelid coloboma, lack of medial eyelashes, middle and outer ear malformations, and conductive hearing loss. The frequency of TCS is estimated at 1: 50,000 live births per year. The genetic trait is autosomal

**Table 2:** Ultrasoundassessment of fetal face by 3D US in the 1st trimester of pregnancy (modified) according to Merzu et al.<sup>59</sup>

Anatomy (3 orthogonal planes)	Section	
3D Surface rendering	Sagittal view	PROFILE: forehead, nose, lips, chin
3D Transparent rendering	Parasagittal view	Nasal bone yes/no Image angle 45°
3D Maximum mode imaging	Coronal view	Forehead, orbits, both nasal bones, maxilla and mandible
	Transverse view	Forehead, orbits, both nasal bones, maxilla and mandible
Biometry	Sagittal view	FMF angle (>85°)
Stored images		Face profile with the nasal bone
		FMF if needed

**Table 3:** Assessment of the fetal face by 3 D US in the second and third trimesters. (modified) according to Merzu et al.<sup>59</sup>

Anatomy (3 orthogonal planes)	Section	
3D Surface rendering	Sagittal view	PROFILE: forehead, nose, lips, chin Nasal bone yes/no
3D Transparent rendering 3D Maximum mode	Coronal view	- forehead with metopic (= frontal) suture – orbits with lenses (symmetry) – lips – palate (3 D surface rendered image and transparent (= maximum intensity) image
	Transverse view	- Orbits with the lenses - palate (transparent) - maxilla - mandible
Biometry	Sagittal view	- nasal bone - FMF angle - inferior facial angle (IFA)
	Coronal view	- orbital diameter – - inner orbital distance - outer orbital distance
	Transverse view	- orbital diameter – - inner orbital distance outer orbital distance maxilla width – mandible width or Jaw index
Stored images		- profile with nasal bone coronal or transverse view with orbits – coronal view

**Figure 13:** First two images- prenatal (left): 3D surface rendering. Notice preauricular tag. Second two images- postnatal (right): postnatal images of the baby with Goldenhar syndrome: notice hemifacial hypoplasia and microphthalmia.<sup>4</sup>



**Figure 14** Postnatal images of the baby with Goldenhar syndrome. Notice Hemifacial hypoplasia with microphthalmia, periauricular tags and sinuses, external ear deformity hemihypoplasia of the mandible.<sup>4</sup>



dominant in 40% of cases with variations of expression. The remaining 60% are the result of new mutations. Cranio-skeletal hypoplasia develops due to insufficient number of neurons as a result of the death of the neuroepithelial progenitor cells.<sup>73</sup> The onset of defects occurs very early between the 4th and 8th week of embryonic development.

**Prenatal diagnosis:**

Prenatal ultrasound TCS diagnosed mainly in the second trimester of pregnancy<sup>73-75</sup> but with sophisticated 3D applications and HDlive technology Pooch reported detection in the 1st trimester of the pregnancy.<sup>16</sup> With the combination of existing anomalies, the established suspicion of this syndrome is very important to informing geneticists who can order the gene sequencing added to the usual amniocentesis panel (AC) to confirm a diagnosis that would otherwise be missed.<sup>75</sup>

**Apert syndrome (AS)**

Apert syndrome shows autosomal dominant mode of inheritance. There is a congenital mutation in the FGFR2 gene, chromosome 10q26.13.<sup>72</sup> This syndrome is also associated with the advanced age of the fathers. The syndrome has several characteristic features that can be recognized during a routine ultrasound examination. Triad of signs to remember would be: strawberry shaped head, flat face, ‘mitten-like’ hands (like baby gloves) (Figure 16)<sup>16</sup>

This syndrome is characterized by:

- Craniosynostosis: is a skull disorder caused by the early fusion of one or more skull bones (early fusion of the sutures). Otherwise, more than

180 different syndromes are known, which include craniosynostosis in the diagnosis. Changing shape of the cranial vault varies, depending on the fused sutures, so that the compensating growth occurs in dimensions that are not limited by the fusion. Apert syndrome occurs in 4.5% of cases of craniosynostosis (Figure16)

- In the case of Apert syndrome, premature fusion of bicoronal suture (metopic suture) occurs and consequently brachycephalic and acrocephalic shaped head forms appear. In other words, an abnormal flathead skull, frontal bossing, mid-face hypoplasia (flat face), ocular hyperthelormism, and swelling of the eyelids (“puffy eyes”) can be detected. (Figure 16)

- Sonographic assessment: Combination of conventional 2D US techniques with 3D maximum mode (for bony structure), 3D surface rendering and 3D HDlive surface mode can be used.

- Mild ventriculomegaly can be detected represented by 3D inversion mode or, if available, with the latest HDLive silhouette display.

- Agenesis of corpus callosum (AGCC) can best be detected by 3D surface imaging in the central (midsagittal) plane with the additional visualization of the 3D sonoangiogram (3D HDlive bi-directional power Doppler) showing the anomaly: the absence of pericallosal artery.

