

Fetal Neurology: Past, Present and Future

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ABSTRACT

Neurological failure is the most apprehension complication of pregnancy, labor and the neonatal period. The origin and outcome correlation is frequently doubtful. The arrival of four-dimensional ultrasonography (4D US) and its function to study fetal behavior patterns have initiated to offer insight into the structural and functional fetal brain development. Although many fetal behavioral studies have been conducted, it is still questionable whether the assessment of continuity from fetal to neonatal behavior could improve our ability of early detection of brain pathology. Neurological assessment of fetus *in utero* is extremely difficult even having such sophisticated equipment like 4D ultrasound. As it is well known that quantity of GMs is not so informative and predictive for neurological impairment, their quality should be assessed. Gestalt perception of premature GMs we are dealing with *in utero* and several weeks postnatally are not as predictive for the detection of neurologically abnormal fetuses or newborns as fidgety GMs. Therefore, some additional parameters should be added to the prenatal neurological examination in order to improve our ability to make the distinction between normal and abnormal fetuses.

Keywords: Neurobehavioral development, Fetal behavior, Four-dimensional ultrasound.

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INTRODUCTION

Understanding the structure and function of the fetal nervous system has been the dream of physicians for centuries. The pioneering efforts of Ian Donald in obstetric ultrasound in the latter part of the 20th century have permitted this dream to become a reality. The initial contribution of obstetric ultrasound focused on normal and abnormal structure. Initially, anencephaly was described and followed by increasingly subtle central

nervous system abnormalities such as agenesis of the corpus callosum. The current and evolving challenge for investigators in obstetric ultrasound is to have similar success with the understanding of fetal neurological function. There are many functional neurological abnormalities such as cerebral palsy (CP) whose causes are poorly understood. There are also an escalating number of results illustrating that a large presence of neurological problems, such as minimal cerebral dysfunction, schizophrenia, epilepsy or autism, upshot at least in part from prenatal neurodevelopmental problems. Clinical and epidemiological studies have revealed that CP most often results from prenatal rather than perinatal or postnatal causes.¹ Currently, although the momentous advances in prenatal and perinatal care, there is no mean to identify or expect the development of these disorders. Therefore, the development of diagnostic strategies to avoid and condense the saddle of perinatal brain damage has turn into one of the most imperative tasks of contemporary perinatal medicine. The application of the new neurobehavioral test Kurjak's antenatal neurodevelopment test (KANET) might improve our understanding of the prenatal neurodevelopmental events and possibly antenatal detection of cerebral palsy and other neurological diseases.

Structural and Functional Development of Central Nervous System (CNS)

The development of central nervous system begins approximately at the end of gastrulation. The generation of the neuroectoderm from ectoderm during the third postconceptional week, results in formation of the neural plate. Thus, the neural epithelium of the embryo, which is a precursor of neurons and glia, is virtually the first part of organism that acquires the separate identity from other cells.² The formation of the neural plate is succeeded by the folding of its edges and formation of a neural tube, whose further growth and reshaping results in formation of structures of CNS. According to O'Rahilly and Muller, forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon) can be distinguished in the rostral portion of the unfused neural folds³ earlier than it is usually referred to, approximately at 22nd postconceptional day. In the rapid succession, during the 4th postconceptional week, the forebrain components-diencephalon and telencephalon can be detected. Three

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embryonic zones, ventricular, intermediary and marginal zone (seen from ventricular to pial surface), are present in all parts of neural tube, while telencephalon contains additional two zones, subventricular and subplate zone. Ventricular zone and subventricular zone of telencephalon are the site of neurogenesis and all the future neurons and glia are born in these structures. During migration toward the pial surface they form other transitional zones before reaching their genetically predetermined final destinations. Those destinations are cortical plate or different nuclei in the brain stem, diencephalon and basal forebrain (for references see(1)). One of the transitional structures, a subplate zone that is a site for transient synapses and neuronal interactions, can play a major role in the developmental plasticity following perinatal brain damage.⁴ Early appearance of interneuronal connections, shown in Table 1, implicates a possibility of an early functional development. However, these first synapses exist only temporarily and disappear due to the normal reorganization processes. Most embryonic zones, types of neurons and glia and early synapses, which play crucial role in certain periods of fetal brain development, eventually disappear, significantly changing structure and function of the brain. Reorganization processes include apoptosis, disappearance of redundant synapses, axonal retraction and transposition, and transformation of the neurotransmitters phenotype.⁴ The Table 1 shows a significant overlap of neurogenesis, migration and synaptogenesis in the embryonic and fetal life. Development of human brain is not completed at the time of delivery. In an infant born at term, characteristic cellular layers can be observed in motor, somatosensor, visual and auditory cortical areas. Although, proliferation and migration are completed in a term infant, synaptogenesis and neuronal differentiation continue very intensively.⁵ Brain stem demonstrates high level of maturity, whereas all histogenetic processes actively persist in cerebellum.⁶

Therefore, only subcortical formations and the primary cortical areas are well developed in a newborn. Associative cortex, barely visible in a newborn, is scantily developed in a 6 months old infant. Postnatal formation of synapses in associative cortical areas, which intensifies between 8th month and 2nd year of life, precedes the onset of first cognitive functions, such as speech. Following the 2nd year of age, many redundant synapses are eliminated. The elimination of synapses begins very rapidly, and continues slowly until the puberty, when the same number of synapses as seen in adults is reached.⁶

The Origins of Fetal Behavior and Fetal Motor Development Assessed by 4D US

The first synapses appear in the spinal cord at 6 to 7 postconceptional weeks⁷ and in the cortical plate at 8 postconceptional weeks.⁸ This is the phase when the first electrical bustle and conduction of information take places. The earliest spontaneous fetal movements can be observed at 7.5 postconceptional weeks. These movements, consisting of slow flexion and extension of the fetal trunk, accompanied by the inactive displacement of arms and legs and emerging in asymmetrical sequences, have been described as 'vermicular'.^{9,10} They are substituted by various general movements, which consist of head, trunk and limb movements, such as 'rippling' seen at week 8, 'twitching' and 'strong twitching' at weeks 9 and 9.5, respectively, and 'floating' 'swimming' and 'jumping' at weeks 10.¹¹ Isolated limb movements emerge almost simultaneously with the general movements. At the same time with the beginning of spontaneous movements, at 7.5 postconceptional weeks, the initial motor reflex activity can be detected, permitting the hypothesis to be made of the existence of the first afferent-efferent circuits.⁷ At that time, head tilting following perioral stimulation was noted. The primary reflex movements are immense and signify a limited number of synapses in a reflex pathway. During the 8th week of gestation, these substantial reflex movements are replaced with local movements, possibly due to an increase in the number of axodendritic synapses. Hands become susceptible at 10, 5 weeks and lower limbs start to contribute in these reflexes at around weeks 14. General movements are the first sign of a supraspinal control on fetal motor activity. Approximately at the 7 postconceptional weeks, the brainstem which consists of the medulla oblongata, pons and midbrain, begins to develop and mature in a caudal to rostral direction. As the medulla matures in advance of more rostral structures of brainstem, reflexive movements of the head, body and extremities, as well as breathing movements and heart rate alterations, appear in advance of other functions.¹² Since, the 10th week onwards,

Table 1: Dynamics of the most important progressive processes in the development of the human brain

	<i>Beginning</i>	<i>Most intensive activity</i>	<i>Ending</i>
Neurogenesis	Early embryonic period (4th week)	8th-12th week	Approx. 20 weeks
Migration	Simultaneously with proliferation	18th-24th week	38th week
Synaptogenesis	6th-7th week—spinal cord 8th week—cortical plate	13th-18th week, after 24th week, 8th month—2 years of postnatal life	Puberty

the amount and incidence of movements increase. By 14 to 19 weeks, fetuses are highly active with the longest period between movements of only 5 to 6 minutes. In the 15th week, 15 singular types of movement can be observed. The general body movements and isolated limb movements, retroflexion, anteflexion and rotation of the head can be seen. Also, face movements, such as mouthing, yawning, hiccups, sucking and swallowing, can be included to an ample repertoire of fetal motor activity in this stage.¹³ But, during the first half of pregnancy, a dynamic pattern of neuronal production and migration, as well as the immature cerebral circuits are considered too immature for cerebral involvement in the motor behavior.⁴ Merely at the end of this period do a quantifiable number of synapses appear in the structures preceding the cerebral cortex, perhaps forming a substrate for the first cortical electric activity, noted at weeks 19.⁴ The spinothalamic tract is established at the 20th week and myelinated by 29 weeks of gestation and the thalamocortical connections penetrate the cortical plate at 24 to 26 weeks. Evoked potentials can be detected from the cortex at the 29th week, indicating that the functional connection between periphery and cortex operates from that time onwards.¹⁴ In the second half of pregnancy, the number of general movements gradually decreases, particularly during the last 10 weeks.¹⁵ Although, this decrease was first explained as a result of the reduction in amniotic fluid volume, it is now believed to be a result of maturation processes in the brainstem.¹² Simultaneously with the decline in the number of generalized movements, an increase in facial movements, as well as opening or closing of the jaw, swallowing and chewing, can be observed. These activities can be seen mainly in the periods of absence of general movements, and this pattern is considered to be a manifestation of the normal neurological development of the fetus.¹⁵ However, alterations not only in the number of movements, but also in their complexity, are revealed to be the result of cerebral maturation processes. It is important to point out that subunits of the brainstem remain the main regulators of all fetal behavioral patterns until delivery.¹² Despite medical reports from 100 years ago and 25 years of systematic research initiated by Prechtl et al,¹⁶⁻¹⁸ the study of prenatal behavior is still in its infancy. One of the most promising advances in the field of ultrasonography has been the new 4D US technology. Its advance has been completed in the last year giving visualizations in almost real-time.¹⁹⁻²² The availability of new diagnostic data has in an extraordinary way raised our knowledge about intrauterine life, substantially modifying some earlier interpretations.²³ First spontaneous fetal movements can be observed

