Advances in Fetal Neurophysiology

[1]Aida Salihagic Kadic, [2]Maja Predojevic, [3]Asim Kurjak

[1]Department of Physiology, Medical School, University of Zagreb, Zagreb, Croatia
[2]Croatian Institute for Brain Research, Medical School University of Zagreb, Zagreb, Croatia
[3]Department of Obstetrics and Gynecology, Medical School University of Zagreb, Sveti Duh Hospital, Zagreb, Croatia

Correspondence: Prof Aida Salihagic Kadic, Department of Physiology, School of Medicine
University of Zagreb, Salata 3, 10 000 Zagreb, Croatia
Tel: 0038514566763, Fax: 0038514590207, e-mail: asalih@mef.hr

Abstract: The human brain function is certainly one of the most amazing phenomena known. All behavior is the result of the brain function. The 100 billion nerve cells are the home to our centers of feelings and senses, pleasure and satisfaction; it is where the centers for learning, memory and creative work are located; where laughing and crying areas and the centers of our mind are. Our cognitive functions, such as thinking, speaking or creating works of art and science, all reside within the cerebral cortex. One of the tasks of the neural science is to explain how the brain marshals its millions of individual nerve cells to produce behavior and how these cells are affected by the environment.[1] The brain function still remains shrouded in a veil of mystery. But what is known is that over 99 percent of the human neocortex is produced during the fetal period.[2] Owing to the employment of state-of-the-art methods and techniques in prenatal investigations, a growing pool of information on the development of the central nervous system (CNS) and behavioral patterns during intrauterine life has been made available. This review outlines these events, along with the development of the fetal sensory system and circadian rhythms, the senses of vision and hearing, fetal learning and memory, and long-term effects of fetal stress on behavior. In brief, this review offers a glimpse of the fascinating world of the intrauterine life.

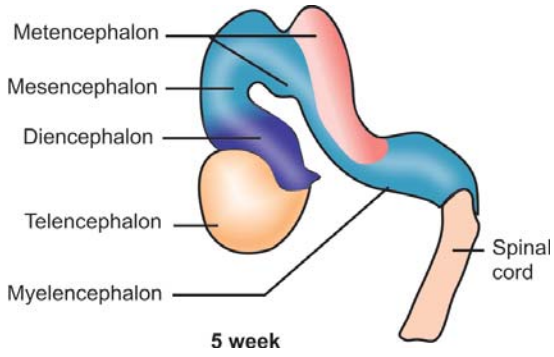
Keywords: Fetal neurodevelopment, behavior, movements, yawning, breathing-like movements, swallowing.

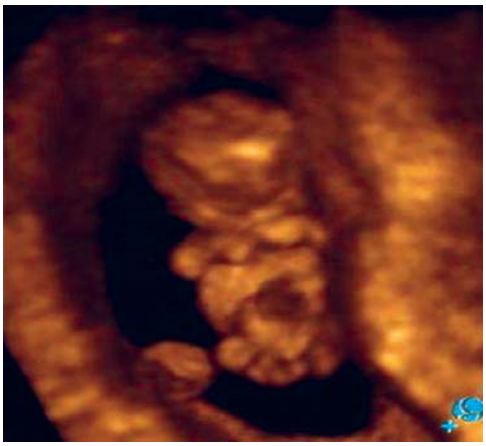
DEVELOPMENT OF THE CNS AND PRENATAL MOTOR DEVELOPMENT

First Trimester

Development of the human CNS begins in the early embryonic period and proceeds through a sequence of very complicated processes long after delivery. Embriology of fetal brain has been extensively described in another article. It is important to point out that the caudal region of the neural tube gives rise to the spinal cord, and the rostral region becomes the brain. The initial development is completed at 7 weeks of gestation (5 weeks after conception), when all five major subdivisions of the brain are clearly visible: the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon (Fig. 1, see reference 3).[4] The earliest interneuronal connections, the synapses, can be detected in the spinal cord shortly before the onset of embryonic motility, at 6-7 weeks of gestation.[5] Therefore, the neural activity leading to the first detectable movements is considered to originate from the spinal motoneurons.[6] Another important prerequisite for the motility is the development and innervation of muscular fibers. It is well known that primitive muscle fibers (myotubes) are able to contract as soon as they are innervated by motor neurons.[7] Between 6 and 8 weeks of gestation, muscle fibers have formed by fusion of myoblasts, efferent and afferent neuromuscular connections have developed, and spontaneous neural activity causing motility can begin.

The main events in the development of the CNS and motor development during the first 8 weeks of pregnancy are presented in Figure 1. The first spontaneous embryonic movements are gross body movements and they can be observed at the 7 to 7.5th weeks of gestation. They consist of slow flexion and extension of the fetal trunk, accompanied by the passive displacement of arms and legs.[8] These, so called, "vermicular" movements appear in irregular sequences.[9] Simultaneously with the onset of spontaneous movements, at the 7.5th week of gestation, the earliest motor reflex activity can be observed, indicating the existence of the first afferent-efferent circuits in the spinal cord.[10] The first reflex movements are massive, and indicate a limited number of synapses in a reflex pathway. General movements are the first complex, well-organized movement pattern, which involve head, trunk and limb movements (Fig. 2). This pattern has been interpreted as the first sign of a supraspinal control on motor activity[11,12] and can be recognized from 8-9 weeks of gestation onwards.[12,13]





3D ultrasound of embryo at 8 weeks of gestation

EARLY FIRST TRIMESTER

Development of the CNS	*Prenatal motor development*
• First interneuronal connections: 6-7 weeks • First neuromuscular connections: 6-8 weeks • First detectable movements—originate from spinal motoneurons • Brain stem: 7 weeks • Basic structures of the diencephalon and cerebral hemispheres: end of the 8th week	• Vermicular movements: 7-7.5 weeks—slow flexion/extension of fetal trunk • First motor reflexes: 7.5 weeks • General movements: 8 weeks—head, trunk and limb movements

Fig. 1: The main events in the development of the CNS and motor development during the first 8 weeks of pregnancy (references are given in the text)

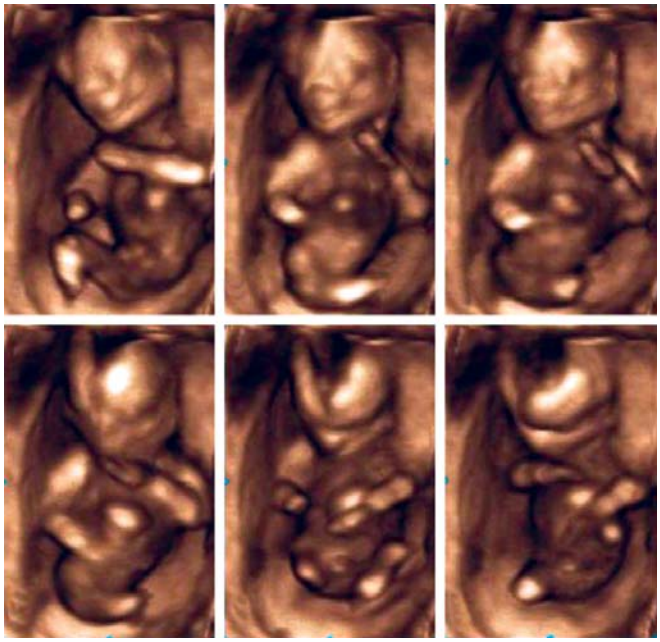


Fig. 2: A sequence of images of the fetus in the 1st trimester recorded by 3D/4D sonography showing general movements