- Very specific for fetuses with Apert syndrome is a defect at the extremities called ‘Mitten-like hands / feet’: syndactily of the second, third and fourth finger (soft and bone tissue) in combination with a broad thumb that has the appear-



**Figure 15:** 3D surface rendering of the fetus with Treacher Collins syndrome. Notice: typical facial dysmorphism: bilateral symmetric otomandibular dysplasia with the hyperplasia of the soft tissue of the face. Low set ears.<sup>4</sup>

ance of a child's gloves). (Figure 16)

Pooh et al.<sup>54</sup> published a prenatally detected case of a fetus with Apert syndrome by using 3D ultrasound diagnostics. Correlation was performed between prenatal 3D USZV image anomalies with identical postnatal appearance (Figures 16)<sup>16,54</sup>. 3D US images could be used when communicating with the parents for better understanding of the extent of abnormalities of the face, skull and extremities. Differential diagnosis should include other syndromes with craniosynostosis such as: Carpenter, Crouzon, Pfeiffer and Seathre-Chotzen syndrome.<sup>5</sup> The important difference is that there is no syndactyly for the fingers and toes.<sup>5</sup>

**Genetic counseling:** AS has autosomal dominant inheritance, but in most cases it is a case of mosaicism. When the mutation is "de novo", the risk of repetition is unlikely, but if one of the parents is the gene carrier, the recurrence risk is 50%!<sup>76</sup>

In order to confirm the diagnosis prenatally, it is necessary to talk to the parents and to offer invasive prenatal testing. Children with Apert syndrome will need multiple surgical procedures to improve their quality of life. A multidisciplinary approach is required.<sup>2,4</sup>

**Some of the sonographic signs and their association with syndromes**

The frontal bossing (Figure 15, 16, 18) may be a

typical finding in Apert syndrome (Figure 15.16) with achondroplasia (autosomal dominant disease with rhizomelic shortening of extremities) (Figure 17), in Russell-Silver syndrome (poor growth, asymmetric IUGR of the skeleton with normal head size).

Asymmetry of the fetal skull, except for a variety of craniosynostosis which may or may not be associated with syndromes, can also be found in a fetus with:

Amniotic Band syndrome: because of the rupture of the amnion, which occurs very early in the first trimester of pregnancy, the amniotic band causes a great variety and severity of destructive fetal malformations depending on the fetal parts that come into contact and become trapped in it. When they affect the skull, it is possible to detect asymmetric anencephaly, encephalocele, facial clefts and micrognathia. Other abnormalities that were also found were limb flaw, constriction rings and limb amputations.

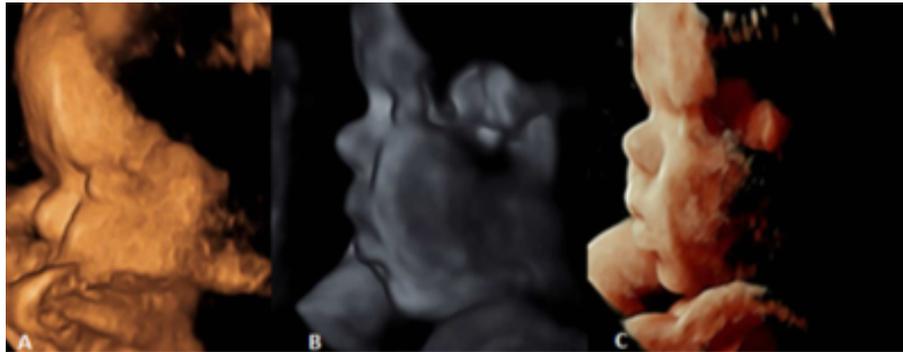
**Other abnormalities of the skull detected by ultrasound are microcephaly and macrocephaly.**

Microcephaly denotes a group of disorders characterized by a small head and is usually associated with abnormal neurological findings and mental disorders.<sup>5</sup> Microcephaly usually also means microencephaly because the size of the head usually determines the size of the brain. Fetuses with prenatal susceptibility to microcephaly have a head circumference (HC) of more than 3 standard deviations below the average for gestational age.<sup>72</sup> The diversity of associated anomalies found by ultrasound depends largely on the etiologic factors that cause microcephaly. Precise etiology in most cases of microcephaly is still unknown. However, this is related to numerous chromosomal abnormalities syndromes such as: Cornelia de Lange, DiGeorge, Wolf-Hirschhorn, Cri du Chat, trisomy 13 and 9, Fetal alcohol syndrome and exposure to some toxic substances (drugs, chlorophene, methotrexate MTX, phenylalanine), mothers' malnutrition, exposure (of pregnant woman) to certain infections during pregnancy such as rubella, toxoplasmosis, varicella, cytomegalovirus (CMV). There are reports of new causes such as exposure (during pregnancy) to Zika virus and unwanted pregnancy outcomes such as microcephaly, other brain, and eye anomalies and increase in loss of pregnancy.<sup>77</sup>

**Figure 16:** Combination of prenatal and postnatal images of the same baby with the Apert syndrome. Prenatal images with 3D surface mode imaging. Notice the similarities of the images: prenatal and postnatal facial dysmorphism, abnormal shape of the scul, frontal bossing hyperthelorism and „mitten like hands/ feet“.<sup>4</sup>



**Figure 17:** A-C: 3D surface rendering of the 34 gestational week fetus, face- profile. Achondroplasia was suspected. Notice different methods of surface rendering. Frontal bossing and depressed nasal bridge.



