

Are We Ready to investigate Cognitive Function of Fetal Brain? The Role of Advanced Four-dimensional Sonography

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ABSTRACT

Human brain is fascinating organ in so many ways. Some of its cognitive functions, such as ability to learn, memorize, think, percept different sensations, such as pain, to have emotion, process audio-visual inputs, and to coordinate reaction and movements have been subjects of studies for many years. Yet, till recently, we could only make assumptions about prenatal activities, interactions and its construction of complex structures in the time frame of antenatal life. With the prenatal assessment (sonoembryology, neurosonoembryology, KANET test, etc.) by latest advanced HDlive, Silhouette and Flow 3D/4D imaging there is possibility to follow in continuity normal structural and functional development from the early beginnings of "life" and on the other hand consider what might be different (not necessarily abnormal) and deviate from normal development and behavior.

On this way, we are able to supplement knowledge of fundamental building blocks of development of fetal cognitive functions, to pay more attention and follow up fetuses at higher risk and finally find some of the possible origins of cognitive dysfunctions which may manifest in childhood or later in life.⁸² With the introduction of different 3D/4D ultrasound modes we have ability to observe all of this *in vivo* while emerging, and make "time-lapse" of fetal neurodevelopment and behavior in correlation to its cognitive functional development.

Keywords: Fetal awareness, Fetal behavior, Fetal facial expression, Fetal neurodevelopment, Four-dimensional ultrasound, Prenatal cognitive function.

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INTRODUCTION

Today, we are witnessing a rapidly changing world. Revolutionary improvements and implementation of new technologies in the last 30 years are amazing. This is very true especially in the medical field. Only few of true visionaries could foresee this.

Advances in functional magnetic resonance imaging and four-dimensional (4D) ultrasound (US) imaging made it possible to study fetal anatomy – structural development as well as fetal intrauterine activities – functional development, so finally we can confirm some of the assumptions and get new insights about fetal behavior in all different stages of intrauterine life.

While observing different fetal behaviors, very similar to what babies do too, we could not help but wonder if fetus is aware of its environment, and if so, in what degree? Is it consciously responding, or is it just a reaction of automatic reflex?

Why is this so important to us?

Human brain is a fascinating organ in so many ways. Some of its cognitive functions, such as ability to learn, memorize, think, percept different sensations, such as pain, to have emotion, process audiovisual inputs, and to coordinate reaction and movements have been subjects of studies for many years. Yet, till recently, we could only make assumptions about prenatal activities, interactions, and the ability to construct complex structures in the time frame of antenatal life. With the introduction of different 4D US modes, we have the ability to observe all of this *in vivo* while emerging, and make "time lapse" of fetal neurodevelopment and behavior in correlation to its cognitive functional development.

Brain structure as well as function has been immensely intriguing to people for ages. Nowadays, brain research accounts for up to 16% of all scientific production in the world and there are 1,730,000 active researchers who have already published nearly 1.79 million scientific papers in the last 5 years (Scopus database).¹

Olesen et al² recently published a report of estimation of annual costs for brain disorders, which accounts for more than 35% of the overall cost and burden to the health care system of the European Union.

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It is not strange that the 21st century is proclaimed as “the century of the mind.” With all these reasons, the greatest challenges of neuroscience remain to be solved out. How does mind work? Question of genetic and evolutionary basis of exclusive human cognitive functions, such as speech, awareness, self consciousness and understanding of the views of others – Theory of mind.¹

FUNDAMENTAL BUILDING BLOCKS OF DEVELOPMENT OF FETAL COGNITIVE FUNCTIONS

Understanding the course and timing of fetal neurodevelopmental events in correlation to motor and sensory system development is essential for determining how environmental effects can affect certain structures as well as functions.³

During the fetal period, brain develops rapidly with a rate of 250,000 neurons per minute.⁴ Neocortex is intensively under construction,⁵ and 99% of it is created during the fetal period of life.^{6,7} Most of the 100 billion neurons and related major pathways found in our adult brains have formed already in fetal period and are present already at birth.^{4,6}

Furthermore, the maturation of cerebral cortex is a very complex and dynamic process dependent and influenced by intrinsic (genetic, biological basis) as well as extrinsic factors (intrauterine as well as extrauterine), stimuli, and environment.⁸ However, the degree of contribution of each is still unclear and is the subject of studies.