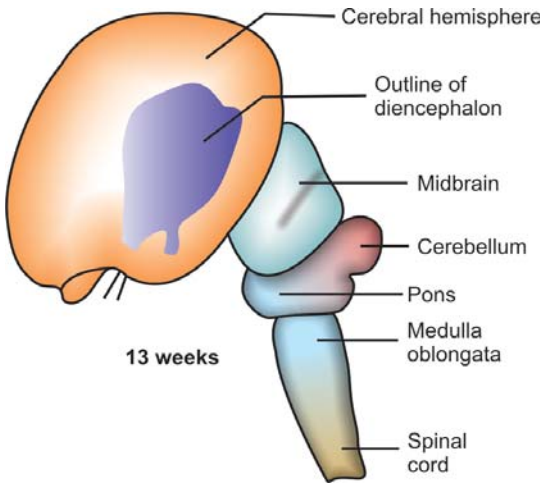
It is very important to note that even at this early stage of development, embryonic and fetal movements appear in recognizable temporal sequences, without any amorphous or random movement. The explanation for this fascinating phenomenon lies in the intrinsic properties of neurons. That means that neural cells begin to generate and propagate action potentials as soon as they interconnect.[14] The interconnected neurons generate patterned activity because of endogenous properties of the neurons.[15] Recent investigations have shown that neurons are able to communicate trough non-synaptic mechanisms even before the onset of synapsogenesis.[16-18]

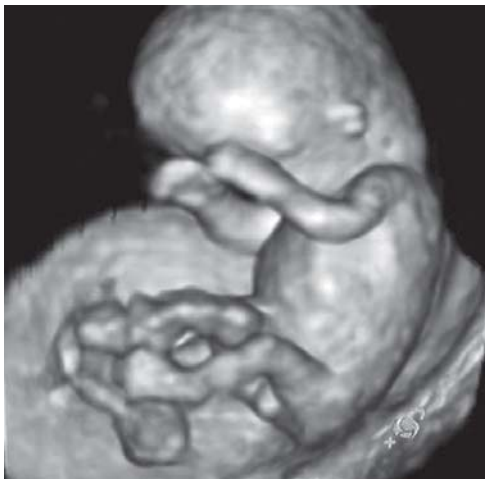
The brain stem is fashioned around the 7th week of gestation[11] and basic structures of the diencephalon and cerebral hemispheres are formed by the end of the 8th gestational week.[19] The remarkable expansion of the cerebral hemispheres follows during the remainder of gestation. The development of synapses in the human cerebral cortex begins after the formation of the cortical plate, at the end of the 10th week of gestation.[16,20]

The brain stem consists of the medulla oblongata, pons and midbrain (Fig. 3). It forms and matures in a caudal to rostral direction. That means that the fillogenetically older structures, such as the medulla oblongata, will form and mature earlier in

the gestation. The major structures of the medulla oblongata are fashioned by the 7-8th week of gestation, and are completely matured by 7 months of gestation.[11] In addition to its many subnuclei, the medulla gives rise to a variety of descending spinal motor tracts which reflexively trigger limb and body movements. It also hosts the five cranial nerves (VIII-XII), which exert tremendous influences on gross body movements, heart rate, respiration and the head turning. As the medulla matures in advance of more rostral structures of the brain stem, reflexive movements of the head, body, extremities, as well as breathing movements (Fig. 3) and alterations in heart rate, appear in advance of other functions. The formation of pons begins almost simultaneously, but its maturation is more prolonged. The structures of the pons include the V-VIII cranial nerves (vestibular nuclei of the nerve VIII) and the medial longitudinal fasciculus (MLF), pontine tegmentum, raphe nucleus and locus coeruleus, which exert widespread influences on arousal, including the sleep-wake cycles. Facial movements, which are also controlled by V and VII cranial nerve, appear around 10-11 weeks.[11]

At 10 weeks of gestation lateralized behavior may be observed, and the fetus begins to show the earliest signs of right- or left-handedness. Stimulation of the brain is known to influence brain organization and it is argued that fetal motor activity may eventually stimulate the brain to develop "handedness" and subsequent lateralization of the function.[21,22] From 10 weeks onwards, the number and frequency of fetal movements increase and the repertoire of movements begins to expand. Qualitative changes in general movements can be also observed. These movements, which are slow and limited amplitude during 8 and 9 weeks, become more forceful at 10 to 12 weeks. After the12th week, they become more variable in speed and amplitude.[23] The isolated limb movements seen at the 9th week of gestation, are followed by the appearance of





3D ultrasound of fetus at 10 weeks of gestation

LATE FIRST TRIMESTER

Development of the CNS	*Prenatal motor development*
• Maturation of brain stem structures • Medulla: VIII -XII cranial nerves • Pons: V-VIII cranial nerves • Midbrain: III-IV cranial nerves; maturation delayed • Synapses in the cerebral cortex: end of the 10th week	• Isolated limb movements: 9 weeks • Hiccups: 9 weeks • Breathing-like movements: 10 weeks • Head flexion and rotation: 10 weeks • Facial movements – jaw opening, yawning: 10-11 weeks • Sucking, swallowing: 11 weeks • Increase in the number and frequency of movements: 10 weeks onwards • Handedness: 10 weeks • Goal orientation: 13 weeks

Fig. 3: The main events in the development of the CNS and motor development during the last 6 weeks of the first trimester (references are given in the text)

the movements in the elbow joint at 10 weeks, changes in finger position at the 11th week, and easily recognizable clenching and unclenching of the fist at 12-13 weeks. Finally, at 13-14 weeks, isolated finger movements can be observed, as well increases in the activity and strength of the hand/finger movements[24] (Fig. 4). Using four dimensional (4D) sonography, Kurjak and collaborators have 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.[25] According to the spatial orientation, they classified the hand movements into several subtypes: hand to head, hand to mouth, hand near mouth, hand to face, hand near face, hand to eye and hand to ear. Our recent longitudinal study, performed by 4 D ultrasound in 100 fetuses from all trimesters of normal pregnancies, has shown increasing frequency of various movement patterns, such as general movements, isolated arm and leg movements, stretching, as well as head movements, during the first trimester. Only the startle movement pattern seemed to occur stagnantly in this period of gestation.[26] Using 4 D sonography, general movements were found to be the most frequent movement pattern between 9 and 14 weeks of gestation.[27] Furthermore, the advanced ultrasonic techniques, three dimensional (3D) and 4D sonography, significantly improve the assessment of structural and functional development of embryonic and fetal CNS.[28-30]

Second and Third Trimester

The second trimester of pregnancy begins at 15 weeks of gestation (13 weeks after conception). Between 15 and 17 weeks, the four lobes of the cerebral cortex have developed.[31] The vast majority of neuron multiplication in the brain is complete by 18 weeks of gestation.[32] The brain stem gradually begins to take the control over fetal movements and behavioral patterns during the first trimester and continues its maturation in the second trimester, resulting in expansion and complexity of the behavioral repertoires.[11] Figure 5 shows the main events in the development of the CNS and fetal motor development in the second trimester. From 14 to 19 weeks of gestation, fetuses are highly active and the longest period between movements last only 5-6 minutes. In the 15th week, 16 different types of movement can be observed. Besides the general body movements and isolated limb movements, retroflection, anteflection, and rotation of the head can easily be seen. Moreover, facial movements such as mouthing, yawning, hiccups, sucking, and swallowing, can be added to the wide repertoire of fetal motor activity in this period.[12] The earliest eye movements appear as sporadic movements with a limited frequency, at 16-18 weeks of gestation.[33,34] The delayed onset of eye movements can be explained with later onset of midbrain maturation. Although the midbrain begins to form at almost the same time as the pons, its maturation does not even begin until the second trimester. It consists of the dopamine producing substantia nigra, the inferior-auditory and superior-visual colliculus, and cranial nerves III-IV, which, together with MLF and cranial nerve VI, control eye movements. Significant trends in fetal eye movement organization can be observed during the second half of pregnancy, especially during the 3rd trimester[33,34] (Table 1).