with conventional two-dimensional ultrasound (2D US) around 8th gestational week, while the newly developed 4D US allows the visualization of fetal motility 1 week earlier (Table 2).²⁴ General movements are the first complex fetal movement patterns observable by 2D US. They can be recognized from the 8th to 9th weeks of pregnancy (Fig. 1) and continue to be present until 16 to 20 weeks after birth.¹⁸ According to Prechtl, these are gross movements, involving the whole body. They wax and wane in intensity, force and speed, and they have a gradual beginning and end.^{13,18} The majority of sequences of extension and flexion of the legs and arms is complex, and may be better assessed with 4D US.²⁴ In the literature, there is a range between the 8th and 12th weeks concerning the first appearance of limb movements.^{13,18,20,25} De Vries found isolated arm and leg movements at the 8th week of gestation.¹³ With 4D US, limb movements at the 8th-9th weeks were found. Using 4D sonography, Kurjak et al found that from 13 gestational weeks onwards, a 'goal orientation' of hand movements appears and a target point can be recognized for each hand movement.¹⁹ More limb joints are active and move simultaneously, such as extension or flexion in arm and elbow or hip and knee. The elevation of the hand, extension of the elbow joint, with a slight change in direction and rotation, can be seen simultaneously.²⁶ The isolated limb movements seen at the 9th week are followed by the appearance of the movements in the elbow joint at 10 weeks, changes in finger position in the 11th week and by easily recognizable clenching and unclenching of the fist at the 12th to 13th weeks. Finally, at the 13th to 14th weeks, isolated finger movements can be seen, as well as an increase in the activity and strength of the hand or finger movements.²⁶ Recent investigation of fetuses in the last trimester of gestation, performed by 4D US, has discovered an even wider range of hand and face movements than was formerly explained.¹⁹ It has been also confirmed that the fetal movement patterns in the second half of pregnancy are about equal to those monitored after birth, while the list of movements in the newborn consists of some patterns that cannot be

Table 2: Developmental sequence of fetal behavioral patterns observed by 4D US in the first trimester of pregnancy

Type of movements	Postconceptional weeks				
	7	8	9	10	11
General movement	+	+	+	+	+
Startle		+	+	+	+
Stretching		+	+	+	+
Isolated arm movement		+	+	+	+
Isolated leg movement		+	+	+	+
Head rotation			+	+	+
Head anteflexion			+	+	+
Head retroflexion			+	+	+



Fig. 1: 4D US imaging demonstrated fetus at 13 weeks of gestation showing general movements pattern

watched in the fetus, such as the Moro reflex.²⁷ As well, study of anencephalic fetuses have presented clear evidence for the influence of supraspinal structures on motor behavior at around the 20th gestational week. In these fetuses the number of movements was normal or even increased, but the complexity of the movement patterns distorted radically and movements were stereotyped and simplified.²⁸

The eminence of fetal movement patterns is distorted in fetuses undergoing intrauterine growth restriction. The activities become slower and monotonous, similar to cramps and their variability in force and amplitude is reduced.²⁹ These changes might designate the subsistence of brain lesions in growth-restricted and possibly hypoxic fetuses. Despite the premature postulations, the changes in the amplitude and complexity of movements in these fetuses do not show to be due to oligohydramnios. In cases of premature rupture of fetal membranes and a subsequently reduced volume of amniotic fluid, movements arise less frequently, but their complexity look likes that of movements achieved in the normal volume of amniotic fluid.¹⁶ Qualitative including quantitative analysis of fetal movements divulged the consistency of the fetal nervous system, and can be applied for the recognition of different cerebral dysfunctions and probably neuromuscular ailments.

The application of the new technology, 4D US, in the examination of fetal facial movements has revealed the existence of a full range of facial expressions including grimacing, tongue expulsion and eye-lid movements (Fig. 2) similar to emotional expressions in adults.^{27,30}

The possibility of studying such subtle movements might open a new area of investigation.³¹

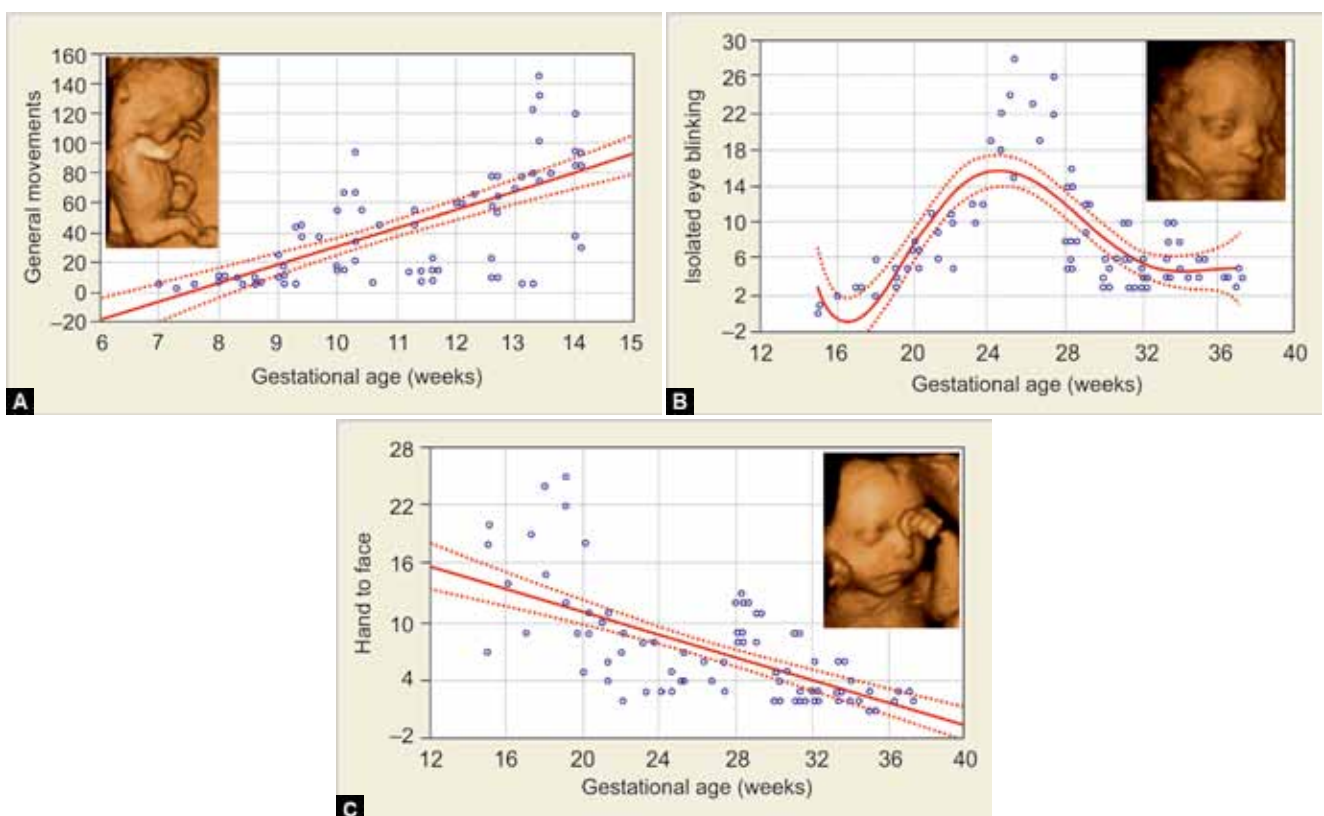
During the first trimester, a tendency toward increased frequency of fetal general movements with increasing gestational age have been noticed (Fig. 3A). While at the beginning of the second trimester, the fetuses began to display a tendency toward increased frequency of observed fetal facial expression up to the end of the second trimester. An oscillation and dispersion of the incidence of the facial expression as seen in the polynomial regression of isolated eye blinking diagram is observable in Figure 3B.³⁰

The most frequent facial movement patterns in the second trimester were isolated eye blinking, grimacing, suckling and swallowing, while yawning, mouthing, tongue expulsion and smiling could be observed less frequently.^{25,30} During the third trimester, the fetuses began to display decreasing or stagnant incidence of fetal facial expression. All of the type of head movements and hand to body contact indicated a tendency to decrease frequency from the beginning of the second trimester to the end of the third trimester (Fig. 3C).³⁰

The investigations of fetal facial expressions established that all mechanisms of the fetal yawning pattern, prolonged jaw opening followed by a quick closure and accompanied by head flexion and elevation of arms, can easily be documented by 4D US in this period (Figs 4 to 10).³² If the fetal yawning in the third trimester was matched up to the yawning in the neonates during the first week of life, no differences were found in the frequencies of this reaction, The frequency of yawning steadily increased between 15th and 24th week when a short



Fig. 2: 3D/4D US provides clear depiction of dynamic changes of fetal facial expression allowing study of fetal behavior during all trimesters of pregnancy



Figs 3A to C: Quantitative analysis of normal fetal behavior patterns using 4D US: (A) General movements, (B) Isolated eye blinking and (C) Hand to face movement