Some of the of different cellular processes that participate in the brain maturation process occur through precise spatiotemporal gene expression and include proliferation, migration, differentiation, synaptogenesis, myelination, synaptic pruning, and apoptosis.⁹⁻¹¹

Even though the number of neurons and related major pathways are present already at birth, their quantitative and qualitative features (synaptogenesis, axon networks, myelination) as well as strength of the connections still need fine-tuning and continue to develop during the postnatal life with continuous interaction with environment.¹² In addition, change and adaptation are lifelong processes. The biological basis of emotional and social behavior is formed by activity of complex local and remote neural networks and related basal ganglia.¹²

Very well-organized and intense process of neuro-maturation of the cerebral cortex is promoted by the subplate (SP), a transient population of neurons that leads the development of cortical and thalamocortical connections.¹³

Between the 8th and 16th weeks of gestation, migrating neurons construct the SP zone, a transitory area of the fetal

brain where increased number of synapses and neuronal interaction are observed, but also earliest cortical electric activity at 19 weeks of gestation.^{14,15} This is also part of the brain involved in developmental plasticity if there is a perinatal brain damage.¹⁶

The most recent study¹⁷ of quantitative assessment of telencephalic wall confirmed the relevance of SP compartment during development of fetal brain. In the period of mid-gestation (13–25 postconceptional weeks, PCW), the SP zone occupies nearly half of the total hemispheric volume. This is when the intensity of neuronal proliferation and relative volume of proliferative zones decrease remarkably; at the same time synapse and extracellular matrix-rich SP compartment continues to grow during the first two trimesters, occupying up to 45% of telencephalon, reaching its maximum volume and thickness around 30 PCW (28 gestational weeks, gw) and being almost four times thicker than the cortical plate (CP). After this period, it decreases again.¹⁷

Subplate zone of the fetal brain disappears after about 34 weeks of gestation (32 PCW). This phenomenon could be related to the pattern of growth of thalamocortical and corticocortical pathways.^{14,17-20}

This could be explained by increased amount of cortical afferents “waiting” within SP, forming transient synapses and then continuing into cortex.^{14,18-20}

After penetration of thalamocortical fibers into the CP, between 24 and 28 PCW (22–26 gw),²¹ an additional convergence of associative and commissural fibers holds up in the SP zone before entering the CP.^{14,21,22} The installation of thalamocortical pathways is essential for cortical analysis of sensory inputs. Functional connection between the periphery and cortex can be verified by evoked potentials from 29 weeks of gestation onward.²³

So, in conclusion, these connections (thalamocortical and corticocortical) are fundamental for cortical processing of sensory information and mental processes that coincide with the age limit for premature baby survival.¹³

Starting from the third trimester, formation of synapses speed up to approximately 40,000 synapses per minute.²⁴ Appearance of six-layered lamination of the neocortex could be seen after 32 gw due to neuronal differentiation and laminar distribution of the thalamocortical axons.¹⁴ Even though neocortex is formed, it still is very immature and subunits of the brain stem remain the main regulators of all fetal behavioral patterns until delivery.²⁵

BEHAVIORAL COMPLEXITY PROGRESSES DURING THE FETAL LIFE

It begins in first trimester with spontaneous fetal movements at around 7.5 weeks of gestation, verifying the

presence of afferent–efferent pathways in spinal cord.²⁶ In late first trimester from 8 to 9 gw onward, more organized and complex movements can be observed, which are the first signs of supraspinal influence on motor control.^{27,28} By mechanical stimulation of the body, fetus can produce reflex movements when sensory nerves have reached the skin.²⁹

Touch is the first sense to develop at 6 to 7 gw by presence of nerve terminals and fibers deep in the skin (process sensations, such as touch, vibration, and temperature). At around 10 gw, the number of nerve terminals increases and moves toward the outer surface of the skin. These are nociceptors which can detect pain, like surgical tissue damage.^{30,31}

Perioral region, hands, lower limbs become touch sensitive at, respectively, 7.5, 10.5, and 14 gw. Development of taste buds starts from 7 gw. Innervations of deeper fetal internal organs start from 13 gw and progress during the gestation. Nociceptors gradually mature during the next 6 to 8 weeks.^{32,33} According to this, it seems that pain is not possible before the presence of nociceptors prior to 10 gw.²⁹ However, presence of nociceptors alone is not sufficient. Input about tissue stimulation can reach the spinal cord from 8 gw, and hormonal stress response at 18 gw induced by needle puncture shows us that input about tissue damage reaches the midbrain.²⁹