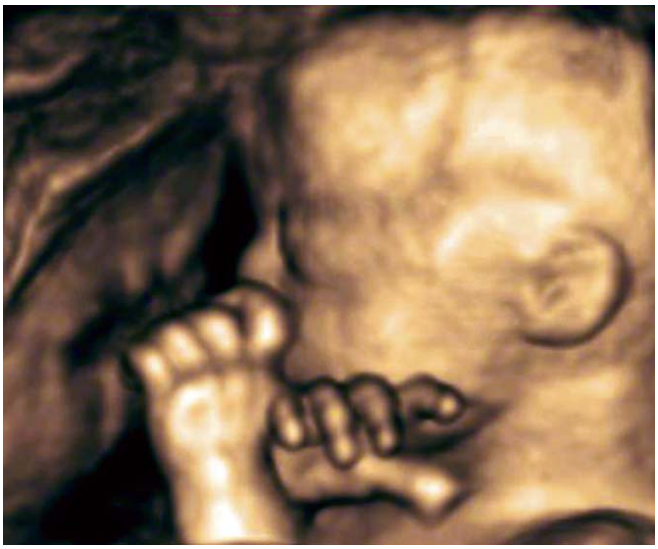
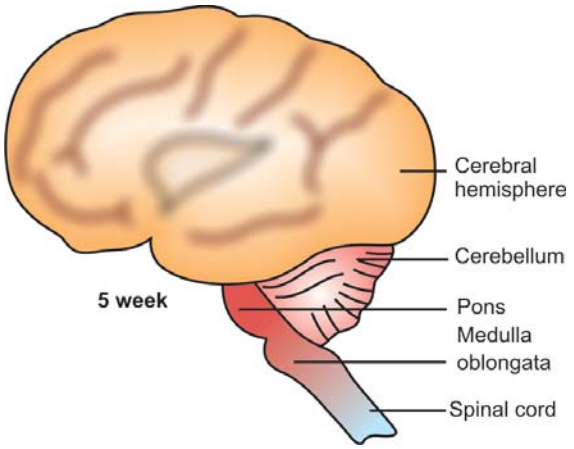


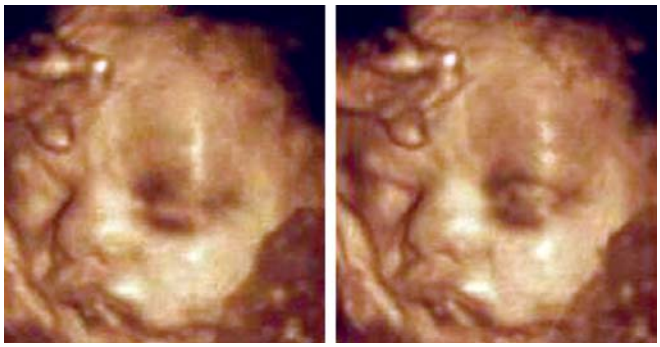
Fig. 4: Image of the fetus recorded by 3D/4D sonography, showing isolated finger movements

Table 1: Trends in fetal eye movements organization during pregnancy (references are given in the text)

	Timing (weeks)	*Characteristics of the eye movements*
Second trimester	**16-18**	Sporadic movements with a limited frequency
	24-26	Movements appear more frequently, periods of eye movements alternate with those without eye movements
Third trimester	**33**	Two types of eye movement can be distinguished during eye movement period, rapid eye movements (REM) and slow eye movements (SEM)
	37-38	Constant mean values of duration of eye-movement and non-eye movement periods are achieved
	36-38	Eye movements are integrated into behavioral states

Fetal human brain has a number of transitory structures, which cannot be observed in the adult human brain. One of the very important zone in the developing cortex is the subplate zone, that is a site for transient synapses and neuronal interactions. The development of subplate zone, between the 15th and 17th week of gestation, is accompanied with an





Fetus at the end of second trimester, taken by 3D/4D sonography. Opened eyelids are clearly visible

SECOND TRIMESTER

Development of the CNS	*Prenatal motor development*
• Maturation of the brain stem continues • Medulla oblongata—almost completely mature by the end of this period • Formation of the subplate zone: 15-17 weeks of gestation • Synaptogenesis: – most intensively: 15-20 weeks • First electrocortical activity: 19 weeks • Spinothalamic tract: 20 weeks • Thalamocortical connections: 24 -26 weeks	• 16 different types of movements can be observed: 15 weeks • High fetal activity: 14-19 weeks; quiescence periods only 5-6 min • Eye movements: 16-18 weeks • Organization of fetal motor patterns: rest-activity cycles - increase in the complexity of movements: 20 weeks • Facial expression patterns (except eye blinking)—a peak frequency: end of the 2 nd trimester

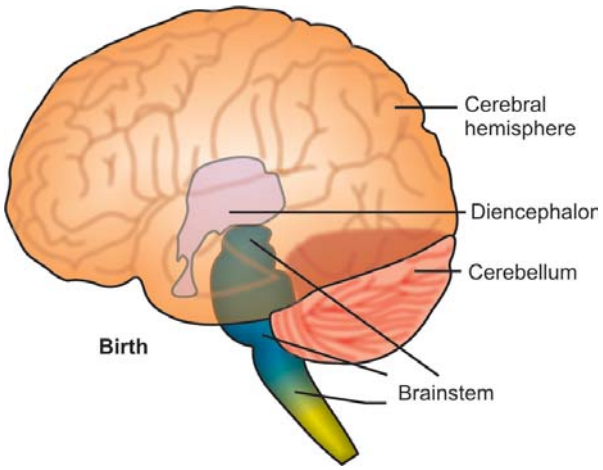
Fig. 5: The main events in the development of the CNS and motor development during second trimester (references are given in the text)

increase in the number of cortical synapses, which probably form the substrate for the earliest cortical electric activity at 19 weeks of gestation.[35] Subplate zone can play a major role in the developmental plasticity following perinatal brain damage.[36] It important to note that by 20 weeks, the cortex has acquired its full complement of neurons[37].

From 20-22 weeks of gestation fetal movements, breathing activity, and heat rate begin to follow daily cycles called circadian rhythms.[38] Main control center of the circadian rhythms, the suprachiasmatic nucleus located in the hypothalamus, is developed by midgestation.[39]

The active and diverse fetal motor behavior in the first half of pregnancy is related to the development of neuronal connections, through axonal in-growth, synaptogenesis and dendrite proliferation. However, we have to emphasize that despite the great diversity of fetal motor patterns in this period of pregnancy, and a dynamic pattern of neuronal production and migration, the cerebral circuits are too immature for cerebral involvement in motor behavior[36]. Nevertheless, the studies of anencephalic fetuses have provided apparent evidence for the influence of supraspinal structures on motor behavior at around the 20th gestational week. In these fetuses the incidence of movements was normal or even increased, but the complexity of movement patterns changed dramatically and movements were stereotyped and simplified.[40] Similar qualitative changes were described at the 17th gestational week in fetuses with cerebral aplasia, and at 18 weeks in fetuses with hydrocephalus.[41]