Fig. 4: 4D US image sequences of facial expression characterized by stereotyped yawning opening

plateau was observed from 24th to 26th week and was followed by a slight decrease toward the term.³⁰ A gestational age-related tendency in the frequency of yawning could be assumed as the maturation of the brain stem and probably the gaining of control of more cranial structures over yawning pattern. These results have offered new data concerning the route of neurodevelopment of this fascinating, but poorly implicit reflex. Whether this is distorted in cases of neurodevelopmental disorders and whether such adaptations can give us impending into the function of fetal nervous system in high risk pregnancies, continues to be determined. It also stays to be determined to what possibility are the facial motoric patterns related to the function and integrity of the CNS. However, the fact that even in the embryonic stage, the identical inductive forces that cause growth and restyling of the neural tube influence the development of facial structures, and that many genetic disorders affecting the CNS are also described by dysmorphology and dysfunction of facial structures, underline the value of these studies.^{2,30,31}

Neonatal Aspect of Fetal Neurology

Obstetric aspects of fetal neurology have been discussed extensively in our previous publications.^{19-25,27,28-31} In order to better understand fetal neurobehavioral patterns, we have learned a lot from basic studies of brain development and from clinical postnatal studies of neonates. Now, when we have reached the edge of fetal behavioral investigation by 4D ultrasonography in

normal fetuses, we intend to find some new ideas and ways of investigation presenting neonatal aspect of fetal neurology.

Cerebral palsy (CP) is an 'umbrella' term for disorders of development, movement and posture, resulting in limitations of activity due to nonprogressive impairment of developing brain.³³ The diagnosis of CP is retrospective and it is exceptionally made before the age of 6 months in only most severely affected infants, and the specificity of the diagnosis will improve as the child ages and the nature of the disability evolves.³⁴ CP does not result from a single event but rather there is a sequence of interdependent adverse events providing to the condition.³⁵ This time frame of evolving adverse events is something which should be taken into account when considering the possibility of CP diagnosis in infants.^{34,35} The understanding of the profile of a child's disability across multiple domains is an ongoing process necessary for appropriate treatment and future planning.³⁴ This theoretical statement is sometimes very difficult to be practically implemented. An attempt to make early diagnosis of CP should be followed with factors related to pathogenesis, impairment and functional limitations in every patient.³⁴ In order to identify pathogenesis of the process, neuroimaging methods should be used, among which cranial ultrasound, magnetic resonance imaging, magnetic resonance spectroscopy and diffusion weighted imaging are the most frequently used in very low birth-weight premature infants and in term infants with encephalopathy.³⁴ Impairment of organs or

systems by clinical assessment of muscle tone, strength, and control of voluntary movements for early detection of infants with the risk for CP has been frustrating, because only 43% of 7-year-old children with CP had a normal newborn neurological examination.^{34,36} Is it possible to change this discouraging fact resulting from our failure to diagnose neurological impairment early enough to intervene? Interests in diagnosis of neurological impairment among ultrasonographers using 4D ultrasound have been recently shifted toward prenatal period.^{23,37} Is there any possibility to improve timing of postnatal diagnosis of neurologically disabled infant? Postnatal assessment is probably easier to perform than prenatal, by using a simple and suitable for everyday work screening clinical test with good reliability, specificity and sensitivity. Such tests are still not widely used, while those complicated and time consuming are used mostly for clinical research purposes. There is a possibility for the early and simple neurological assessment of the term and preterm newborns with the aim to detect associated risks and anticipate long-term outcome of the infant, and to establish a possible causative link between pregnancy course and neurodevelopmental outcome.³⁸ As CP is a disorder of movement and postural control resulting in functional limitations, its diagnosis could be helpful in detection of early impairment.³⁴ Clinical neurological assessment proposed and practiced by Amiel-Tison could be very useful in the early detection of newborns at risk.³⁸ As development of central nervous system (CNS) is very complex and long-lasting process, the assessment of its developmental optimality is something which should be assessed in order to investigate whether the infant is neurologically normal or damaged. Neurological assessment at term by Amiel-Tison is taking into account neurological maturation exploring so called lower subcortical system developing earlier from the reticular formation, vestibular nuclei and tectum, and upper cortical system developing from the corticospinal pathways.³⁹ The role of lower system is to maintain posture against gravity, while the upper system is responsible for the control of erect posture and for the movements of the extremities.³⁹ At the corrected age of 40 gestational weeks optimality assessment consists of: head circumference measurement, assessment of cranial sutures, visual pursuit, social interaction, sucking reflex, raise-to-sit and reverse, passive tone in the axis, passive tone in the limbs, fingers and thumbs outside the fist, and autonomic control during assessment.³⁹ The Amiel-Tisson neurologic assessment at term is increasing accuracy in assessing CNS function in the neonate by using simple scoring system, focusing on the most meaningful items, promoting a clinical synthesis at term, for term and preterm infants.³⁹ It was

recognized that clinicoanatomic correlations using high resolution neuroimaging techniques could be helpful in the neurological assessment of newborns, while the neurological examination and the functional assessment of the developing CNS are bringing a new perspective of CNS status in neonatal period.⁴⁰

General Movements

In the last 30 years, objective assessment of videotaped general movements by Precht's method has been shown to be predictive of later CP.^{9,16,18,41} The quality of general movements at 2 to 4 months post-term (so-called fidgety GM age) has been found to have highest predictive value in the detection of the infants at risk for CP development.⁴² It seems that assessment of the quality of GM is a window for early detection of children at high risk for developmental disorders.^{17,42} Method is simple and it is based on the so called Gestalt evaluation of GM complexity and variation.^{17,41,42} Assessment of GMs at 2 to 4 months post-term at so called fidgety GM age has been found to have the highest predictive value for development of CP if abnormal.^{17,41,42}

Heinz Prechtl's work enabled that spontaneous motility during human development has been brought into focus of interest of many perinatologists prenatally and developmental neurologists postnatally.^{9,16,18,41,42} According to the research preceding Prechtl's ingenious idea, during the development of the individual the functional repertoire of the developing neural structure must meet the requirements of the organism and its environment.⁴¹ This concept of ontogenetic adaptation fits excellently to the development of human organism, which is during each developmental stage adapted to the internal and external requirements.⁴¹ Prechtl stated that spontaneous motility, as the expression of spontaneous neural activity, is a marker of brain proper or disturbed function.^{41,42} The observation of unstimulated fetus or infant which is the result of spontaneous behavior without sensory stimulation is the best method to assess its central nervous system capacity.⁴¹ All endogenously generated movement patterns from unstimulated central nervous system could be observed as early as from the 7 to 8 weeks of postmenstrual age, with developing a reach repertoire of movements within the next 2 or 3 weeks, continuing to be present for 5 to 6 months postnatally.¹³ This remarkable fact of the continuity of endogenously generated activity from prenatal to postnatal life is the great opportunity to find out those high risk fetuses and infants in whom development of neurological impairment is emerging. The most important among those movements are GMs involving the whole body in a variable sequence of arm, leg, neck and trunk movements,

with gradual beginning and the end. They wax and wane in intensity, force and speed being fluent and elegant with the impression of complexity and variability. GMs are called fetal or preterm from 28 to 36 to 38 weeks of postmenstrual age, while after that we have at least two types of movements: writhing present to 46 to 52 weeks of postmenstrual age and fidgety movements present till 54 to 58 weeks of postmenstrual age.^{18,41,42} Lack of fluency and existence of considerable variation and complexity are the main characteristics of mildly abnormal GMs.⁴³ When complexity, variation and fluency are absent, than we are dealing with definitely abnormal GMs.⁴³

The quality of each individual movement includes speed, amplitude and force combined in one complex perception.^{13,18,41-44} Investigation of normal and neurologically impaired preterm infants showed that except for higher incidence of cloni in the abnormal group, there was no marked difference in the quantity of different motor patterns studied.^{44,45} However, video analysis of another group of sick preterm infants revealed a 'reduction of elegance and fluency as well as variability, fluctuation in intensity and speed rather than any change in incidence of distinct motor patterns'.⁴⁴⁻⁴⁶ Based on postnatal studies, it would be very important to seek for abnormal quantity and quality of prenatal movements in order to find fetuses neurologically at risk.⁴⁶

Some facts are very important in the assessment of GMs. The first is that evaluation of GMs should be based on the video recorded movements either pre- or postnatally. The second fact is that when assessing GMs one should use so called 'gestalt perception', which could be described as overall impression of GMs with standardized procedure.⁴¹ During the perception one should recognize the movement patterns of GMs, than assess their complexity, variability and fluency.^{41,42} According to Hadders-Algra, GMs could be classified as normal-optimal, normal-suboptimal, mildly abnormal and definitely abnormal.⁴² This modality of GM assessment is important for the prenatal and postnatal observation of GMs. It is not so important to assess the quantity of GMs, while the assessment of their quality is of utmost importance in terms of the prognosis of neurodevelopmental outcome. They can better predict neurodevelopmental outcome than classical neurologic examination alone.⁴⁷

We can conclude that prenatal and postnatal assessment of GMs according to Prechtl's method gives quite new insight on the function and development of central nervous system. This important modality is time consuming and requires some technology and expertise to be practiced, but advantages of its implementation in prenatal and postnatal life are very promising and

encouraging in terms of its prognostic value. Prenatal assessment of GMs is well-developed and established, while prenatal assessment needs sophisticated real time 4D ultrasonographic or other technology in order to enable more precise assessment of GM quality in fetuses.