It has been shown that noxious stimuli can initiate physiological, hormonal, and metabolic responses, but these neither imply nor preclude suffering, pain, or awareness.^{29,34}

When electrical energy reaches the threshold, action potential is induced and driven toward the central nervous system. Intact pathway from periphery (skin) nerve fibers to spinal cord, thalamus, primary sensory cortex, insular cortex, and anterior cingulate cortex is mandatory.

At the age of 18 gw, local spinal cord or brain stem reflexes control movement and, even as movement becomes more coordinated from 24 weeks, it does not require the involvement of higher brain centers. The lack of cortical connections before 24 weeks, therefore, implies that pain is probably not possible until after 24 weeks and continuing development and elaboration of intracortical networks. Of course, there is controversy, someone could argue that even a simple sense of pain involves more than reflex activity and, therefore, requires connection to functional, higher regions of the cortex. At this point, there is no simple answer, and this will remain controversial until there would be a way of resolving these empirically.^{29,35–37}

Nevertheless, even more important, scientists now know that exposure to maternal stress depending on the cause, timing, duration, and intensity of stress can have

very harmful effects on the fetus. The role of stress is not straightforward.³ The individual's biological stress responsivity, which is necessary to complete the picture, is not yet included.

Long-term effects of unfavorable intrauterine environment have been extensively studied, and unconscious reactions to the pain and stress, such as the secretion of stress hormones and their effect are probably more dangerous for the development of the fetus than possible conscious experience and memory of pain.³⁸

There has been a significant number of published papers and studies (mainly by Croatian and Japanese group)^{39–56} describing 4D US assessment of fetal behavior during prenatal development through different gestational stages. With the introduction of 4D US modes, we have the chance to present observations of fetal facial expressions of smiling, yawning, and swallowing, in real time. The universal perception of intrauterine life was brought on a totally new level. Yawning develops at 11.5 to 15.5 gw.⁵⁷ However, the physiological role of yawning during intrauterine life remains speculative,^{58–60} it is presumed that it can be used as an index of dopaminergic system functioning.⁶¹

Some of the more recent observational studies proved that yawning can be reliably distinguished from other forms of mouth opening, using 4D US, and can be used as an index of fetal healthy development.⁶²

Many studies are done on twins. The best way to get some interesting new insights into the origin of some disorders is to study them on monozygotic (monochorionic preferably) twins. Some of the studies (a.o.) confirmed the genetic basis for autism.

Studies of twins with 4D US are even more interesting, as we can observe their interaction with each other *in vivo*. To our knowledge, the first study to observe twin behavior with 4D US during all three trimesters of pregnancy was published by Kurjak et al.⁴⁷ Observations had been made on the first intertwin contacts in the 1st trimester and behavior and Kurjak Antenatal Neurodevelopmental Test (KANET) score of the twins in the 2nd and the 3rd trimesters.

First intertwin contacts were observed at 61 postmenstrual days (8+5 weeks). Complex movements were seen from 68 postmenstrual days onward (9+5 weeks). These results differ from those reported with two dimensions, probably due to a better visualization of fetal movements with 4D. The complexity of intertwin contacts increases from 84 postmenstrual days (12 weeks) onward. With advancing gestational age and diminishing distance between twins, more complex contacts were seen. The more complex interactions develop just a few days after the simplest ones. This implies that either the central and peripheral nervous system already has the appropriate maturation

or that the learning process is very fast.⁴⁷ Different facial expressions were observed and recorded. Probably the most impressive one was of the twin painful facial expression after being kicked in the face with the leg of the cotwin.^{47,65}

In the 4D US study of Myowa-Yamakoshi and colleagues,⁶⁶ it was observed that opening of the mouth proceeded the hand-to-mouth movement. This can be seen as anticipatory behavior of the fetus which tries to reach the mouth, and somehow realize what is the way to move the hand in order to do that. This could be explained as movement based on knowledge intersensory motor relations in fetal bodies.⁶⁶