The spinothalamic tract is established at the 20th week and myelinized by 29 weeks of gestation,[42] and thalamocortical connections penetrate the cortical plate at 24-26 weeks.[43,44] The main events in the development of the CNS and fetal motor behavior in the third trimester are presented in Figure 6. At the 29th week, evoked potentials can be registered from the cortex, indicating that the functional connection between periphery and cortex operates from that time onwards.[45] The establishment of thalamocortical connections seems to be the prerequisite for cortical analysis of sensory inputs. Approximately between 24-34 weeks, cortical areal differentiation begins and continues until the end of gestation[36]. Neuronal differentiation and the laminar distribution of the thalamocortical axons lead to the appearance of six-layered lamination throughout the neocortex after 32 weeks of gestation.[46] However, it is important to point out that the cerebral cortex is still very immature despite the appearance of adult-like lamination pattern and initial areal differentiation. It should be emphasized that until delivery, subunits of the brainstem remain the main regulators of all fetal behavioral patterns.[11]



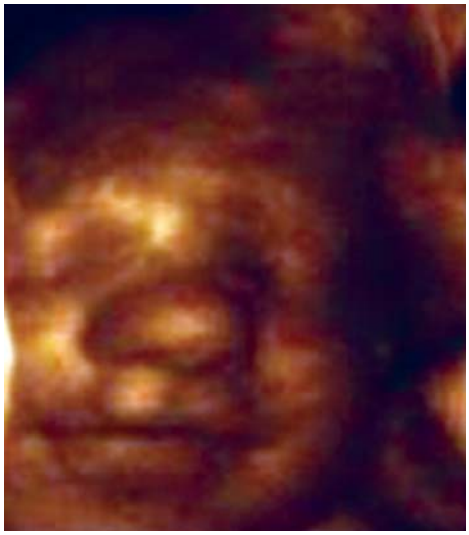


Image of the fetus taken by the 3D sonography, showing a smiling movement

THIRD TRIMESTER

Development of the CNS	*Prenatal motor development*
• Maturation of the brain stem (pons, mesencephalon) continues • Myelinization of the spinothalamic tract: 29 weeks • Evoked potentials from the cerebral cortex: 29 weeks • Beginning of the cortical areal differentiation: 24 -34 weeks • Lamination of the neocortex: 32 weeks onwards	• Eye blinking pattern—a peak frequency: 28 weeks • Facial expression patterns—decreasing or stagnant incidence • General movements—decrease in number, increase in complexity • Eye movements; increase in complexity: 33-38 weeks • Integration of fetal body movements, heart rate, eye movements and breathing—like movements into behavioral states: 36-38 weeks

Fig. 6: The main events in the development of the CNS and the motor development during third trimester (references are given in the text)

The second half of pregnancy is characterized by organization of fetal movement patterns and increase in complexity of movements. The periods of fetal quiescence begin to increase, and the rest-activity cycles become recognizable. Hardly any new movement pattern emerges in this period. The number of general body movements, which tends to increase from the 9th week onwards, gradually declines during the last 10 weeks of the pregnancy.[47-49] By term, the average number of general movements per hour was found to be 31 (range 16-45), with the longest period between movements ranging from 50 to 75 minutes.[50] Although this decrease was first explained as a consequence of the decrease in amniotic fluid volume, it is now considered to be a result of cerebral maturation processes. As the medulla oblongata matures, myelinates, and stabilizes, these spontaneous movements are less easily triggered, and begin to be controlled by more stable intrinsic activities generated within the brainstem.[11] It is very important to point out that general movements are characterized by large variation and complexity in the third trimester.[51] Simultaneously with the decrease in the number of general movements, an increase in facial movements, including opening/closing of the jaw, swallowing and chewing, was observed using 2D sonography. These movements appeared mostly in the periods of absence of generalized movements, and such pattern was considered to be a reflection of the normal neurologic development of the fetus.[47] However, a revolutionary improvement in the study of fetal facial movements came with the development of 3D and 4D sonography (Fig. 7). Our results confirmed the potential of 3D/4D sonography for the investigation of structural and functional development of the fetal face.[52] The application of 4D sonography in the examination of fetal facial movements has revealed the existence of a full range of facial expressions, including smiling, crying, and eye-lid movements[26,53,54] (Fig. 8), similar to emotional expressions in adults, in the 2nd and 3rd trimesters (Fig. 9). Other facial movements, such as yawning, sucking, swallowing and jaw opening can also be observed in this period by 4D ultrasound (Fig. 10). Recent study demonstrated that the most frequent facial movement patterns in the 2nd trimester were isolated eye blinking, grimacing, sucking and swallowing, whereas mouthing, yawning, tongue expulsion and smiling could be seen less frequently.[53] Mouthing was the most frequent facial movement during early third

trimester.[55] Our longitudinal analysis of the frequencies of different facial movements in the 2nd and 3rd trimester revealed some interesting results. Contrary to the declining trend of head movement and hand movement patterns from the beginning of the second trimester to the end of the third trimester, a constant increase in the frequencies of almost all facial movement patterns was observed during the 2nd trimester. Various types of facial expression patterns displayed a peak frequency at the end of 2nd trimester, except eye blinking pattern, which displayed a peak frequency at 28 weeks of gestation. During the reminder of pregnancy, decreasing or stagnant incidence of facial expression patterns was noted.[26] Obviously, this developmental trend provides yet another example of the maturation of the medulla oblongata, pons, and midbrain, or

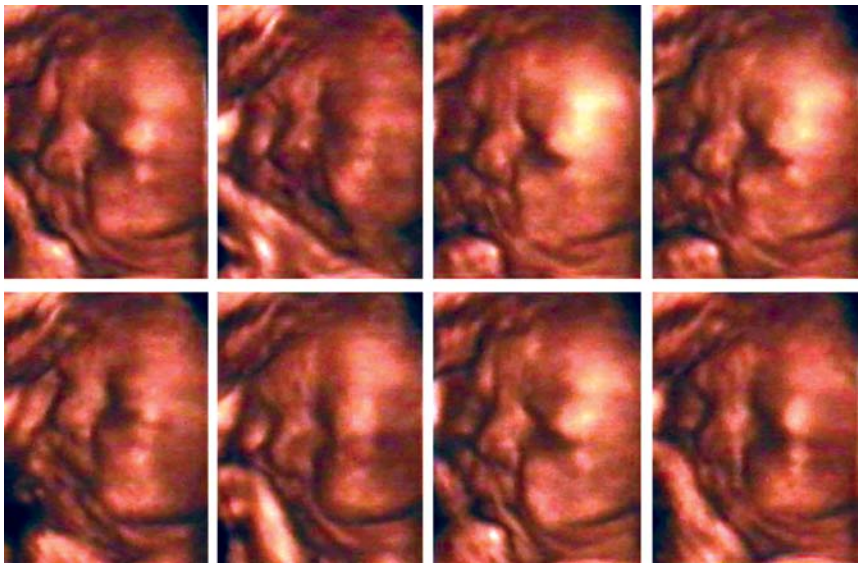


Fig. 7: A sequence of images of the fetus recorded by 3D/4D sonography, exhibiting grimacing