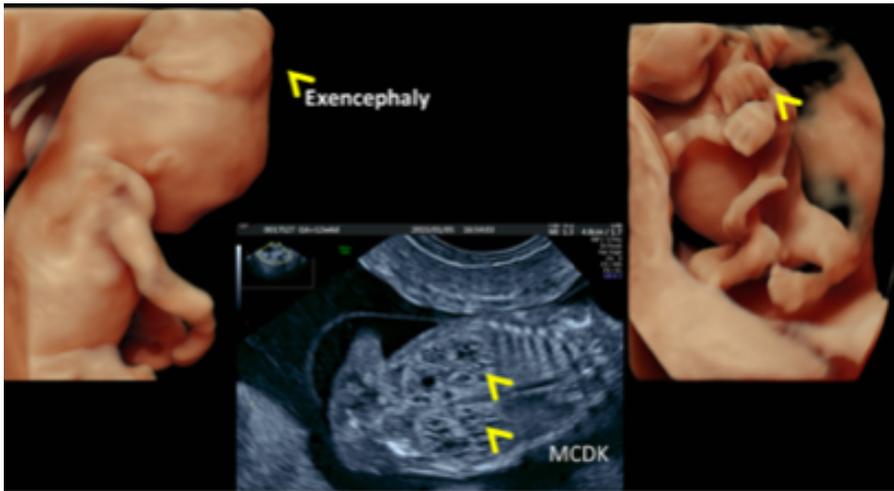
Zika Congenital Syndrome is generally characterized by cerebral atrophy which may interfere with formation and with the neuronal migration during early cerebral embryogenesis.<sup>78</sup> Other features of this syndrome are the following: expressed microcephaly, lysencephaly, microphthalmia, contractures, and arthrogriposis<sup>72, 77,78</sup> Viruses such as CMV or Zika have been shown to attack brain cells, particularly neural progenitors, infecting and destroying the primary stem cells (radial glial cell) of the brain, and therefore missing new neuronal ‘daughters’. The severity of depends largely on the time of infection during pregnancy.<sup>63-65, 79</sup> Microcephaly is mainly a result of a small cerebral cortex. In addition, the infection can cause scars and calcifications in the brain tissue which can be depicted by ultrasound. Infection should be confirmed by a real rev. by polymerase transcription reaction (rRT-PCR)<sup>79</sup> Recent research from the endemic region of Brazil has shown that although some babies are born with normal size of head, postnatal development of microcephaly may occur, as well as significant neurological disorders leading to arthrogyroses, conditions leading to deformity of joints and disabilities.<sup>78</sup>

Abnormalities such as periventricular and intraparenimal calcification, ventricular hypertrophy, secondary cerebral atrophy, cerebellar hypoplasia and cortical abnormalities are seen and detected much earlier than the microcephaly itself.<sup>79</sup>

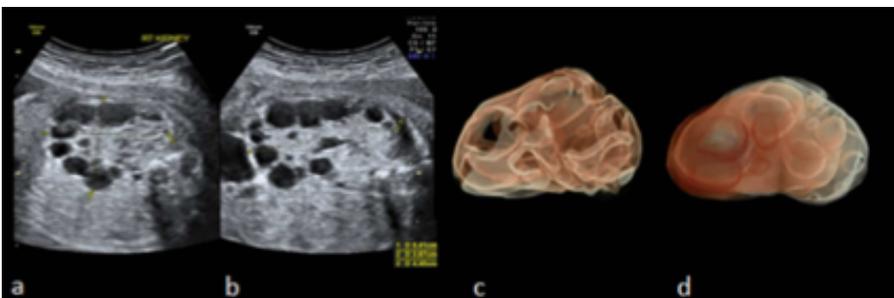
When needed, in addition to the standard ultrasound examination by 2D US, 3D/ 4D US may be used to enhance the accuracy. Fetal neurosonography with 3D advanced US techniques should be included in prenatal assessment if abnormalities are suspected. Different displays of 4D US can be used to evaluate the fetal dynamics and functionality of some organs and different organ systems.

Kurjak Antenatal Neurodevelopmental Test (KANET) can be used to evaluate fetal brain function between 28 and 38 gestational weeks.<sup>80-85</sup> KANET (Figure 25) can be very useful tool, easily performed and used to detect the fetuses at risk for neurological impairment. If score of the test is borderline or abnormal, this test should be repeated at intervals of every 2 weeks until delivery. Prenatal results can be compared with the neonatal ones. Fetuses that are found to be at risk should be followed-up during at least first 2-3 years of life, as suggested by pediatricians, to be able to exclude the neurological damage to same extent and cerebral palsy (CP). Clinical usefulness of the KANET test was suggested by many authors over the past decade, and the exact data of the meta-analysis are underway, nevertheless, preliminary reports are promising.

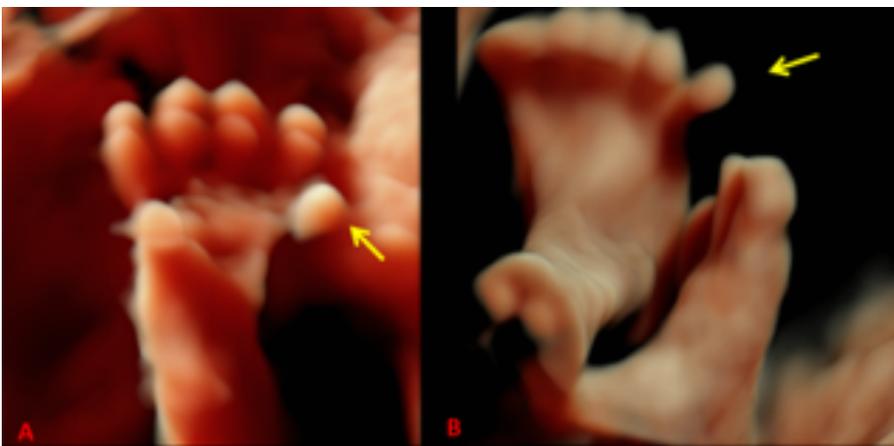
During the routine ultrasound examination, abnormalities of fetal kidneys and bladder can be detected (Figure 18,19) and abnormalities of the anterior abdominal wall in the form of ompha-