In the study, movements toward the cotwin and self-directed ones toward the eye region were noted (Fig. 1, our observation). It was concluded that movements directed at the cotwin are not coincidental: Twin fetuses already perform movements aimed at the cotwin from the 14th gw.⁶⁷

Monozygotic twins do have (except for some rare cases) identical genetic basis, but they do not have identical brains and that is because learning leads to anatomical changes in the brain, and even identical twins will have different social experiences, different learning experiences, and therefore will end up having different brains.^{68,69}

In addition, and according to our knowledge so far, we believe that fundamentals of this process of learning start already in prenatal life and speed up after birth. Finally, neural elements involved in emotional, social, and cognitive functioning develop structurally by 3 years of life; however, functional organization of neuronal networks of the cerebral cortex continues to develop in interaction with environment during later life.¹²

From the simple spontaneous fetal movements observed in the first trimester, progression in behavior

continuous to the development in further period in more complex movements and activities and rise to crescendo with presumed preferences for the sound of mother's voice, reflecting maturational events that take place in the brain stem, followed by forebrain structures.³⁸

Furthermore, the fetus is able to learn and remember familiar auditory stimuli. The intrauterine origins of memory and learning have been studied profoundly implementing habituation methods, classical conditioning and exposure learning to assess fetal ability to learn, using different stimuli such as vibroacoustic ones and sound of the mother's voice.

Prenatal olfactory learning is again another aspect of fetal memory, which seems to show preference for certain taste. For example, prenatal exposure to sweet taste (low-dose sucrose solution) stimulates swallowing, while bitter taste of poppy seed extracts (Lipiodol) used as contrast into the amniotic fluid does the opposite.^{28,70} All of these findings could be interpreted as evidence for fetal rudimentary, learning-related cognitive-like activity.³⁸

Between 36 and 40 gw, maturation of the visual cortex starts and is characterized by the appearance of surface-positive evoked potentials. This process continues after birth.²⁸ Amygdala is the unifying center for emotions, emotional behavior, and motivation and as such receives inputs from all senses as well as visceral inputs. Stimulation of the amygdala causes intense emotion.⁷¹

Very interesting is that prenatally, there is already established connection between cerebral cortex and amygdala, allowing good emotional interaction with mother by birth. In addition, there is basic recognition of outlines of face, which is at that point predominantly subcortical (amygdala), and during later months there is

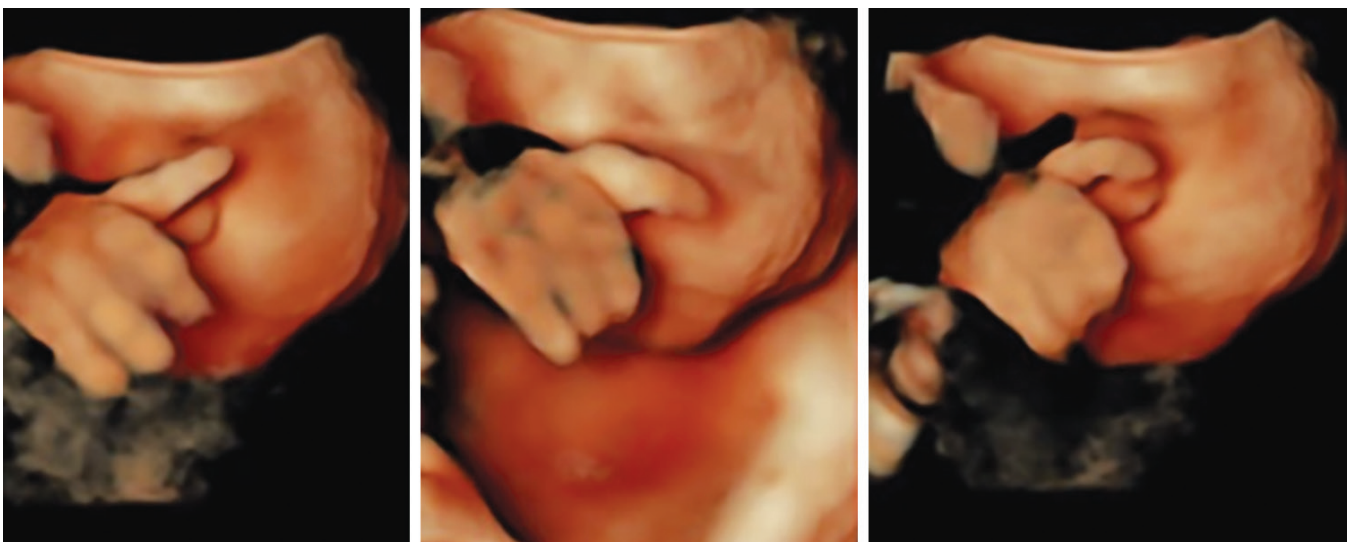


Fig. 1: HDlive sequence of images of the fetus 38 gestational weeks, touching his eye with the finger