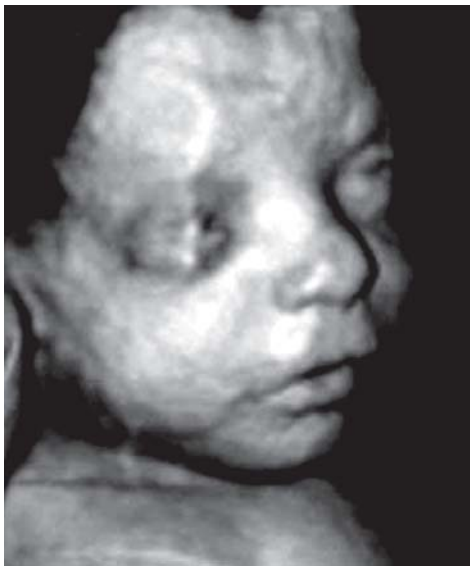


Fig. 8: Image of the fetus recorded by 3D/4D sonography, showing opened eyelids

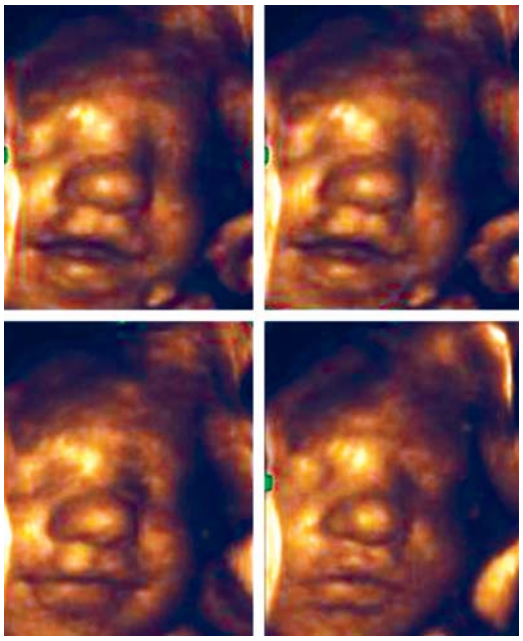


Fig. 9: A sequence of images of the fetus in the 3rd trimester recorded by 3D/4D sonography, exhibiting smiling movements

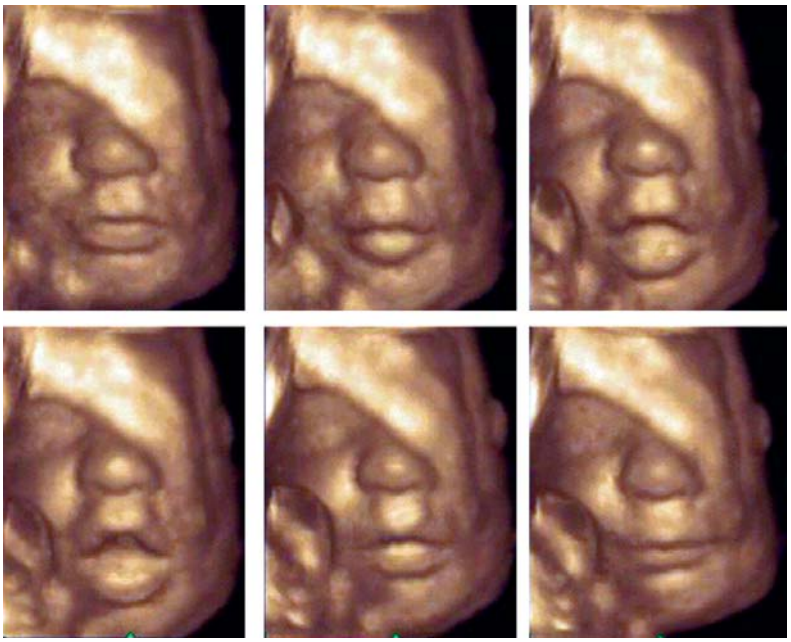


Fig. 10: A sequence of images of the fetus in the 3rd trimester recorded by 3D/4D sonography, exhibiting mouthing movements

perhaps even the establishment of control by more cranial structures. The facts that even in the embryonic period same inductive forces that cause the growth and reshaping of the neural tube influence the development of facial structures, and that many genetic disorders affecting the CNS are also characterized by dysmorphology and dysfunction of facial structures, emphasize the importance of structural and functional evaluation of the fetal face.[19,56]

At 36-38 weeks of gestation, eye movements become integrated with other parameters of fetal activity, such as heart rate and fetal movements, into organized, coherent behavioral states.[57,58] Our recent study has demonstrated that there were no movements observed in fetal life that were not present in neonatal life. Furthermore, prenatal-neonatal continuity exists even in subtle, fine movements such as facial mimics.[59]

The diverse repertoire of fetal movements present during intrauterine life raises the question of their function and significance for normal fetal development. The finding that intrauterine motor activity exists in different animal species, even including invertebrates, implicates their importance in neurodevelopment. The "neuronal group selection" theory suggests the existence of genetically determined neural networks at the onset of development.[60] These networks undergo substantial variations through dynamic epigenetic regulation of histogenetic processes. Development than proceeds with selection on the basis of afferent information produced by the movements, and this selection is accomplished by the retention of the most favorable neural networks and motor patterns.[61] According to this theory, fetal movements could be important for the regulation of some histogenetic processes in the brain and spinal cord, such as the programmed cell death (apoptosis), or the fine-tuning of the connectivity in the nervous system. For instance, breach presentation at the end of pregnancy may have long-lasting effects on the motility of the lower limbs. Mechanical restriction of fetal leg movement in these cases can affect the neurological maturation of the leg reflexes and later motility.[61,62] Hence, fetal motor activity appears to be crucial for the development of most parts of the nervous system and the muscles.

The fine interaction between external influences and endogenous fetal activity is revealed in the fact that fetal behavior may be influenced by a number of external factors. Cigarette smoking or injection of corticosteroids for fetal lung maturation have been shown to decrease the number of spontaneous fetal movements.[63] Furthermore, fetal activity is increased in mothers suffering emotional stress.[64] It is known that qualitative alterations of spontaneous general movements can be observed in preterm and term newborns with cerebral impairment. Their movements seem to lose the characteristic fluency and complexity, and become cramped and unsynchronized. Similar qualitative alterations in fetal general movements have been observed in several conditions, including maternal diabetes mellitus, fetal anencephaly and intrauterine growth restriction (IUGR).[65] Recent data on IUGR fetuses obtained by 4D sonography during the 3rd trimester of pregnancy have shown that IUGR fetuses have less behavioral activity than normal fetuses in hand to head, hand to face and head retroflexion movements. Statistically significant differences could be shown in the five qualitative categories of head and hand movements.[66] Further, in fetuses suffering IUGR, fetal movements become slower and monotonous, resembling cramps, and their variability in strength and amplitude is reduced. The alterations in amplitude and complexity of movements in these fetuses do not appear to be due to the oligohydramnios. In cases of premature rupture of fetal membranes and a subsequently reduced volume of amniotic fluid, movements occur less frequently, but their complexity resembles that of movements performed in the normal volume of amniotic fluid.[65] The rapidly expanding pool of evidence show that the qualitative assessment of general movements has a high predictive value for cerebral dysfunctions. Assessment of neonatal behavior often seems more informative about brain function than functional testing does, despite the availability of various neurologic, physiologic, and other methods of investigation.[67] We produced a new scoring system for fetal neurobehavior based on prenatal assessment by 3D/4D sonography.[68] A comprehensive description of spontaneous motor assessment as a diagnostic tool for detection of brain dysfunction in newborns was given by Einspieler et al.[67] The application of 4D sonography have facilitated the development of such diagnostic method in the prenatal period.[68]

The 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, somatosensory, visual, and auditory cortical areas. Although neuronal proliferation and migration are completed in a term infant, synaptogenesis and neuronal differentiation continue very intensively.[69] Brain stem demonstrates high level of maturity, whereas all histogenetic processes actively persist in cerebellum.[70] Therefore, only subcortical formations and primary cortical areas are well developed in a newborn. Associative cortex, barely visible in a newborn, is scantily developed in a 6-month-old infant. Postnatal formation of synapses in associative cortical areas, which intensifies between the 8th month and the 2nd year of life, precedes the onset of first cognitive functions, such as speech. Following the 2nd year of life, many redundant synapses are eliminated. Elimination of synapses begins very rapidly, and continues slowly until puberty, when the same number of synapses as seen in adults is reached.[70]

FETAL YAWNING AND DEVELOPMENT OF SPECIALIZED MOVEMENT PATTERNS

The fact that spontaneous yawning movements occur as early as at the end of the first trimester of gestation and are consistently

present until delivery, suggests its evolutionary importance and indicates that research into prenatal yawning patterns may help illuminate the physiological role of this reflex. Another finding of interest is the connection between an altered yawning frequency and various brain dysfunctions observed in adults,[71,72] giving way to the conclusion on the possibility of such a relation existing in human fetuses as well.