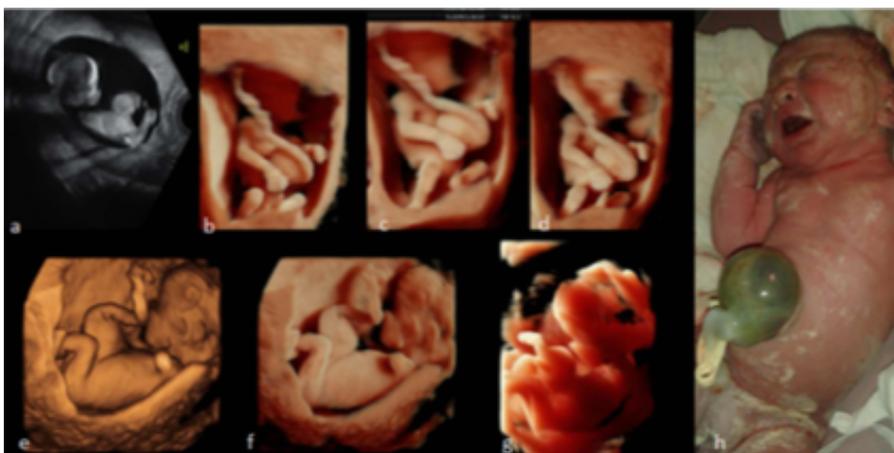
**Figure 18:** Typical triade of findings: Encephalocele( 80%), Renal cystic dysplasia-bilateral MCDK (95%); Polydactyly( 75%); Lethal syndrome: Meckel Gruber Syndrome!<sup>16</sup>



**Figure 19**  
A, b: Multicystic Dysplastic Kidneys: (MCDK) in a fetus of 26 gestational weeks.  
C, d: 3D HDlive Silhouette imaging: extraction of the volume of the MCDK kidney!<sup>16,4</sup>



**Figure 20**  
3D HDlive surface rendering of the fetal hand and the feet. Notice the polydactily in both cases! Fetus with Meckel Gruber Syndrome.



**Figure 21:**  
Sequence of images with a: 2D US: fetus with the omphalocele in the second trimester,  
b-g: 3D HDlive modality in the assessment of the fetus. Notice the difference in image illumination and angle of imaging. Notice the similarities of imaging with postpartum image.<sup>4</sup>

locele (Figure 21). It can be seen a wide range of structural and functional abnormalities.

Dysplastic kidneys with multiple cysts (Multi-cystic dysplastic kidney - MCDK) (Figure 18,19) that vary in size can be found as an anomaly in some very severe syndromes. Because MCDK is dysfunctional, if found bilaterally, it indicates a lethal outcome and is often associated with Meckel Gruber's syndrome (Figure 18,19) with autosomal recessive inheritance.<sup>86</sup> Specifically, a triad of anomalies is found: occipital encephalocele, MCDK (Figure18) and polydactyly (Figure 20). A newborn dies in the first few days of life due to lung hypoplasia and kidney failure. Detection of occipital encephalocele in the first trimester is easier because of a better examination and normal amount of amniotic fluid. Later in pregnancy, there is a progressive oligohydramnios which can be the cause that encephalocele is missed. Special attention should be paid to the evaluation of both fetal kidneys because the normal ultrasound finding of a kidney excludes lethal Meckel Gruber syndrome!



Figure 22: 3D HDlive surface image of the fetus with Trisomy 18. Notice the triade of the anomalies: omphalocele, contracted wrists/hands and hygroma colli (arrows)<sup>16</sup>

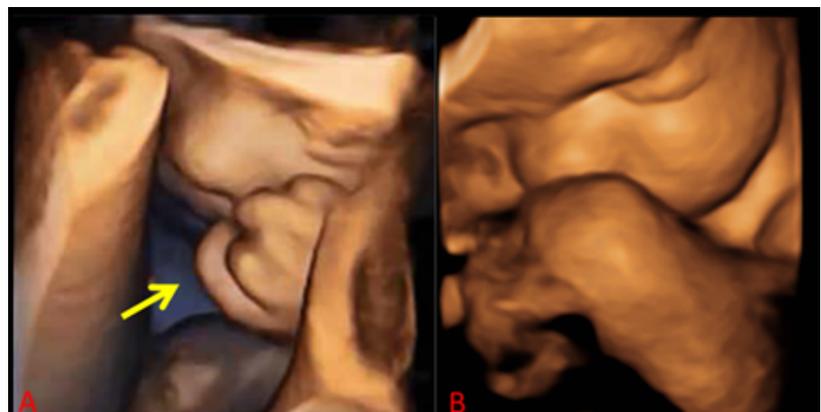
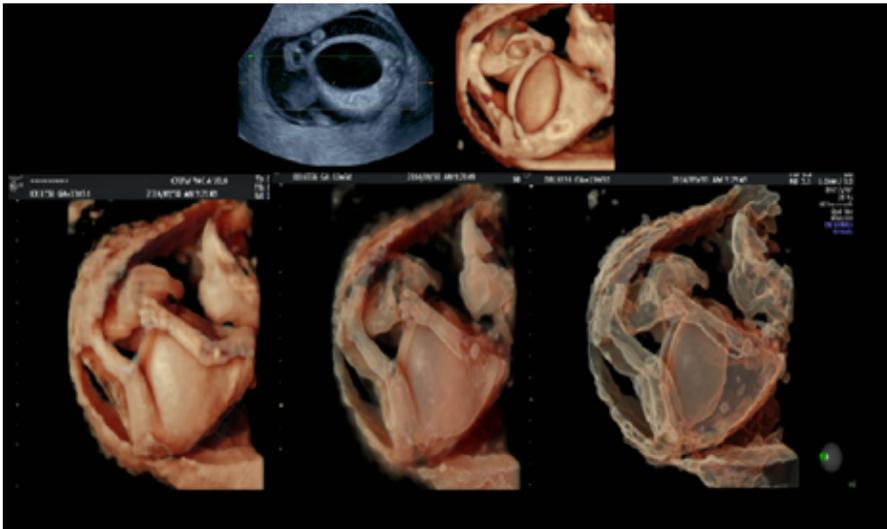


Figure 23: 3D surface rendering; trisomy 18 (Edwards syndrome) in 2 cases. Notice the clenched haand (arow) and overlapping fingers in both.