development of predominantly cortical mechanisms of face recognition.¹⁶

Face-processing models in modern neuroscience propose the presence of feedback loops between neural structures (temporal, occipital, and frontal brain areas). Certain cortical regions are selectively engaged in face perception: the fusiform face area (FFA) in the fusiform gyrus along the ventral part of the brain, the occipital face area (OFA) in the lateral occipital area, and the posterior region of the superior temporal sulcus. Especially FFA and OFA are involved in face identification but not facial expression.⁷²

There are also some reports on attempts to study the fetal phenotype expression of the fetal temperament.^{63,64,65}

Recognition of face expression is the most important transcultural element of social interaction in humans. Enigmatic functions of brain in resting and “default” phase are present in preterms, which can be explained as beginning of self- and preconsciousness.¹²

The 4D US studies of fetal awareness by Kurjak et al in 2005 enhanced the depiction of all different facial expressions and movements such as scowling, smiling, isolated eye-blinking, tongue expulsion, mouth and eye squeezing, and so on, as these facial expressions or activities might represent fetal awareness³⁴ (Fig. 2).

In the study of Reissland et al,^{73,74} there have been some observations of fetal facial expressions in the

third trimester assessed by 4D US. Fetuses developed complex facial mimics that define a “cry-face-gestalt” or a “laughter-gestalt,” expressing emotions that will communicate powerfully immediately after birth in the regulation of parental care^{75,76} (Figs 3 to 6).

Hata et al⁷⁷ recently published results of the study done by 4D HDlive in the assessment of the twin fetal development in the first and second trimesters of pregnancy, intertwin contacts in the first trimester, the KANET in twin fetuses, and prediction of twin temperament. HDlive facilitates natural and amazingly realistic images which give even better insight in developmental pathways, fetal structural, and functional development.⁷⁷

CONCLUSION

If we compare ourselves as the creation of an amazing puzzle, by putting the pieces together, we will have to go all the way back starting from the first ones, our unique genetic basis, and go on with assembling our embryonic and fetal, prenatal life exactly the way that fits in the puzzle.

How this structural and functional development with interaction of intrauterine environment in our prenatal life takes place, as we discover and confirm in many studies, is of great influence and consequences for development and shaping of behavior in our future