The earliest yawning movements were observed by 2D sonography around 11 weeks of gestation as an infrequent and non-repetitive movement pattern.[12] This early onset of fetal yawning and other jaw movements coincides with the development of the lower portions of the brain stem, medulla oblongata and pons.[11] Maturation of medulla and pons could also be associated with the changes of the yawning pattern. Sepulveda et al[73] described repetitive sequences of yawning movements in the 27-week-old fetus in normal pregnancy, pointing to the shift in the yawning pattern—from non-repetitive to repetitive sequences.

The 4D sonography has opened new possibilities in the field of fetal behavior research, especially in the investigations of facial movements (Fig. 11). Early reports of yawning movements in the 20-week-old fetus indicated that 4D sonography might facilitate the investigation of this infrequent movement pattern.[74] In this report, fetal yawning was described as a sudden opening of the jaw, accompanied by stretching of the fetal upper limbs and flexion of the head, which distinguished it from the more frequently observed swallowing pattern. Still, aside from this early report, very little systematic research into fetal yawning and other facial movement patterns has been conducted, with the first scientific papers being published only recently. Our results have pointed to technical limitations of investigation of fetal yawning movements in early pregnancy. The study compared the frequencies of embryonic and fetal movements during the first trimester of normal gestation recorded by 4D sonography with those recorded in the same period with 2D sonography. 4D sonography proved inadequate in detecting several movements which could clearly be observed by 2D sonography, including fetal yawning.[27] Yawning movement appeared at the end of the 1st trimester and was one of the most infrequent movement patterns. Apparently, very infrequent movements, as well as discrete or short rapid movement patterns could not be observed by 4D sonography because the repetition time for data acquisition was not sufficient for capturing images of satisfactory quality. Yet other studies have demonstrated that yawning movement could clearly be recognized in the 2nd and 3rd trimester [75] and that all elements of the fetal yawning pattern-prolonged jaw opening followed by rapid closure and accompanied by head flexion and elevation of the arms - can easily be observed in this period using 4D sonography[26,53,59] (Fig. 12). Furthermore, a comparison between fetal yawning in the 3rd trimester and yawning in neonates during the 1st week of life has revealed no difference as to the frequencies of this reflex. The components of the yawning pattern were also identical in the neonates and the fetus.[26,53] Our recent study revealed a gradual increase in the frequency of yawning between the 15th and 24th week, followed by a short plateau from the 24th to 26th week and a slight decrease towards term.[26] This was the first study to demonstrate a clear gestational age-related trend in the frequency of yawning movements. This may be associated with maturation of the brainstem and possibly with the more cranial structures assuming control over the yawning pattern. These findings have

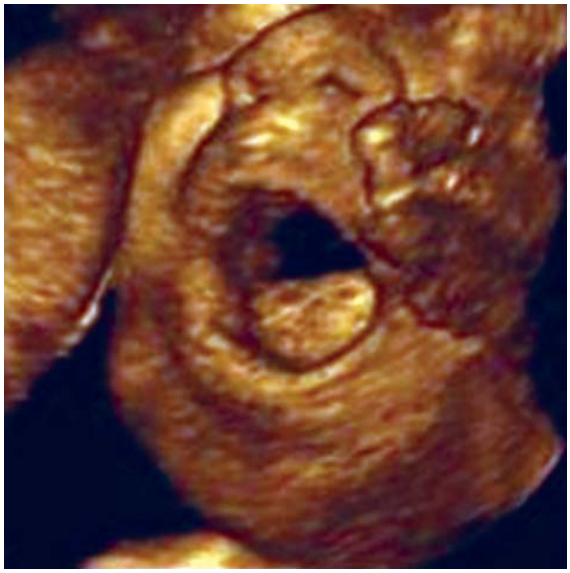


Fig. 11: Image of the fetus recorded by 3D/4D sonography, showing yawning

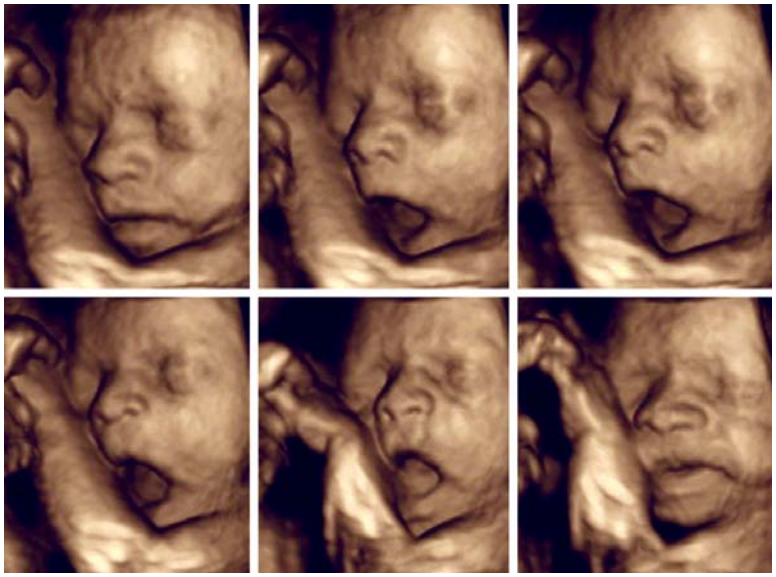


Fig. 12: A sequence of images showing the components of the yawning pattern recorded by 4D sonography

shed some light on the neurodevelopment of this specific reflexive behavioral pattern.

Fetal yawning is, just like the yawning in adult humans and animals, still quite a mysterious phenomenon. A possible connection between yawning and certain pathological conditions, particularly those affecting the fetal CNS has not been investigated so far, despite an observed connection between an altered incidence of yawning and a wide spectrum of CNS disorders in adults.[71,72] An increased frequency of yawning movements has been reported in fetuses suffering Rhesus immunization, fetal erythroblastosis, and severe anemia. This phenomenon was interpreted as a fetal attempt to increase the venous return and in that way improve the delivery of oxygenated blood to the vital organs.[76] However, these findings should be confirmed on a larger number of high-risk fetuses.

Specialized movement patterns, crucial for the survival of newborns, such as swallowing and respiratory movements, develop and mature during gestation. Furthermore, it has been established that these movements play an important role during intrauterine life.