Figure 25: 3D/ 4D HDlive fetal assessment by KANET test: Notice open eyes of the fetus at 28 gestational weeks.<sup>4</sup>



**Figure 24**  
Sequence of images. From 2D to 3D surface and Silhouette display with increasing Silhouette intensity. Notice: Megacystic bladder in the fetus with Prune Belly syndrome.<sup>16,2</sup>

## CONCLUSION

The 3D ultrasound technique with different visualization and manipulation capabilities of stored volume, provides a unique opportunity for a detailed view of normal and abnormal fetal development. If facial anomaly is suspected, this technique will help in the evaluation and gained images will give some answers to question about severity and extent of anomalies. Particularly handy tool may be when communicating the neonatologist, pediatrician, plastic and reconstructive head and neck surgeon and especially when consulting with the parents of the child. However, as in any imaging technique (ultrasound, MSCT, MRI), you need to know the dome and limitations of 3D / 4D rendering and to be aware of possible artifacts and traps.

From the first trimester to the next, as soon as it becomes possible to detect congenital anomaly by prenatal ultrasound, the question arises- what can and should be done. Many ethical dilemmas present at the time.<sup>2,4</sup> Contemporary medicine faces some major problems when it has the ability to prolong life of severely sick baby with potentially lethal congenital syndromes.

Taking specific ultrasonic diagnostics into account, the idea is to find the balance between the advantages and limitations of sonographic assessment. At the same time, it should be possible to optimize recommendations with the expectations of parents of potentially seriously ill baby. Given the complexity of prenatal diagnosis of syndrome, everything involved in the process is also complex. This includes confor-

mation of prenatal diagnosis postnatally and determination of the short and long term prognosis if possible to assist parents who are facing a baby with syndrome.<sup>2,4</sup> It is essential to point out the necessity of complex, lifelong and costly multidisciplinary care for severely ill baby.

All the aforementioned, 3D / 4D US techniques promise to improve the accuracy of clinicians in detection of fetal abnormalities and detecting fetal syndromes as early as possible. There are many advantages in prenatal detection of fetal syndromes already described, but there is also a great room for improvement. Since new 3D / 4D ultrasound technology becomes more available in everyday clinical practice, the clinician should remain well-informed, well trained and monitor new diagnostic capabilities. Continuous education is necessary. In this way, the number of fetal abnormalities and syndromes detected prenatally will probably increase over time.<sup>4</sup> Auxiliary tools such as network databases ("online databases") that integrate all the necessary information should be included and used for better diagnostic precision.<sup>2,4,87</sup>

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None.

## CONFLICT OF INTEREST

None.