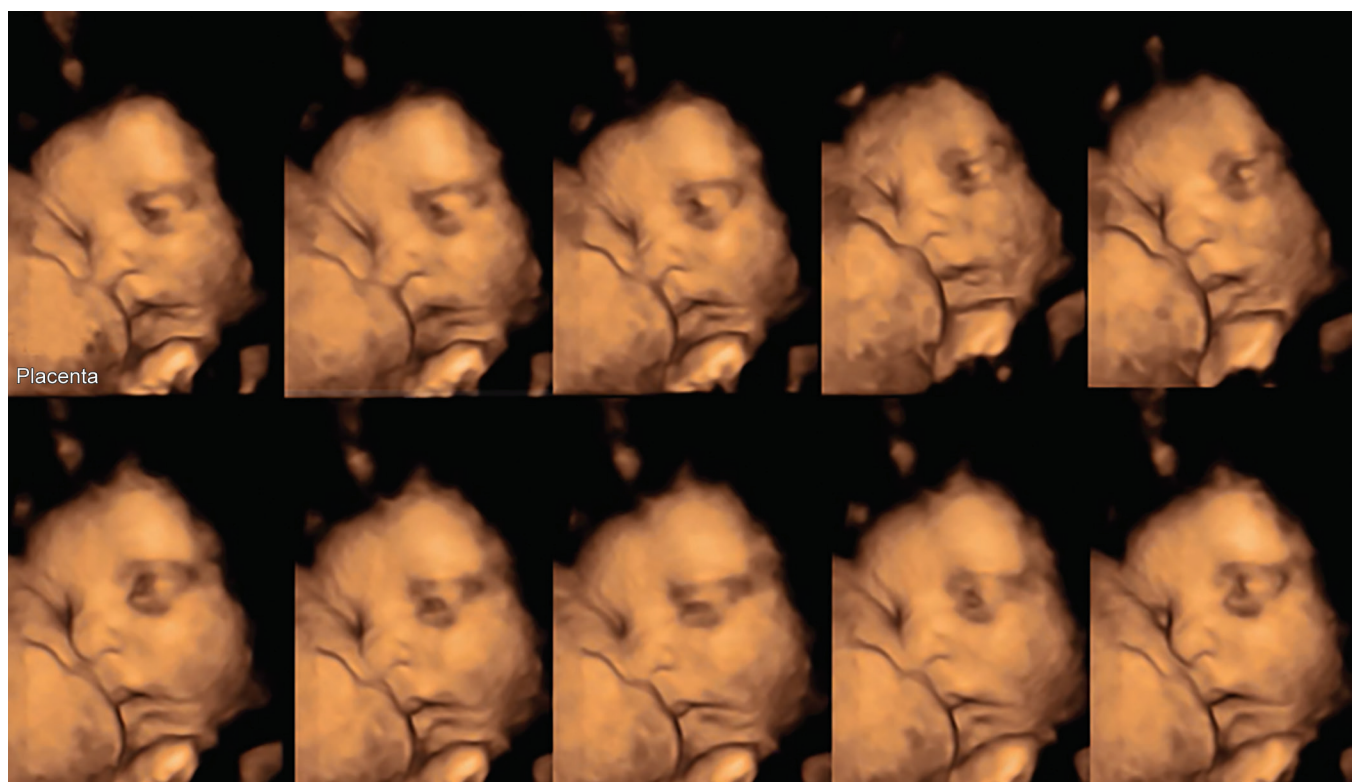


Fig. 2: Sequence of 4D ultrasound images. The 33 gestational week-old fetus. Notice the different position of the eye and facial expression



Fig. 3: Sequence of 4D ultrasound images. Following Figure 2, observation of 33 weeks of gestation old fetus exploring and sucking on the placenta. Notice the awake fetus with different eye movements. We could not help but notice some similarities with neonates searching for the mother's breast and facial expression of some "disappointment" while not finding it. Mother of the child commented just before the examination that she skipped the lunch and she was feeling hungry