Continuity of GMs from Prenatal to Postnatal Life

Postnatal studies of neonatal behavior have taught us that the assessment of behavior is a better predictor of neurodevelopment disability than neurological examinations.⁴⁶ It is important to mention that postnatal observation of movement patterns was introduced by Prechtl et al in the way that they have been observing spontaneous movements of the infant using video typing and 'off-line' analysis of both quantity and quality of the movement.^{17,48} They proved that assessment of general movements in high risk newborns has significantly higher predictive value for later neurological development than neurological examination.^{46,47,49} Kurjak et al conducted a study by 4D ultrasound and confirmed earlier findings made by 2D ultrasonography, that there is behavioral pattern continuity from prenatal to postnatal life.²⁷ Assessment of neonatal behavior is a better method for early detection of CP than neurological examination alone.⁵⁰ It is being speculated that intrauterine detection of encephalopathy would improve the outcome. Although, many fetal behavioral studies have been conducted, it is still questionable whether the assessment of continuity from fetal to neonatal behavior could improve our ability of early detection of brain pathology. Early detection could possibly rise an opportunity to intervene and even prevent the expected damage.

Could Some Postnatal Signs of Neurological Disability be used Prenatally?

It has been proven by now that ultrasonography is a powerful tool in the assessment of fetal behavior. 4D sonography brought up to light visual observation of the fetus, particularly in two especially important domains: fetal finger movements and facial expressions.^{19,51} This new technology is not only a toll of fetal observation but a very useful toll to evaluate the development of fetal CNS in normally developing fetuses and those at high risk. A basic understanding of fetal neurology includes: defining of motor pathways involved, chronology of their maturation and direction of myelination.^{52,53} This information helps clinician in better interpretation of fetal movements. The experience acquired with the Amiel-Tison's Neurological Assessment at Term (ATNAT) helps us in interpretation of fetal movements.^{39,54,55}

The domain of fetal neurology is already too extensive, but the focus of interest is mainly second trimester, despite the fact that spontaneous fetal mobility emerges and has already become differentiated at a very early age.⁵⁶ This means that we will take into a consideration period of pregnancy from 20 till 40 weeks of gestation, including the end of the neuronal migration and the postmigratory phase corresponding to the development of neocortex.^{4,57}

As it was already mentioned, CP describes a group of disorders of the development of movement and posture, causing activity limitations, which are attributed to nonprogressive disturbances occurring at the time of fetal brain development.⁵⁸⁻⁶¹ Motor disorders which occur in patients with CP are often accompanied by disturbances of sensation, cognition, communication, perception, behavior, and/or with seizure disorder.⁵⁸⁻⁶¹ 'Disturbances' is a term that refers to events or processes that in some way influence the expected pattern of brain maturation.⁵⁵ Those events or processes are many, with consequences varying from very conspicuous to very subtle. It should be kept in mind what many neurologists emphasize, that morphology does not always correspond to neurological outcome.^{39,54,55} The opposite view is the one from pediatricians and neurophysiologists, who are involved in long-term follow-up studies, and they are certainly not that optimistic. It would be wise to consider long run prognosis, for each specific type of fetal brain damage and make appropriate decisions for conservative management.

Hopes have been headed toward MR, but in many cases brain changes can not be detected as early as the first year of life, like for example pathological gliosis which causes secondary hypomyelination.

While examining the fetal head by 4D, sonographer should examine bony structures and fetal cranial sutures, if they are folding over one another, it is considered to be a bad sign previously described by Amiel-Tison.^{39,55} The same sign should be searched for postnatally, as a part of neurological examination.⁶²

The majority of pediatricians believe that the main obstacle for early prediction of CP based on a functional observation of the fetus such as visual observation by 4D sonography, is due to the 'precompetent' stage of most of the motor behavior observed *in utero*.^{39,55} One of the possible signs detected could be high arched palate, described by Amiel-Tison, in clinical assessment of the infant nervous system.^{39,55} What was believed as undetectable became visible by 4D. Recently, the 3D 'reverse face technique' has been described. This technique overcomes shadowing the fetal face by rotating the frontal facial image through 180° along the vertical axis, so that the palate, nasal cavity and orbits become visualized.^{63,64}

In their early work, Pooh and Ogura examined⁶⁵ normal fetuses by 3D/4D. The purpose of their study was to investigate the natural course of fetal hand and finger positioning.²⁶ During 9th and on the beginning of 10th week fetal hands were located in front of the chest and no movements of wrists and fingers were visualized. From the middle of 10th week, active arm movements were observed.²⁶ This study is very important, because it is showing that finger and thumbs movements begun in the early stage of human life, long before the maturation of the upper system. Therefore this motor activity depends on the lower system and not before 30 to 32 weeks switches to the upper control.

Amiel-Tison also described so called neurologic thumb squeezed in a fist. Clenched fingers can also be detected by 4D sonography, as well as overlapping cerebral sutures.^{19,26}

Head anteflexion becomes visible during 10th and 11th gestational week, according to de Vries et al.⁵⁶ However, the activity of flexor muscles will depend on the upper system since 34 weeks of gestation. The absence of active head flexion explored by the raise-to-sit maneuver is one of the major neurological signs at 40 weeks of gestation.^{39,54,62}

Indeed, recent advances of technology and especially of three-dimensional (3D) and four-dimensional (4D) ultrasound, have given us the opportunity to study in real time and with explicit detail parts of fetal anatomy and fetal activity, that we could not imagine it would be possible even a few years ago. As we already described, we now know that there is a specific fetal behavioral pattern that corresponds to each week or trimester of fetal life and this pattern reflects the steps of human brain development and maturation.^{12,66-68} It is clear that the development of the human brain is a long lasting procedure that with specific developmental stages, starting from the first few weeks of *in utero* life and continues long after birth, in such a way that it remains incomplete at the end of *in utero* life and continues to evolve for decades after birth⁶⁸ (Table 3). Human brain development is also very sensitive and affected by wide variety of factors and defects at any of stage of fetal or neonatal life. For example in neonates born very prematurely the brain development *ex utero* as much as we try to resemble the intrauterine environment cannot follow the genetically programmed growth pattern that the brain should have *in utero*.^{69,70} Genetic factors, external stimuli, pathological conditions or even environmental changes, can affect the fetal human brain up to a degree that may be difficult to assess, especially prenatally. Many times we cannot detect fetal brain impairment, and even in cases when we suspect that fetal brain impairment



Table 3: Major events in neural development

<i>Developmental event</i>	<i>Peak time of occurrence</i>
• Primary neurulation (dorsal induction)	3-4 weeks antenatally
• Prosencephalic cleavage (ventral induction)	5-6 weeks antenatally
• Neuronal proliferation	
– Cerebral	2-4 months antenatally
– Cerebellar	2-10 months postnatally
• Neuronal migration	
– Cerebral	3-5 months antenatally
– Cerebellar	4-10 months antenatally
• Neuronal differentiation	
– Axon outgrowth	3 months—birth
– Dendritic growth and synapse formation	6 months—1 year postnatally
• Synaptic rearrangement	Birth—years postnatally
• Myelination	Birth—years postnatally

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may exist, in most cases we are unable to clarify how will the fetus be affected. Neurological compromises may occur prenatally, perinatally and/or even postnatally or neonatally, and their clinical picture, varies ranging from mild behavioral and learning disabilities to severe cerebral palsy (CP).⁶⁸ Indeed, neurological disability is one of the most feared complications in obstetrics and its diagnosis antenatally is one of the greatest challenges. The assessment fetal neurobehavior both in normal and abnormal cases is a major project, since first of all we need to study and understand the normal cases before we can compare them with the pathological ones in order to draw safe conclusions.^{70,71}

CAN WE ASSESS FETAL BEHAVIOR WITH ULTRASOUND?

We know that fetal behavioral patterns reflect the degree of development and maturation of the fetal nervous system, and it has also been proved that the quality and quantity of fetal movements reflect the neurological integrity of the fetus.^{1,48,72-79} The introduction of two dimensional ultrasound allowed the assessment of fetal anatomy, but also the direct monitoring of fetal activity.¹⁸ Fetal movements were studied and analyzed with 2D even 30 years ago,^{18,80-82} and further studies suggested that the assessment of fetal behavior in the specific periods of *in utero* life could make it possible to distinguish between normal and abnormal brain developmental patterns.^{50,82-84} Advances in ultrasound technology introduced 3D and 4D ultrasound, offering exceptional images of the fetus *in utero* and in real time, allowing to evaluate even the movements and the behavior of the fetus *in utero*, overcoming the problem of subjective

poorer quality images obtained with 2D ultrasound.⁸⁴⁻⁸⁸ 4D ultrasound allows imaging of details of fetal face and fetal expressions (e.g. smiling, crying, mouthing and blinking), something that cannot be achieved with 2D ultrasound. Studies have shown that with 4D ultrasound it is feasible to distinguish between normal and abnormal behavioral patterns of the fetus, which could eventually lead to early diagnosis of brain impairment, enabling us to produce measurable parameters for the assessment of normal neurobehavioral development.^{20,89-91}

Fetal movements occur much earlier than when pregnant women can feel them, even during the embryonic period starting with gross, a synchronized movements of the whole embryo and finally leading to organized and detailed movements, as well as facial expressions towards the end of the pregnancy.^{92,93} Regarding neonates the assessment of neonatal behavior is a better predictor of neurodevelopmental disability than neurological examination. So, the question would be whether by studying fetal behavior we could identify cases of normal and abnormal brain development, and also whether we could achieve earlier diagnosis of various structural or functional abnormalities of the fetal nervous system.^{12,18,50,66-68,80-83,109} The first structured and systematic way for assessing the integrity of the fetal central nervous system, by using 4D ultrasound, was called KANET, and its innovation is that it assesses the fetus *in utero* in the same way that neonates are examined postnatally for brain damage, incorporating parameters from neonatal neurological tests (Amiel-Tison), with the use of 4D ultrasound.^{37,38,94,95}

WHAT IS KURJAK'S ANTENATAL NEURO-DEVELOPMENTAL TEST (KANET)?