## REFERENCES

- Merz E. 25 Years of 3D ultrasound in prenatal diagnosis (1989-2014). *Ultraschall Med.* 2015 Feb;36(1):3-8. doi: 10.1055/s-0034-1398866.
- Spalldi Barišić L, Kurjak A, Pooh K. R et al. Antenatal detection of fetal syndromes by ultrasound: From a single piece to a complete puzzle. *Donald School J Ultrasound Obstet Gynecol* 2016;10(1):63-77.
- Merz E, Abramowicz JS. 3D/4D ultrasound in prenatal diagnosis: Is it time for routine use? *Clin Obstet Gynecol* 2012;55:336-351.
- Spalldi Barišić L, Stanojević M, Kurjak A, et al. Diagnosis of fetal syndromes by three- and four- dimensional ultrasound: is there any improvement? *J. Perinat. Med.* 2017;45(6): 651-665.
- Benacerraf BR. *Ultrasound of fetal Syndromes.* 2nd ed. Churchill Livingstone, 2008. Chapter 1, Differential Diagnoses; Chapter 2, Syndromes.
- Lanna M, Rustico MA, Pintucci A, Spaccini L, Lalatta F, Nicolini U. Three-dimensional ultrasound and genetic syndromes. *DSJUOG* 2007 Jul-Sep;1(3):54-59.
- Jones, KL. *Smith's Recognizable Patterns of Human Malformation.* 5th ed. Philadelphia: Elsevier Saunders, 1997. Chapter 1, Introduction including dysmorphology approach and classification.
- Jones, KL. *Smith's Recognizable Patterns of Human Malformations.* 6th ed. Philadelphia, PA: Elsevier Saunders; 2006.
- Pashaj S, Merz E. Tree-dimensional ultrasound for detection of fetal syndromes. *Donald School textbook of ultrasound in obstetrics & gynecology*, 4th edition. Edited by Kurjak A, Chervenak FA. 2017. 800-16.
- Pooh RK, Kurjak A. Novel application of three-dimensional HDlive imaging in prenatal diagnosis from the first trimester. *J Perinat Med.* 2015;43:147-58
- Pooh, RK. Neuroanatomy visualisation by 2D and 3D. In: Pooh RK, Kurjak A, editors. *Fetal neurology.* New Delhi: Jaypee Brothers Medical Publisers (P) Ltd; 2009. p. 15-38.
- Pooh RK, Kurjak A. 3D/4D sonography moved prenatal diagnosis of fetal anomalies from the second to the first trimester of pregnancy. *J Matern Fetal Neonatal Med.* 2012;25(5):433-455.
- Benacerraf BR. *Ultrasound of fetal Syndromes.* 2nd ed. Churchill Livingstone, 2008. Chapter 1, Differential Diagnoses; Chapter 2, Syndromes.
- Kurjak A, Carrera JM, Stanojevic M, et al. The role of 4D sonography in the neurological assessment of early human development. *Ultrasound Rev Obstet Gynecol* 2004; 4: 148-159.
- Salihagic-Kadic A, Kurjak A, Medić M, Andonotopo W, Azumendi G. New data about embryonic and fetal neurodevelopment and behavior obtained by 3D and 4D sonography. *J Perinat Med* 2005; 33: 478-490.
- Pooh RK, Kurjak. *Donald School Atlas of Advanced Ultrasound in Obstetrics and Gynaecology.* 1st edition. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd: 2015. Images with the permission of the publisher.
- Andonotopo W, Kurjak A, Kosuta MI. Behavior of an anencephalic fetus studied by 4D sonography. *J Matern Fetal Neonatal Med* 2005; 17: 165-168.
- Abo-Yaqoub S, Kurjak A, Mohammed AB, Shadad A, Abdel-Maaboud M. The role of 4-D ultrasonography in prenatal assessment of fetal neurobehaviour and prediction of neurological outcome. *J Matern Fetal Neonatal Med* 2012; 25: 231-236.
- Talic A, Kurjak A, Ahmed B, et al. The potential of 4D sonography in the assessment of fetal behavior in high-risk pregnancies. *J Matern Fetal Neonatal Med* 2011; 24: 948-954.
- Miskovic B, Vasilj O, Stanojevic M, Ivanković D, Kerner M, Tikvica A. The comparison of fetal behavior in high risk and normal pregnancies assessed by four dimensional ultrasound. *J Matern Fetal Neonatal Med* 2010;23:1461-1467.
- Andonotopo W, Kurjak A. The assessment of fetal behavior of growth restricted fetuses by 4D sonography. *J Perinat Med* 2006;34: 471-478.
- Talic A, Kurjak A, Stanojevic M, Honemeyer U, Badreldeen A, DiRenzo GC. The assessment of fetal brain function in fetuses with ventrikulomegaly: the role of the KANET test. *J Matern Fetal Neonatal Med* 2012; 25:1267-1272.
- Morokuma S, Fukushima K, Yumoto Y, et al. Simplified ultrasound screening for fetal brain function based on behavioral pattern. *Early Hum Dev* 2007;83:177-181
- Kurjak A, Miskovic B, Stanojevic M, et al. New scoring system for fetal neurobehavior assessed by three- and four-dimensional sonography. *J Perinat Med* 2008; 36: 73-81.
- Kurjak A, Abo-Yaqoub S, Stanojevic M, et al. The potential of 4D sonography in the assessment of fetal neurobehavior-multicentric study in high-risk pregnancies. *J Perinat Med* 2010; 38: 77-82.
- Stanojevic M, Talic A, Miskovic B, et al. An attempt to standardize Kurjak's antenatal neurodevelopmental test: Osaka Consensus Statement. *Donald School J Ultrasound Obstet Gynecol* 2011; 5: 317-329.
- Stanojevic M, Antsaklis P, Salihadic-Kadic A et al. Is Kurjak Antenatal Neurodevelopmental Test Ready for Routine Clinical Application? *Bucharest Consensus Statement. DSJUOG July-September 2015(9);3:260-265*
- Kurjak A, Antsaklis P, Stanojević M, Vladareanu R, Vladareanu S, Moreira Neto R, Spalldi Barišić L, Porović S, Delić T. Multicentric studies of the fetal neurobehavior by KANET test. *J. Perinat. Med.* 2017;45(6):717-727.
- Kurjak A, Spalldi Barišić L, Stanojević M, et al. Are We Ready to investigate Cognitive Function of Fetal Brain? The Role of Advanced Four-dimensional Sonography. *Donald School Journal of Ultrasound in Obstetrics and Gynecology.* 2016;10(2):116-124. DOI 10.5005/jp-journals-10009-1453
- Salihagic Kadic A, Kurjak A. Cognitive functions of the