Fetal Breathing-like Movements

During the early embryonic period, the rhythmic respiration-related neuronal network has been located within the hindbrain (the rhombencephalon), and after that period in the pons and medulla oblongata.[77] The hindbrain neuroepithelium becomes divided into several cellular segments, called rhombomeres, in the second half of the first postconceptional month (in chick embryo between stages 9 and 24), and it is believed to regulate spatial distribution of neurons. Electric activity of the hindbrain, composed of simultaneous burst discharges that occur spontaneously in the different cranial nerves, appears at the end of the segmentation period in a cheek embryo.[77] The onset of breathing movements on the human fetus coincides partly with the end of the segmentation period.[78] The fetus will take its first **«breath»** and by the 10th week of gestation spontaneous **«breathing»** (chest and abdominal) movements has been observed by 2D sonography.[12] It has been demonstrated that these movements can not be observed by 4D sonography, but only by 2D sonographic technique during the first trimester of pregnancy.[27]

Early in gestation fetal breathing activity is variable and isolated event, but the frequency and complexity of the breathing patterns change over the ensuing weeks and months (Table 2). At 24-28 weeks of gestation, fetuses breath about 14% of the time in a 24 hour-period, and by weeks 32-40, fetal breathing activity increases to about 30% of the time.[79,80] Between 25-32 weeks, the episodes of breathing-like movements that last less than 10 seconds decrease, whereas episodes that last longer than 30 seconds increase. In addition, breathing episodes are interspersed with apneic periods, which alter in length from 14 minutes in premature fetuses to 122 minutes at term.[79,80] It has been recently reported that at 33-36 weeks of gestation, length of a entire respiratory cycle, the inspiratory phase and the expiratory phase tends to be shorter than in younger and older fetuses.[81] Fetal breathing-like movements are brainstem reflexes that occur more frequently as the medulla oblongata matures.[11] Moreover, changes in breathing patterns are consequences of the maturation of the fetal lungs as well as the respiratory and sleep centers in the CNS. During the 38th and the 39th week of gestation, the frequency of movements decrease to 41 respirations per minute and the movements become as regular as in the postnatal period.[82]

Table 2: Trends in fetal breathing-like movement patterns during pregnancy (references are given in the text)

Timing (weeks)	*Characteristics of the breathing-like movements*
10	The onset of breathing movements
24-28	Respiratory response to CO_2 level
	Breathing—like movements occur 14 percent of the time
33-36	Length of entire cycle—shorter than in younger and older fetuses
32-40	Breathing—like movements occur 30 percent of the time
38-39	Frequency of movements decrease to 41 respirations/ min

A number of internal and external factors can influence fetal breathing movements during the second half of pregnancy (Table 3). At 24-28 weeks the fetal respiratory rate can rise as high as 44 inhale/exhale cycles per minute.[79] This rate changes according to maternal carbon dioxide (CO_2) levels, strongly suggesting that respiratory center in the brainstem of the fetus already detects and responds to changes in CO_2 levels in the blood. This respiratory response to CO_2 is similar to that seen in newborns and adults.[83] Furthermore, an increased number of fetal respiratory movements following the elevation of the glucose concentration in the maternal blood has been observed at the 34th week of gestation.[84,85] Recent investigation has shown that intermittent maternal fasting is connected with a considerable alteration in the frequency and pattern of fetal breathing movements from the 30th week of gestation onwards.[86] Following premature rupture of membranes,[87,88] during the three days prior to the initiation of labor, a decrease in fetal breathing has been recorded.[89,90] However, similar maturation patterns in breathing and spontaneous fetal body movements were demonstrated among low-and high-risk fetuses threatening to deliver prematurely, which suggests normal functional development in the high-risk fetal group.[91] Some studies have shown that maternal consumption of alcohol, methadone, as well as cigarette smoking decrease the incidence of respiratory movements.[92-94] Conversely, aminophylline, conjugated estrogens and betamethasone are responsible for an increase in frequency of breathing movements.[95,96]

Table 3: Influence of external and internal factors on fetal breathing-like movements (references are given in the text)

Frequency of fetal breathing-like movements	
Increased	*Decreased*
• Carbon dioxide	• Premature rupture of membranes
• Hyperglycemia	• Alcohol
• Aminophylline	• Methadone
• Conjugated estrogens	• Cigarette smoking
• Betamethasone	

The functions of breathing-like movements during intrauterine life are the development of respiratory muscles, widening of the alveolar spaces and maintenance of lung liquid volume.[97-99] Animal investigation has shown that absence of respiratory movements (due to destruction of the brainstem nuclei above the phrenic nucleus) leads to hypoplasia of the lungs.[100] Furthermore, recent data have indicated the role of fetal breathing-like movements in lung organogenesis. These movements have important role on biochemical differentiation of Clara cells, type I and type II pneumocytes. It has been confirmed that even though type II pneumocytes are able to synthesize surfactant-associated proteins, in the complete absence of fetal breathing-like movements, they are incapable to compile, store and release the surfactant. Likewise, in the absence of these movements, type I pneumocytes are unable to flatten in order to allow the gas exchange in the lungs.[101] Moreover, recent study on the amyogenic mouse embryos has demonstrated significantly reduced number of airway smooth muscle cells in their hypoplastic lungs, suggesting that the number of these cells is primarily regulated by the fetal breathing-like movements.[102] All these findings indicate that respiratory movements are crucial for the normal lung development.

Fetal Swallowing and Development of Fetal Thirst, Appetite and Satiety

Appetite and thirst-mediated ingestive behavior develop and are likely programmed during prenatal life, thus preparing for newborn and adult ingestive behavior.[103] Fetal swallowing activity was observed as early as 11 weeks of gestation,[104] with daily swallowing rates of 500-1000 mL near term.[105] Fetal swallowing and ingestive behavior contribute significantly to the regulation of the amniotic fluid volume and composition, acquisition and potential recirculation of solutes from the fetal environment. A major pathway for amniotic fluid resorption is fetal swallowing.[105,106] This fetal swallowing activity also contributes to the development and maturation of the fetal gastrointestinal tract.[103,105]

It has been well known that a normal amniotic fluid volume is critical for normal fetal growth and development.[106] Disordered fetal swallowing has been associated with both a decrease and increase in amniotic fluid volume.[103] These conditions are associated with a significant increase in perinatal morbidity and mortality. Further, in some fetuses with esophageal atresia, the volume of amniotic fluid is increased. It is important to note that this is the case in some, but not all fetuses with esophageal atresia. Namely, this anomaly is often accompanied by tracheoesophageal fistula, a shortcut to the gastrointestial tract. Therefore, intake of liqiud during the respiratory movements might explain the nonappearence of polyhidramnios in some of these cases.[105] Polyhidramnios sometimes, although not always, develops in anencephalic fetuses. Some of these fetuses have an intact swallowing reflex. Cases with normal amniotic fluid volume and decreased fetal swallowing were also described.

Assessment of fetal swallowing with gray-scale and color Doppler sonography has demonstrated that there is fetal trend towards development of increased coordinated swallow-related movements and more functional nose-mouth flow with increasing gestational age. These investigators have postulated that knowledge of the physiologic mechanism involving swallowing development could contribute identification of altered swallow-related movements in fetuses with malformations of the gastrointestinal tract or with neurologic disorders.[107] Our recent investigation, performed by 4D ultrasound, has shown that swallowing pattern (Fig. 13) displays a peak frequency at the end of the second trimester. At the beginning of the third trimester, decreasing incidence of this pattern was recorded.[26] Some studies have shown that fetal swallowing activity may be modulated in accordance with neurobehavioral state alterations (stimulation of swallowing with shifts from quite to active sleep). Furthermore, fetal swallowing is influenced by the volume of amniotic fluid, hypoxia, hypotension and plasma osmolality changes.[105]

Experiments in fetal lambs have indicated that dipsogenic mechanisms begin to regulate swallowing during intrauterine life. Swallowing and arginine-vasopressin (AVP) secretion increase following the central administration of hypertonic saline and angiotensin II.[108,109] Studies in near-term ovine fetuses have also proven that hypertonicity-activated neurons were detected by determination of the Fos protein in dipsogenic hypothalamic nuclei. Intensive production of Fos protein, indicating activation of c-Fos genes, was detected in putative dipsogenic nuclei, parvocellural and magnocellural divisions of the paraventricular nucleus (PVN), and supraoptic nucleus (SON).[105,110-112] The fetus swallows about 6 times more liquid in comparison an adult and mechanisms underlying the high rate of human fetal swallowing are regulated, in part, by tonic activity of central angiotensin II, glutamate N-methyl-D-aspartate receptors, and neuronal production of the nitric oxide.[103] Reduced NMDA receptor expression within the forebrain dipsogenic neurons contributes to observed differences in drinking activities between the fetus/neonate and