Kurjak's antenatal neurobehavioral test (KANET) is a new scoring system for the assessment of fetal neurobehavior based on prenatal evaluation of the fetus by 3D/4D ultrasound.⁹⁴ It is a combination of some parameters consisting of fetal general movements (GM) and of postnatal Amiel-Tison neurological assessment at term (ATNAT) signs, which can be easily visualized prenatally by using 4D ultrasound.^{25,38} The following parameters have been incorporated in the KANET test: isolated head anteflexion, overlapping cranial sutures, head circumference, isolated eye blinking, facial alterations, mouth opening (yawning or mouthing), isolated hand and leg movements and thumb position, Gestalt perception of general movements (overall perception of the body and limb movements with their qualitative assessment).

Several papers have shown that there is a continuity of behavior from pre- to postnatal life and it has been observed that all movements which are present in

neonates are also present in fetal life, with the exception of Moro's reflex, which cannot be demonstrated in fetuses.⁹⁶ This is probably due to a different environment to which fetus and neonate are exposed. The fetus lives in an environment of microgravity, while the newborn is exposed to full gravity, which creates certain obstacles for neurodevelopment in the first months of life.⁷⁰ The parameters were chosen based on developmental approach to the neurological assessment and on the theory of central pattern generators of general movements emergence, and were the product of multicentric studies conducted for several years.^{25,30} KANET is a combination of assessments of fetal behavior, general movements and three out of four signs which have been postnatally

considered as symptoms of possible neurodevelopmental impairment (neurological thumb, overlapping sutures and small head circumference).⁹⁷

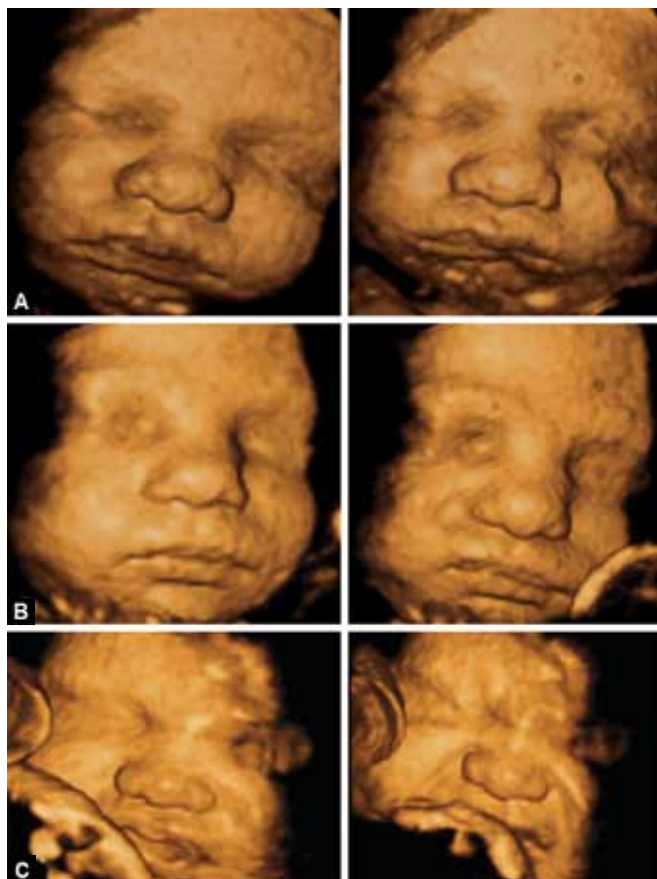
KANET test has been standardized, it is reproducible and easily applied by fetal medicine specialists.⁹⁷ KANET should be performed in the 3rd trimester of pregnancy, between 28 and 38 weeks. The duration of the examination should last between 15 and 20 minutes, and fetuses should be examined while they are awake. If the fetus is in the sleeping period, the assessment should be postponed for 30 minutes or for the following day, at a minimum period of 14 to 16 hours. In cases of grossly abnormal or of borderline score, the test should be repeated every 2 weeks until delivery. Special attention should be paid



Fig. 5A: Normal KANET score at 34 weeks of pregnancy



Fig. 5B: Normal KANET score at 32 weeks of pregnancy—the impact of the evolution of ultrasound technology on the quality of fetal assessment



Figs 6A to C: Face grimacing

to the facial movements and to eye blinking, which are prenatally very informative and important ('the face is the mirror of the brain'). Overall number of movements should be defined in very active or inactive fetuses and compared with normal values of previous studies^{25,30} (Figs 5 and 6). All the examiners should have extensive hands-on education for the application of KANET test, both in low and in high-risk pregnancies. Interobserver and intraobserver variability should be available. It is advisable to use 4D ultrasound machines, with frame rate of minimum 24 volumes/second. KANET consists of eight parameters (Table 4). A score range of 0 to 5 is characterized as abnormal, a score calculated from 6 to 13 is considered borderline and a score range of 14 to 20 is normal (Table 5). After that neonates should be followed up postnatally for neurological development for a 2 years period.

The test evaluates quantitative as well as qualitative aspects of fetal motor behavioral patterns. The parameters examined by this test are a combination of general movements (GMs) and parameters adopted from ATNAT.^{98,99} The criterion of quality and quantity of spontaneous GMs is believed to have excellent reliability in evaluating the integrity of fetal CNS.^{83,100} Furthermore a continuity of behavioral patterns from prenatal to the postnatal period has been proven.^{27,101,102} Both those facts


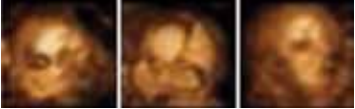







justify the choice of the parameters used in this test, making KANET theoretically appropriate for the assessment of fetal behavior. According to previous reports^{28,29,103-106} KANET easily recognizes serious functional impairment associated with structural abnormalities. Studies have shown that application of KANET in both low and high risk populations has given good results and especially in high risk populations, KANET may provide useful information regarding the neurological outcome of these fetuses.¹⁰⁷ KANET is the first test which is based on 4D ultrasound, with an original scoring system and has been standardized, so it can be implemented in everyday practice, overcoming the practical difficulties and covering the gaps of methods that were used in the past for the evaluation of fetal behavior.^{16,108-110} Studies show that KANET is easily applicable to most pregnancies, the learning curve is reasonable for physicians who already have training in obstetrical ultrasound and the actual duration of KANET ranges from 15 to 20 minutes, showing strong evidence that it can be widely implemented in everyday clinical practice.¹⁰⁶

WHAT HAVE STUDIES ABOUT KANET SHOWN SO FAR?

One of the first studies to use a preliminary form of the KANET scoring system was that by Andonotopo et al in 2006. They aimed to assess fetal facial expression and quality of body movements and examine if they are of diagnostic value for brain impairment in fetuses with growth restriction. In that prospective study of 50 pregnancies with IUGR fetuses in the 3rd trimester of pregnancy, a tendency of less behavioral activity in IUGR than normal fetuses has been noted. The results of the study encouraged future investigation of the use of 4D ultrasound for quantitative and qualitative assessment of fetal behavior as possible indicators of the neurological condition in IUGR fetuses.²⁹

The Zagreb group in 2008, were the first to introduce the KANET for the assessment of neurological status of the fetus, aiming to the detection of fetal brain and neurodevelopmental alterations due to *in utero* brain impairment. In order to develop the new scoring system they identified severely brain damaged neonates and neonates with good neurological condition and then compared the neonatal findings, with corresponding findings *in utero*. In the group of 100 low-risk pregnancies they retrospectively applied KANET. After delivery, postnatal neurological assessment ATNAT was performed and all neonates assessed as normal reached a score between 14 and 20, which was assumed to be the score of optimal neurological development. New scoring system was applied in the group of 120 high-risk pregnancies in

Table 4: Proposal for the new KANET assessment tool consisting of eight parameters

Sign	Score		
	0	1	2
Isolated head anteflexion 	Abrupt	Small range (0-3 times of movements)	Variable in full range, many alteration (> 3 times of movements)
Cranial sutures and head circumference 	Overlapping of cranial sutures	Normal cranial sutures with measurement of HC below or above the normal limit (-2 SD) according to GA	Normal cranial sutures with normal measurement of HC according to GA
Isolated eye blinking 	Not present	Not fluent (1-5 times of blinking)	Fluency (> 5 times of blinking)
Facial alteration (grimace or tongue expulsion)  Or mouth opening (yawning or mouthing) 	Not present	Not fluent (1-5 times of alteration)	Fluency (> 5 times of alteration)
Isolated leg movement 	Cramped	Poor repertoire or small in range (0-5 times of movement)	Variable in full range, many alternation (> 5 times of movement)
Isolated hand movement  Or hand to face movements 	Cramped or abrupt	Poor repertoire or small in range (0-5 times of movement)	Variable in full range, many alternation (> 5 times of movements)
Fingers movements 	Unilateral or bilateral clenched fist, (neurological thumb)	Cramped invariable finger movements	Smooth and complex, variable finger movements
Gestalt perception of GMS	Definitely abnormal	Borderline	Normal