- fetus. *Ultraschall in der Medizin* 39(2):181-189 DOI 10.1055/s-0043-123469
31. Pooh RK. A New Field of 'Fetal Sono-ophthalmology' by 3D HDlive Silhouette and Flow. *Donald School J Ultrasound Obstet Gynecol.* 2015;9(3):221-222.
  32. Kurjak A, Pooh RK, Merce LT, Carrera JM, Salihagic-Kadic A, Andonotopo W. Structural and functional early human development assessed by three-dimensional and four-dimensional sonography. *Fertil Steril.* 2005;84:1285-99.
  33. Hata T, Mashima M, Ito M, Uketa E, Mori N, Ishimura M. Three-dimensional HDlive rendering images of the fetal heart. *Ultrasound Med Biol.* 2013;39:1513-7.
  34. Hanaoka U, Tanaka H, Koyano K, Uematsu R, Kanenishi K, Hata T. HDlive imaging of the face of fetuses with autosomal trisomies. *J Med Ultrasonics.* 2014;41:339-42.
  35. Basel-Vanagaite L, Wolf L, Orin M, Larizza L, Gervasini C, Krantz ID, et al. Recognition of the Cornelia de Lange syndrome phenotype with facial dysmorphology novel analysis. *Clin Genet.* 2016;89:557-63.
  36. Gripp K. Face2Gene RESEARCH for Deep Phenotyping of Novel Syndromes. ACMG. April 18, 2018. [http://www.fdna.com/blog/acmgtalk\\_gripp](http://www.fdna.com/blog/acmgtalk_gripp).
  37. FDNA Face2Gene: <http://www.face2gene.com>
  38. Phenotip Online Database: <http://www.phenotip.com>
  39. Dorland's Illustrated Medical Dictionary online. Elsevier. Available at: [www.dorlands.com/wsearch.jsp](http://www.dorlands.com/wsearch.jsp)
  40. Lyons KJ, Crandall MJ, del Campo M. Smith's recognizable patterns of human malformation, 7th ed. Philadelphia, PA: Elsevier Saunders; 2013
  41. Shprintzen RJ, Siegl-Sadewitz VL, Amato J, et al. Anomalies associated with cleft lip, cleft palate or both. *Am J Med Genet.* 1985;29:585-95.
  42. Gonçalves LF, Lee W, Espinoza J, Romero R. Three- and 4-dimensional ultrasound in obstetric practice: does it help? *J Ultrasound Med.* 2005;24(12):1599-624.
  43. Merz E, Welter C. Two-dimensional and three-dimensional ultrasound in the evaluation of normal and abnormal fetal anatomy in the second and third trimesters in a level III center. *Ultraschall Med* 2005;26(1):9-16. 22.
  44. Benoit B, Levaillant J.M. Voluson GE healthcare technology. available at [www.volusonclub.net](http://www.volusonclub.net) (accessed on December 10, 2016)
  45. Bonilla-Musoles F, Raga F, Castillo JC, Bonilla FJr, Climent MT, Caballero O. High definition real-time ultrasound (HDlive) of embryonic and fetal malformations before week 16. *Donald School J Ultrasound Obstet Gynecol.* 2013;7:18,11
  46. Kagan KO, Pintoff K, Hoopmann M. First-trimester ultrasound images using HDlive. *Ultrasound Obstet Gynecol.* 2011;38 : 607.
  47. Hata T. HDlive rendering image at 6 weeks of gestation. *J Med Ultrason.* 2013;40:495 - 6. 26.
  48. Hata T, Mashima M, Ito M, Uketa E, Mori N, Ishimura M. Three-dimensional HDlive rendering
  49. Pooh RK. Novel Application of HDlive Silhouette and HDlive Flow: Clinical Significance of the 'See-through Fashion' in Prenatal Diagnosis. *Donald School J Ultrasound Obstet Gynecol.* 2016; 10(1):90 – 98
  50. Pooh RK. Clinical significance of 3D HDlive silhouette/flow in neurosonoembryology and fetal neurosonography. *Ultrasound in Obstetrics & Gynecology* 2016; 48 (Suppl. 1): 51-166.
  51. Tonni G, Castigliero AP, Grisolia G, Lituania M, Meagher S, Da Silva Costa F, Araujo Júnior E. Three-dimensional ultrasonography by means of HDlive rendering in the first trimester of pregnancy: A pictorial review. *J Turk Ger Gynecol Assoc* 2016; 17(2): 110-119. Published online 2016 Jan 12. doi: 10.5152/jtgga.2016.15201
  52. Hata T, Kanenishi K, Akiyama M, Tanaka H, Kimura K. Real-time 3-D sonographic observation of fetal facial expression. *J Obstet Gynaecol Res* 2005;31: 337-340.
  53. Kurjak A, Azumendi G, Andonotopo W, Salihagic-Kadic A. Three- and four-dimensional ultrasonography for the structural and functional evaluation of the fetal face. *Am J Obstet Gynecol* 2007; 196: 16-28.
  54. Pooh RK, Kurjak A. Three-dimensional Ultrasound in Detection of Fetal Anomalies. *Donald School J Ultrasound Obstet Gynecol.* 2016;10(3)376-390
  55. The third cycle of the Give A Face To A Syndrome research program. Research Proposals Issued at the American College of Medical Genetics and Genomics (ACMG) Annual Meeting 2016. <http://www.FDNA.com>
  56. Sperber GH. Craniofacial embryology. 4th edition, London: Wright; 1989.
  57. Johnston MC. Embryology of the fetal head and neck. In JG. McCarthy (Ed) Plastic surgery. Philadelphia: WB. Saunders Co; 1990;4:2451-95
  58. Paladini D, Morra T, Teodoro A, Lamberti A, Tremolattera F, Martinelli P. Objective diagnosis of micrognathia in the fetus: the Jaw index. *Obstet Gynecol* 1999 Mar;93(3):382-386.
  59. Merz E et al. 3D imaging of the fetal face - Recommendations from the International 3D Focus Group. *Ultraschall in Med* 2012; 33: 175-182
  60. Maarse W, Berge SJ, Pistorius L, van Barneveld T, Kon M, Breugem C, Mink Van der Molen AB. Diagnostic accuracy of transabdominal ultrasound in detecting prenatal cleft lip and palate: a systematic review. *Ultrasound Obstet Gynecol* 2010 Apr;35(4):495-502.
  61. Godbout A, Leclerc JE, Arteau-Gauthier I, Leclerc LD. Isolated Versus Pierre Robin Sequence Cleft Palates: Are They Different? *Cleft Palate Craniofac J* 2014 Jul;51(4):406-411.
  62. Evans KN, Sie KC, Hopper RA, Robin, Hing AV, Cunningham ML. Robin Sequence: From Diagnosis to Development of an Effective Management Plan. *Pediatrics* 2011 May;127(5): 936-948.
  63. Nowakowski, TJ, Pollen AA, Di Lullo E, Sandoval-Espinosa C, Bershteyn M, Kriegstein AR. Expression