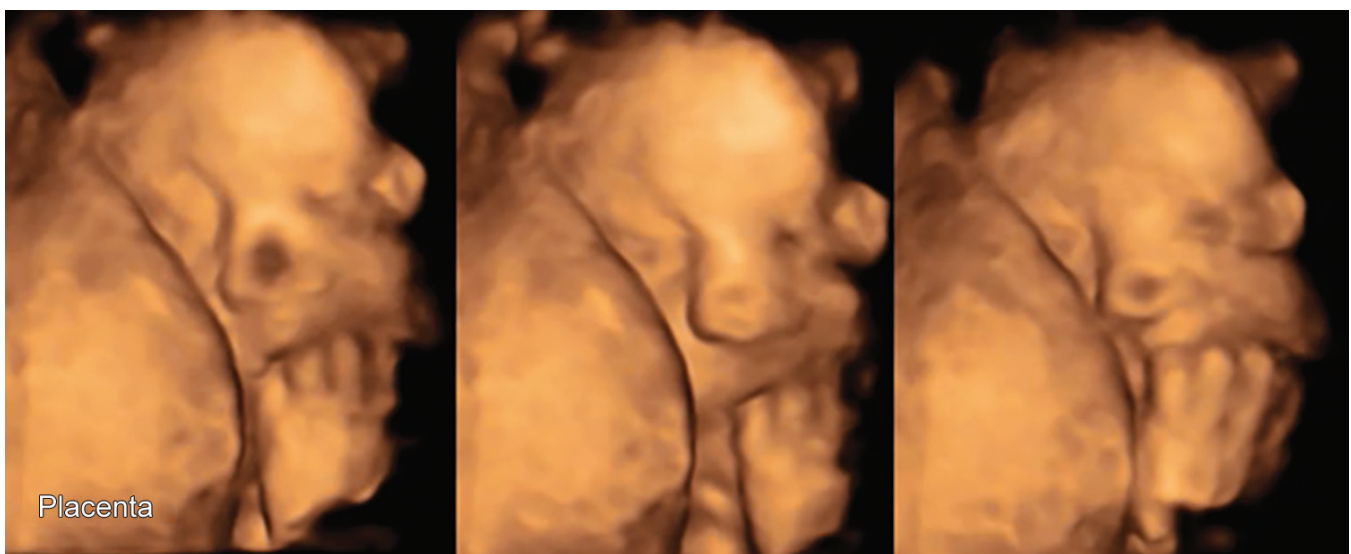


Fig. 4: Following the sequence of images of Figure 2. The same fetus placed his hand in the previously opened mouth and kept on sucking on his fingers

adult life.^{28,38} Roots and triggers for many disorders (behavior, mood, and addictive), metabolic, psychiatric (schizophrenia, autism) and other illnesses, chronic diseases (hypertension, diabetes, and cardiovascular diseases), and even cancer (xenobiotic exposure) have been traced to adverse events and effects on the fetus during the intrauterine life.⁷⁸

In addition, prenatal exposure to maternal stress has long-term neurodevelopmental consequences for future human beings. Recent studies have found profound structural changes leading to functional impairment of hippocampus influencing cognitive functions, such

as learning (disability) and memory (impairment) and temperament formation.⁷⁸⁻⁸⁰ Furthermore, it seems that maternal stressful life events during pregnancy are associated even with increased risk for some birth defects, such as cleft lip/palate and cardiovascular defects (tetralogy of Fallot, transposition of the great arteries).⁸¹

Prenatal US imaging, especially new advanced modes such as 4D HDlive and silhouette mode, have brought the possibilities of prenatal assessment of fetal structural, functional, and behavioral development on a totally new level and gave us the chance and ability to closely follow progression of life from its very first beginning.

Showing the mother realistic images of her unborn baby can have even deeper significance and psychological consequences. Allowing better maternal–fetal bonding, reassuring the mother, and making her feel better, as we do positively, will influence her mood and indirectly make stress-free environment for the fetus.

Our aim is to use our tools, such as new 4D US technology, in the best possible ways to learn more and at the same time create an environment that displays the importance of safe and happy environment as the basis for optimal structural, functional, and behavioral development of future conscious human beings.

A multidisciplinary approach will become increasingly necessary to balance the delicate relationship between our knowledge and possibilities.

Entering a world of neuroscience research, we realize that many dilemmas are rather old and we can agree with Aristotle by saying: “As more you know, (you realize), the more you know you do not know.” So, we keep up with the good work.

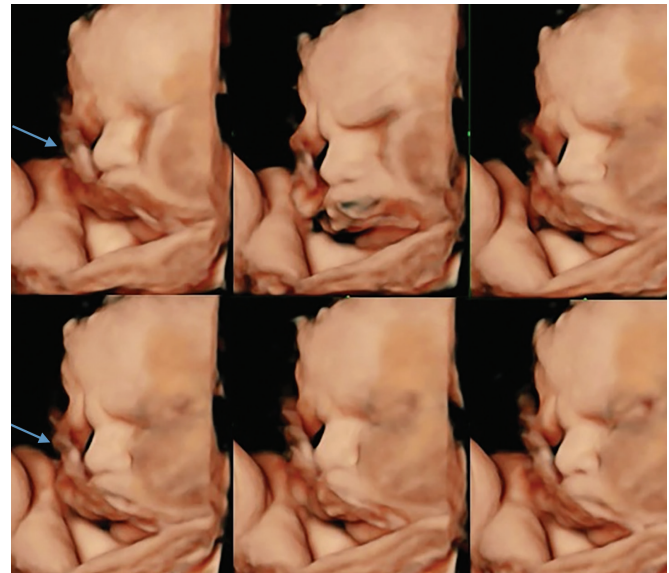


Fig. 5: HDlive mode, sequence of images. Fetus of 40 gestational weeks. Facial expression of “being angry” or “something is bothering him.” Notice the umbilical cord (blue arrow) in front of the face and mouth



Fig. 6: HDlive mode, sequence of images. The same fetus (40 gestational weeks) as in Figure 5, few minutes later, trying to blow the obstacle (umbilical cord) away

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