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Table 5: Interpretation of KANET scores

Total score	Interpretation
0-5	Abnormal
6-9	Borderline
10-16	Normal

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which, based on postnatal neurological findings, three subgroups of newborns were identified: normal, mildly or moderately abnormal and abnormal. Based on this, a neurological scoring system has been proposed. All normal fetuses reached a score from 14 to 20. Ten fetuses who were postnatally described as mildly or moderately abnormal achieved a prenatal score of 5 to 13, while another ten fetuses postnatally assigned as neurologically abnormal had a prenatal score 0-5. Among this group four had lobar holoprosencephaly, one had severe hypertensive hydrocephaly, one had thanatophoric dysplasia and four fetuses had multiple malformations. This study inspired a large series of multicenter studies (Table 6) that used the KANET in order to assess the usefulness of this promising new scoring system for the assessment of neurological status in fetuses and the recognition of signs of early brain impairment *in utero*.^{85,91}

The results of the first multicenter study, which included 288 high risk pregnancies, from four different centers, were published in 2010. They identified seven cases with abnormal KANET and twenty five cases with borderline KANET score, yielding 32 fetuses at neurological risk. There were also 11 cases with abnormal KANET, of which 6 fetuses died *in utero* and 5 were terminated. The seven remaining neonates with abnormal KANET were followed up postnatally at 10 weeks and out of these seven cases, three were found to have abnormal ATNAT scoring postnatally. These were a case of arthrogryposis, a case of vermis aplasia and a fetus whose previous sibling had verified cerebral palsy. The fetuses in these three cases had especially reduced facial movements – the faces were like masks during the ultrasounds. The remaining four cases were considered normal (ventriculomegaly, pre-eclampsia, thrombophilia, oligohydramnios). Out of 25 borderline KANET there were 22 borderline newborns by ATNAT, whereas three were normal (ventriculomegaly, syndrome of intra-amniotic infection, maternal thrombocytopenia). Those who were abnormal prenatally and normal postnatally had the following prenatal risk-factors: ventriculomegaly, Dandy-Walker malformation, skeletal dysplasia, polyhydramnios, gestational diabetes, hydrocephaly, thrombophilia, pre-eclampsia, achondroplasia, oligohydramnios, non-immune hydrops, intra-amniotic infection, IUGR, trisomy 21, thrombocytopenia. Out of three abnormal neonates, neonates after ATNAT

assessment two had definitely abnormal Prechtl's premature GMs (arthrogryposis and vermis aplasia) and an additional six were considered abnormal (neonate of the mother with the previous child with CP, Dandy-Walker syndrome, hydrocephaly, trisomy 21, ventriculomegaly, non-immune hydrops). The remaining 21 children had normal optimal or normal suboptimal GMs. During their study they also followed the pregnancy of a fetus with acrania, which the mother had refused to terminate due to religious reasons, documenting the evolution of the fetal behavior from 20 weeks and as the motor control was shifting from the lower to the upper control center the fetus ended up with a very low KANET score. The authors reached the conclusion that there is a potential for antenatal detection of serious neurological conditions, especially in identifying the fetuses from high-risk pregnancies at neurological risk.¹⁰³

Miskovic et al applied KANET in 226 cases, both high and low risk pregnancies and compared the results. They found three cases of abnormal KANET, that had chromosomal abnormalities and all three had abnormal ATNAT, as well. The KANET scores from both groups were compared to the results of the ATNAT tests, and found statistically significant difference among the low and the high risk groups, for eight out of the ten KANET parameters (isolated anteflexion of the head, eye blinking, facial expressions – grimacing, tongue expulsion, mouth movement such as yawning, jawing, swallowing – isolated hand movements, hand to face movements, fist and finger movements and general movements). Comparison of KANET and ATNAT showed statistically significant, moderate correlation between the two tests, which means that the neuropsychiatric exam ATNAT confirmed the prenatal findings of 4D ultrasound examination KANET. The authors concluded that these preliminary results were promising and stated that further studies are needed before the test could be recommended for wider clinical practice.¹⁰⁶

Talic et al around the same period, in a multicenter study, published the largest series of KANET so far, with 620 singleton pregnancies, both low- and high-risk cases (100 low risk and 520 high risk cases), excluding however fetuses with structural abnormalities, that were studied between 26 and 38 weeks of gestation. Fetuses with congenital anomalies multiple pregnancies were excluded from the study. The high-risk group of patients consisted of the following subgroups: threatened preterm delivery with or without preterm rupture of membranes (PPROM), previous child diagnosed with CP, hypertension in pregnancy with or without pre-eclampsia, diabetes before pregnancy or gestational diabetes, intrauterine growth restriction, polyhydramnios, Rhesus isoimmunization, placental bleeding and maternal fever >39°C. Analysis

Table 6: List of studies that have applied KANET test to different populations

Author	Year	Study	Study design	Study population	Indication	No	GA (weeks)	Time (mins)	Result	Summary
Kurjak et al ¹⁰⁹	2008	Cohort	Retro-spective	High-risk	Multiple	220	20-36	30	Positive	A new scoring system was proposed for the antenatal assessment of fetal neurological status
Kurjak et al ¹⁰³	2010	Multi-center	Prospective	High-risk	Multiple	288	20-38	30	Positive	KANET appeared to be prognostic of antenatal detection of serious neurological fetal problems. KANET also identified fetuses with severe structural abnormalities, especially associated with brain impairment
Miskovic et al ¹⁰⁶	2010	Cohort	Prospective	High-risk	Multiple	226	20-36	30	Positive	Correlation between antenatal (KANET) and postnatal (ATNAT) results was found. KANET showed differences of fetal behavior between high and low-risk pregnancies
Talic et al ¹⁰⁵	2011	Multi-center cohort	Prospective	High-risk	Multiple	620	26-38	15-20	Positive	KANET test had a prognostic value in discriminating normal from borderline and abnormal fetal behavior, in normal and in high-risk cases. Abnormal KANET scores were predictable of both intrauterine and postnatal death
Talic et al ¹⁰⁷	2011	Multi-center cohort	Prospective	High-risk	Ventriculomegaly	240	32-36	10-15	Positive	Statistically significant difference was identified in KANET scores between normal pregnancies and pregnancies with ventriculomegaly. Abnormal KANET scores and most of the borderline-scores were noted in fetuses with severe ventriculomegaly, especially associated with additional abnormalities
Honemeyer et al ¹¹¹	2011	Cohort	Prospective	Unselected	Unselected	100	28-38	N/A	Positive	Normal prenatal KANET scores had a significant predictive value of a normal postnatal neurological evaluation
Lebit et al ⁸⁷	2011	Cohort	Prospective	Low-risk	Normal 2D examination	144	7-38	15-20	Positive	A specific pattern of fetal neurobehavior corresponding to each trimester of pregnancy was identified
Abo-Yaqub et al ¹⁰⁴	2012	Cohort	Prospective	High-risk	Multiple	80	20-38	15-20	Positive	Significant difference in KANET scores was noted. All antenatally abnormal KANET scores had also an abnormal postnatal neurological assessment