- Analysis Highlights AXL as a Candidate Zika Virus Entry Receptor in Neural Stem Cells. *Cell Stem Cell*. 2016;18(5):591 – 596.
64. Li, C, Xu D, Ye Q, Hong S, Jiang Y, Liu X, Zhang N, Shi L, Qin CF, Xu Z. Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice. *Cell Stem Cell*. 2016;19(1):120 – 6.
65. Society for Maternal-Fetal Medicine (SMFM) Publications Committee. SMFM Statement. Ultrasound Screening for Fetal Microcephaly Following Zika Virus Exposure. *AJOG*. 2016;214 (6):B2– B4.
66. Teoh M, Meagher S. First-trimester diagnosis of micrognathia as a presentation of Pierre Robin syndrome. *Ultrasound Obstet Gynecol* 2003 Jun;21(6):616-618.
67. Johnson JM, Moonis G, Green GE, Carmody R, Burbank HN. Syndromes of the first and second branchial arches, part 2: syndromes. *American Journal of Neuroradiology*. 2011;32(2):230 – 237.
68. Breugem CC, Courtemanche DJ. Robin Sequence: Clearing Nosologic Confusion. *Cleft Palate Craniofac J* 2010 Mar;47(2):197-200.
69. Evans AK, Rahbar R, Rogers GF, Mulliken JB, Volk MS. Robin sequence: a retrospective review of 115 patients. *Int J Pediatr Otorhino laryngol* 2006 Jun;70(6):973-980.
70. Martinelli P, Maurotti GM, Agangi A, Mazzarelli LL, Bifulco G, Paladini D. Prenatal diagnosis of hemifacial microsomia and ipsilateral cerebellar hypoplasia in a fetus with oculoauriculovertebral spectrum. *Ultrasound Obstet Gynecol* 2004; 24:199 - 201. DOI:10.1002/uog.1118.
71. Wang R, Martinez-Frias ML, Graham JM Jr. Infants of diabetic mothers are at increased risk for the oculo-auriculo-vertebral sequence: A case-based and case-control approach. *J Pediatr*. 2002;141(5):611 - 7.
72. Chervenak FA, Rosenberg J, Brightman RC, Chitkara U, Jeanty P. A prospective study of accuracy of ultrasound in predicting fetal microcephaly. *Obstet Gynecol*. 1987;69(6):908 - 1043.
73. Paul A Trainor PA, Dixon J, Dixon MJ. Treacher Collins syndrome: etiology, pathogenesis and prevention. *European Journal of Human Genetics*. 2009;17:275 – 283; doi:10.1038/ejhg.2008.221.
74. Tsai MY, Lan KC, Ou CY, Chen JH, Chang SY, Hsu TY. Assessment of the facial features and chin development of fetuses with use of serial three-dimensional sonography and the mandibular size monogram in a Chinese population. *Am J Obstet Gynecol*. 2004;190(2):541 – 546
75. Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, Glynn EF, Ellington L, Du C, Dixon J, Dixon MJ, Trainor PA. Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. *Nat Med*. 2008;14(2):125 – 33
76. Toriello HV, Meck JM. Statement on guidance for genetic counseling in advanced paternal age. *Genet. Med*.2008.10 (6): 457 – 60.
77. De Araújo TV, Rodrigues LC, de Alencar Ximenes RA, de Barros Miranda-Filho D, Montarroyos UR, de Melo AP.. et al. Association between Zika virus infection and microcephaly in Brazil, January to May, 2016: preliminary report of a case-control study. *Lancet Infect Dis*. 2016;(16)12:1356 - 1363.
78. van der Linden V, Pessoa A, Dobyns W, Barkovich AJ, van der Linden HJ, Rolim Filho EL. et al. Description of 13 Infants Born During October 2015–January 2016 With Congenital Zika Virus Infection Without Microcephaly at Birth, Brazil. *MMWR*. 2016;65(47);1343 – 1348
79. Melo AS de O, Aguiar RS, Amorim MMR, Tanuri A, Melo FO, Ribeiro ST. et al. Congenital Zika Virus Infection. *JAMA Neurol*. Published online October 3, 2016. Corrected on October 24, 2016.
80. Kurjak A, Miskovic B, Stanojevic M, Amiel-Tison C, Ahmed B, Azumendi G. et al. New scoring system for fetal neurobehavior assessed by three- and four-dimensional sonography. *J Perinat Med* 2008;36(1):73-81.
81. Kurjak A, Abo-Yaqoub S, Stanojevic M, Yigiter AB, Vasilj O, Lebit D. et al. The potential of 4D sonography in the assessment of fetal neurobehavior-multi-centric study in high-risk pregnancies. *J Perinat Med*. 2010;38(1):77-82.
82. Stanojevic M, Antsaklis P, Kadic AS. et al. Is Kurjak Antenatal Neurodevelopmental Test Ready for Routine Clinical Application? Bucharest Consensus Statement. *Donald School J Ultrasound Obstet Gynecol*. 2015;9(3):260-265.
83. Stanojevic M, Talic A, Miskovic B, et al. An attempt to standardize Kurjak's antenatal neurodevelopmental test: Osaka Consensus Statement. *Donald School J Ultrasound Obstet Gynecol* 2011; 5: 317-329.
84. Kurjak A, Barisic LS, Stanojevic M, Kadic AS. Porović S. Are we ready to investigate cognitive function of fetal brain? The role of advanced four-dimensional sonography. *Donald School J Ultrasound Obstet Gynecol*. 2016;10(2):116-124.
85. Kurjak A, Antsaklis P, Stanojević M, Vladareanu R, Vladareanu S, Moreira Neto R, Spalldi Barišić L, Porović S, Delić T. Multicentric studies of the fetal neurobehavior by KANET test. *J. Perinat. Med*. 2017;45(6):717-727.
86. Barišić I, Odak LJ, Loane M, Garne E, Wellesley D, Calzolari E. et al. Prevalence, prenatal diagnosis and clinical features of oculo-auriculo-vertebral spectrum: a registry-based study in Europe. *European Journal of Human Genetics*. 2014;22:1026 – 1033; doi:10.1038/ejhg.2013.287.
87. Barišić Spalldi L, Kurjak A, Pooh RK. 3D sonography in fetal syndromes. In: Merz E, Kurjak A (eds) *Donald School Textbook: Current status of clinical use of 3D-4D sonography in Obstetrics and Gynecology*, Jaypee Brothers (New Delhi) 2019, in press