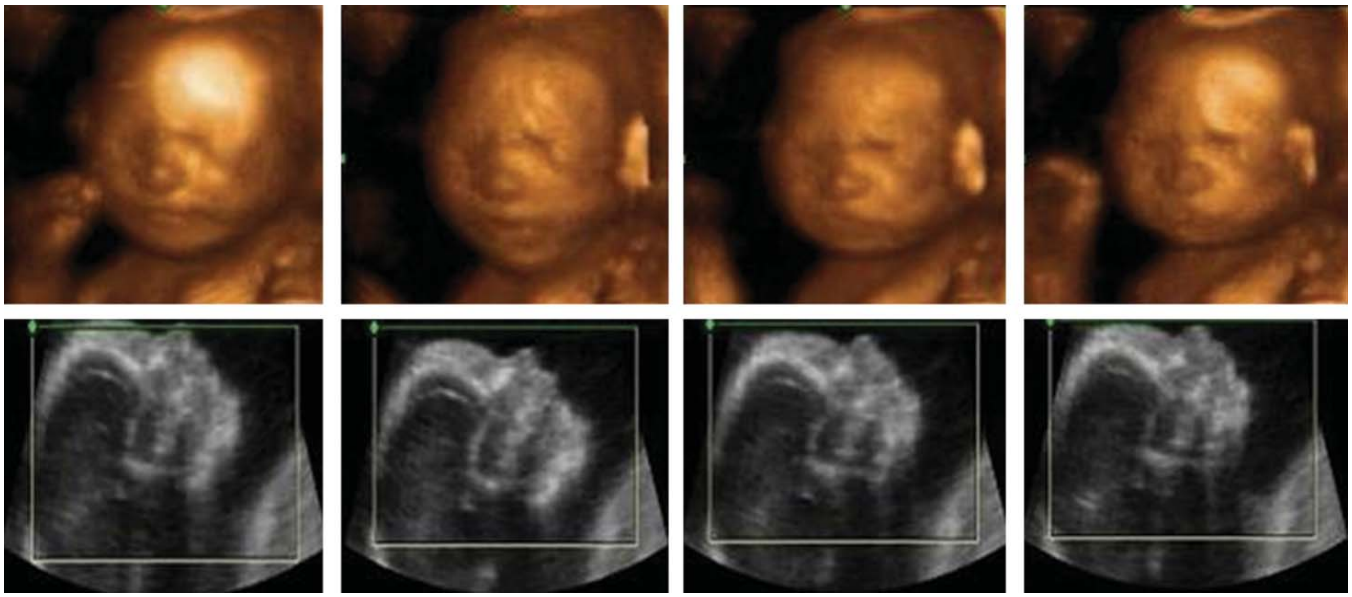


Fig. 13: A sequence of images of the fetus recorded by 4D sonography showing swallowing movements

the adult.[113] Furthermore, the fetus seems to have a extensively reduced sensitivity to osmotic stimuli when compared the adult,[114-116] despite the intact dipsogenic nuclei. Reduced swallowing activity during the systemic hypotension, despite elevated renin levels in plasma, provides further evidence that the fetal dipsogenic response is markedly different from that of the adult.[117] It is possible that dipsogenic responses develop *in utero* in the human fetus to provide thirst stimulation for appropriate water intake during the immediate neonatal period.[105] According to some studies, an altered intrauterine osmotic environments may modulate not only fetal swallowing activity, but also the development of adult sensitivities for thirst, AVP secretion, and AVP responsiveness.[103,105,118]

Similar to dipsogenic mechanisms, peripheral and central fetal orexic mechanism also develop during intrauterine life. Prenatal ingestive behavior is manifested as swallowing and intake of amniotic fluid. Amniotic fluid proteins and growth factors contribute to the growth and maturation of the fetal gastrointestinal tract, and possibly to fetal somatic growth.[119] Amniotic fluid proteins provide 10-15% of the nitrogen requirement in the normal fetus, and esophageal atresia is often associated with the lower birth weight.[120] It is generally believed that appetite and satiety mechanisms develop during the intrauterine period in all precocious species. By the 7th week of gestation, human embryos demonstrate taste buds.[121] Sweet taste, such as that of a low-concentration sucrose solution, stimulates swallowing in the human fetus, whereas the incidence of swallowing movements decreases following the injection of Lipiodol, a bitter extract of poppy seeds used as a contrast into the amniotic fluid.[121] The main feeding regulatory factors, neuropeptide Y (NPY) and leptin, are secreted in the human fetuses as early as 16 and 18 weeks, respectively.[122-124] NPY is the most potent known inducer of food intake and a leptin is a primary satiety factor. In some animal experiments, increased fetal swallowing has been demonstrated upon central NPY administration.[125] The role of leptin in regulating ingestive behavior is interesting because, contrary to function in adults, leptin does not suppress fetal ingestive behavior.[103] Fetal swallowing was significantly increased following the injection of leptin.[126] Therefore, some investigators have postulated that the lack of leptin-inhibitory responses might potentiate feeding and facilitate weight gain in newborns, despite high body fat levels.[119] Some data suggest a possible role of leptin in the development of the fetal gastrointestinal tract.[127] Recent animal experiments have shown that perirenal adipose tissue could be a main source of leptin in the fetal circulation, and that leptin gene expression is regulated by both glucocorticoids and thyroid hormones. Developmental changes in circulating and perirenal adipose tissue leptin may mediate prenatal maturational effects of cortisol and have long-term consequences for appetite regulation and the development of obesity.[128] Another study suggests that the placenta could be an important source of leptin in fetal circulation.[129]

According to some other recent studies, the potential *in utero* imprinting of appetite and satiety mechanisms may effect infant, childhood and ultimately adult appetite "set-points". Recent studies have also shown that the mechanisms by which environmental factors modulate the physiologic systems that control body weight may have their roots before birth.[130] An adverse intrauterine environment, with altered fetal orexic factors, could change the normal set-points of appetitive behavior and potentially lead to programming of adulthood

hyperphagia and obesity.[103,119] However, further investigations are needed to delineate precisely the relationship between the intrauterine environment and the development of the set-points of adult appetite and thirst.

CONCLUSION

The prenatal period of life has considerable influence in shaping future development and behavior. Functional development of the fetal brain begins as early as the late embryonic period. During the nine months of gestation, a repertoire of fetal activities constantly expands, correlating precisely with the structural development of the CNS. Major developmental events, such as the establishment of neural connections in different regions of the brain, are accompanied by the occurrence of new patterns of fetal activities or by the transformation of existing patterns. Extensive studies into the movement patterns have been conducted, tracing all newborn movement patterns back to the prenatal period. The integration of random and abundant fetal activity into the organized behavioral states indicates the maturation of the control centers in the CNS. Moreover, many investigations have confirmed the role of fetal motility in the maturation of motor functions as well as revealed its involvement in the development of other organs, such as the CNS, lungs, gastrointestinal tract. Some light has also been shed on the purpose of specific reflexive behavioral patterns, such as fetal yawning.

Investigations carried out using 4D sonography have produced invaluable details of fetal behavior and its development, opening the door to a better understanding of the prenatal functional development of the CNS.

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