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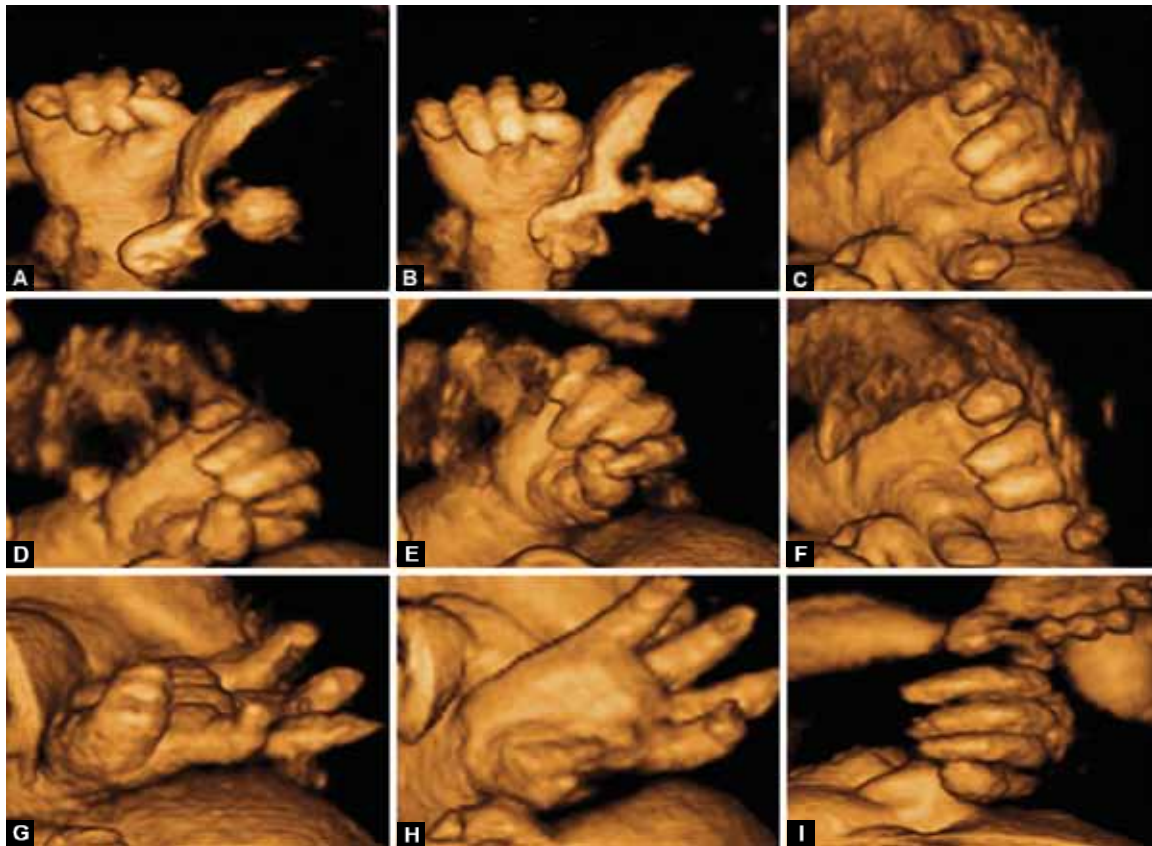
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Vladareanu et al ¹¹²	2012	Cohort	Prospective	High-risk	Multiple	196	24-38	N/A	Positive	Most fetuses with normal KANET → low-risk, those with borderline → IUGR fetuses with increased MCA RI and most fetuses with abnormal KANET → threatened PTD with PPROM. Difference in fetal movements was identified between the 2 groups. For normal pregnancies → 93.4% of fetuses achieved normal score, for high-risk pregnancies → 78.5% of fetuses had a normal score
Honemeyer et al ¹¹³	2012	Cohort	Prospective	High and low-risk	Multiple	56	28-38	30	Positive	Introduction of the max average KANET score → combination of the mean value of KANET scores throughout pregnancy. Revealed a relationship of fetal diurnal rhythm with the pregnancy risk
Kurjak et al ¹²³	2013	Cohort	Prospective	High and low-risk	Multiple	869	28-38	20	Positive	Statistically significant differences in the distribution of normal, abnormal, and borderline KANET scores between low-risk and high-risk groups were found. Fetal behavior was significantly different between the normal group and the high-risk subgroups
Predojevic et al ¹²⁴	2013	Case study	Prospective	High-risk	IUGR	5	31-39	30	Positive	KANET could recognize pathologic and borderline behavior in IUGR fetuses with or without blood flow redistribution. Combined assessment of hemodynamic and motoric parameters could enable in better diagnosis and consultation
Athanasiadis et al ¹²⁵	2013	Cohort	Prospective	Unselected (High and low-risk)	Multiple (IUGR, PET, GDM)	152	2nd and 3rd trimester	N/A	Positive	The neurodevelopmental score was statistically significant higher in the low-risk group compared to the high-risk group ($p < 0.0004$). The diabetes subgroup score was statistically significantly higher compared to the IUGR and the pre-eclampsia subgroup ($p = 0.0001$)

KANET: Kurjak's antenatal neurological test; No: Number of patients; IUGR: Intrauterine growth restriction; MCA: Middle cerebral artery; PTD: Preterm delivery; PPROM: Preterm premature rupture of membranes; PET: Pre-eclampsia; GDM: Gestational diabetes mellitus

of the data confirmed statistically significant difference in the distribution of fetal KANET scores between the two populations. Impressively the largest incidence of fetuses with abnormal KANET was noticed in the subgroup of participants with a previous child diagnosed

with cerebral palsy (23.8%) and the largest incidence of fetuses with borderline KANET was observed in the subgroup of mothers with fever (56.4%). The following parameters of KANET test significantly differed between the fetuses from low and high-risk pregnancies:



Figs 7A to I: Hand and finger movement



Fig. 8: KANET—facial alterations mouthing, eye blinking and hand movement

overlapping cranial sutures, head circumference, isolated eye blinking, facial expressions, mouth movements, isolated hand movements, isolated leg movements, hand to face movements, finger movements and general movements. The authors observed that a low KANET score is predictive of both intrauterine or neonatal death

– they had two intrauterine deaths in fetuses with low KANET (scores of 3 and 4 respectively) and one neonatal death (with a KANET score of 2). In 10 out of 36 fetuses with abnormal KANET after 2 and 6 months, postnatal neurological examination indicated severely abnormal finding: four of them had severe generalized spasticity.

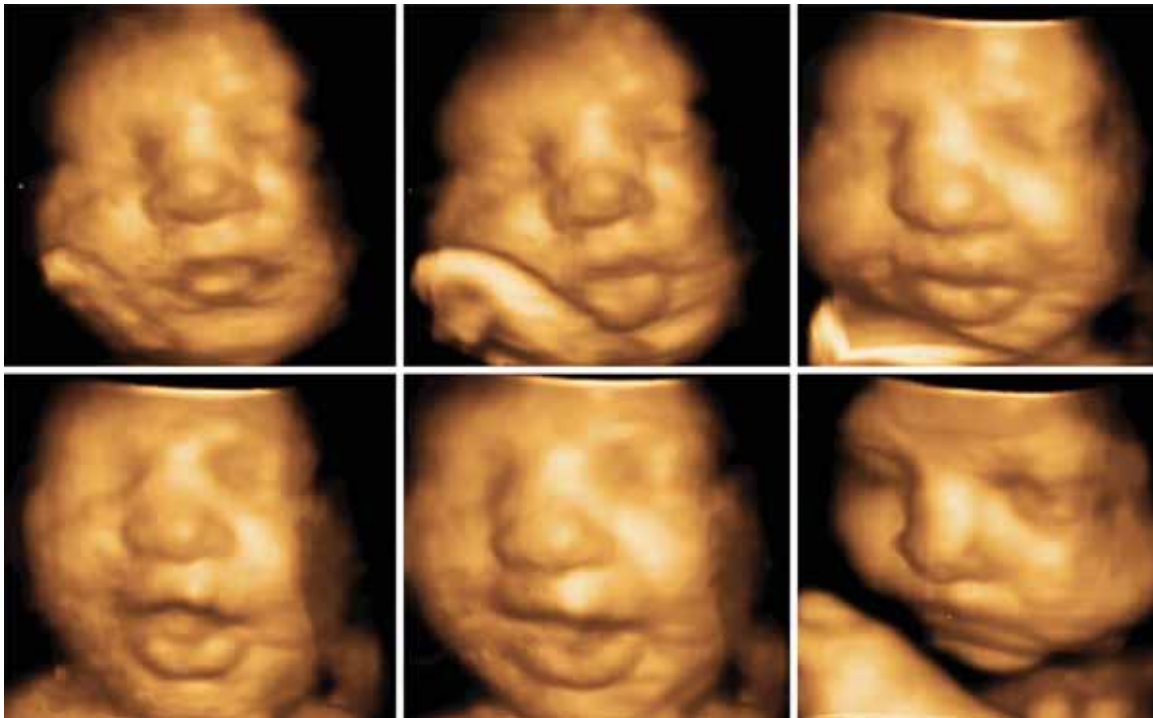


Fig. 9: Tongue expulsion and mouthing

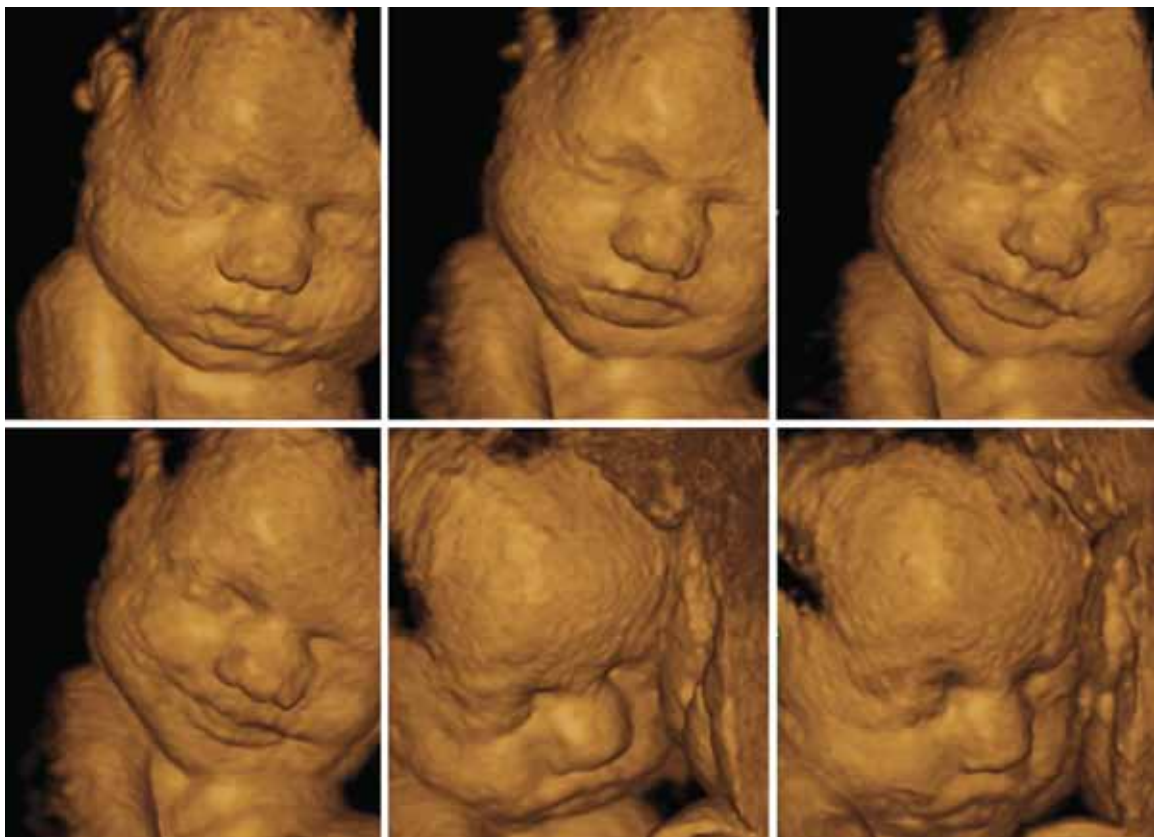


Fig. 10: Smiling

The study demonstrated the potential of KANET to detect and discriminate normal from borderline and abnormal fetal behavior in normal and in high-risk pregnancies. Other neonates are still followed up in this study, in order to reach safe conclusions.¹⁰⁵

Honemeyer et al studied 100 fetuses, who underwent, between 28 and 38 weeks of gestation, up to 3 times during their pregnancy assessment by KANET. The fetuses were followed-up postnatally, immediately after delivery and again at 12 weeks of life, with systematic

neurological assessment by the neonatologist. The results from the scoring systems of pre- and postnatal evaluation were compared. Results showed that a normal prenatal KANET score is significantly predictive of normal postnatal neurological assessment of the newborn immediately after delivery and at 12 weeks of life. The authors concluded that that normal antenatal KANET scores is a very good predictor of a normal postnatal neurological outcome.¹¹¹

Lebit et al used part of the KANET to assess fetal movements throughout pregnancy in 144 low-risk pregnancies, between 7 and 38 weeks of gestation, concluding to a specific pattern of fetal behavior for each trimester of pregnancy.⁹³ The authors noticed that in the first trimester fetal movements grow rapidly in frequency and complexity, while in the second half of pregnancy the motor behavior significantly increases in frequency and variability. Facial expressions and eye movements also appear in second trimester, with the first eye movements starting at about 18 weeks. In late pregnancy fetal movements show a decline and the periods of rest start to grow. This decrease is rather a consequence of the brain maturation process rather than reduced amount of amniotic fluid.^{86,92} They concluded that dynamic evaluation of fetal behavior reflects directly the processes of maturation and development of the central nervous system and that KANET test has much to offer in the assessment of fetal behavior.⁹³

An important study was that by Talic et al which aimed to assess the differences in fetal behavior in both normal fetuses and fetuses with cerebral ventriculomegaly, by using KANET. They studied 240 fetuses between 32 and 36 weeks of gestation, 140 fetuses with ventriculomegaly and 100 normal fetuses. Six percent of the fetuses from the low risk-control group had pathological KANET scores, while 34.9% of the fetuses with ventriculomegaly had pathological KANET. The largest number of abnormal KANET scores was found in 22 fetuses with severe ventriculomegaly, accompanied by other structural abnormalities (Dandy-Walker, Arnold-Chiari, agenesis of the corpus callosum, holoprosencephaly, encephalocele, spina bifida, choroid plexus cyst, osteogenesis imperfect type II, thanatophoric dysplasia type I and Meckel Gruber syndrome). There were no fetuses with abnormal KANET in the group of isolated mild and moderate ventriculomegaly. The authors concluded that prenatal neurological findings of the fetuses by application of KANET test is in concordance with their postnatal outcome and that evaluation of fetal behavior by KANET in fetuses with cerebral ventriculomegaly had the potential to detect fetuses with abnormal behavior, adding a functional dimension of the central nervous system evaluation to the brain morphology. Also, the degree

of ventriculomegaly and the presence of coexisting congenital malformations, appeared to be important factors determining the final KANET score. The results of this study were very positive and showed that KANET could provide useful information for the correct assessment and counseling of patients with a common finding, such as ventriculomegaly, the significance of which is not well defined.¹⁰⁷

More recently, our collaborators from Qatar, Abo-Yaqoub et al studied 40 pregnant women with high risk pregnancies for neurological abnormalities, between 20 and 38 weeks of gestation using KANET scoring system and compared the results with 40 low-risk cases, in order to determine the role of 4D ultrasound in prenatal assessment of fetal neurobehavior and in the prediction of adverse neurological outcome. The difference in the range of KANET score was significant between the 2 groups and all cases with abnormal KANET proved to be abnormal postnatally, whereas those with normal or borderline KANET scores were neurologically normal at least in the early neonatal period that they were assessed. The parameters that were significantly different between the two groups were: isolated head anteflexion, isolated eye blinking, facial expressions, mouth movements, isolated hand movements hand-to-face movements, finger movements and general movements. For isolated leg movements and cranial sutures, the difference was not significant.¹⁰⁴

Vladareanu et al applied KANET in 196 singleton pregnancies (61 low risk and 135 high-risk patients) between 24 and 38 weeks of gestation in a period of 3 years. Most fetuses in the study who obtained normal KANET score belonged to the low-risk pregnancies, those who obtained borderline score were fetuses with intra-uterine growth restriction (IUGR) and with increased resistance index (RI) of middle cerebral artery (MCA) and most fetuses with abnormal KANET score derived from pregnancies complicated by threatened preterm delivery with PPROM. There was statistical significant difference in fetal movements in the two groups. In normal pregnancies, most fetuses (93.4%) achieved a normal KANET score compared to 78.5% of the fetuses from high-risk pregnancies. Borderline and abnormal scores were dominant in high-risk pregnancies. In the high-risk pregnancy group, most abnormal KANET scores were in pregnancies complicated by threatened preterm delivery with PPROM (25%). Most fetuses with pregnancies complicated by IUGR with MCA RI index changes and with hypertension above 160/100 mm Hg achieved borderline score (50%). The highest percentage of normal fetal movements was found in pregnancies complicated by Rh isoimmunization without hydrops fetalis (96%). The characteristics of reduced speed and amplitude were

found in the threatened preterm delivery group. There was a reduction of both number and duration of general movements in the IUGR group. The IUGR fetuses moved less and their general movements were poorly organized. Alterations in the quality of fetal movements were accompanied by considerable decrease in the quantity of fetal movements. The authors concluded that KANET can be useful for early diagnosis of neurological disorders that become manifest in perinatal and postnatal period.¹¹²

Honemeyer et al studied 56 singleton pregnancies (24 low-risk and 32 high-risk cases) between 28 and 38 weeks of gestation and applied serial KANETs on them, performing a total of 117 tests in total. They did not identify any abnormal KANET scores, but two thirds of the borderline scores occurred in the high-risk pregnancies. Because they performed more than one KANET in each pregnancy they introduced the average KANET score, which derived from the scores of each fetus during pregnancy. Only one fetus had a borderline average KANET score, and this fetus who belonged to the high-risk group, was the only one out of 56 pregnancies who had an abnormal early neurological outcome. When the authors compared all the 18 borderline KANET scores with fetal diurnal rhythm based on maternal observation, they noticed that 89% of the borderline scores of the at-risk group were recorded at times that the mothers characterized them as active periods, compared with 33.3% in the low-risk pregnancies. The authors concluded that KANET is suggestive of expressing the risk for neurodevelopmental fetal disorders, but the connection of fetal diurnal rhythm and pregnancy risk status should be investigated further.¹¹³

Kurjak et al studied 869 high and low-risk singleton pregnancies taking under consideration the results of the Doppler studies of umbilical and middle cerebral arteries, and noticed that fetal behavior was significantly different between the normal group and the following subgroups of fetuses: fetal growth restriction (FGR), gestational diabetes mellitus, threatened preterm birth, antepartum hemorrhage, maternal fever, sibling with cerebral palsy, and polyhydramnios.¹²³ The authors concluded that their study showed a new clinical application of the KANET test in early identification of fetuses at risk for adverse neurological outcome.

CONCLUSION

One of the greatest challenges of obstetrical ultrasonography is the better understanding of fetal neurological function.^{95,114} Neurological problems, such as cerebral palsy are poorly understood and often falsely attributed to intrapartum events, while for the majority of cerebral palsy cases it has been proven that the causative

pathway starts long before delivery.¹¹⁵⁻¹¹⁷ Several attempts have been made in order to define normal and abnormal fetal neurological function and to develop a method of assessment of the integrity of the fetal nervous system, but still without satisfactory sensitivity.^{103,116-118}

Fetal behavioral patterns are directly reflecting developmental and maturational processes of fetal central nervous system.¹¹⁶⁻¹¹⁸ It has been suggested that the assessment of fetal behavior during different periods of gestation may provide valuable information about normal and abnormal brain development, and contribute to the early diagnosis of various structural or functional neurological abnormalities.⁸⁰ The introduction of three and four dimensional ultrasound (3D and 4D) allowed real time assessment of fetal behavior. Details of the fetal face, and especially movements of mouth, eyes (facial expressions) and fingers have been made possible with the introduction of 4D ultrasound.^{31,119-122} KANET is the first method that attempted to use 4D ultrasound in order to assess and combine parameters of fetal behavior and form a scoring system that would assess the fetus in a comprehensive and systematic approach, in the same way that neonatologists perform a neurological assessment in newborns during the first days of their life, in order to determine their neurological status.⁹³ KANET appears to be able to identify functional characteristics of the fetus that predict normal and abnormal neurological development and hopefully future results of the prospective multicentric studies that are taking place at the moment in the next few years it will provide more information on fetal neurology. Such information will be of great value in counseling mothers of high-risk pregnancies, like for example in cases with previous child with cerebral palsy and also provide valuable evidence for cases of litigation.

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