73 Fertilization and normal embryonic and early fetal development

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The anatomy and physiology of placental and embryonic development is a field where medicine exerts its greatest impact on early pregnancy at present time, and it opens fascinating aspects of embryonic differentiation. Clinical assessment of those stages of growth relies heavily on three-dimensional (3D) and four-dimensional (4D) sonography, one of the most promising forms of noninvasive diagnostics today. The embryologic phenomenon, once matters for textbooks, is now routinely recorded with outstanding clarity.

It is clear that with the use of 3D and 4D ultrasound, both structural and functional developments in the first 12 weeks of gestation can be assessed more objectively and reliably. New technology moved embryology from postmortem studies to the *in vivo* environment. It is the purpose of this chapter to illustrate the potential of 3D/4D sonography in the study of structural and functional early human development (1–11).

FROM FERTILIZATION TO IMPLANTATION— THE FIRST 4 WEEKS

Introduction of the color Doppler made it possible to visualize blood vessels that enabled detailed examination of small vessels such as arteries supplying preovulatory follicle, corpus luteum, or endometrium (Figs. 1–5). Perifollicular vascularization can help in the identification of follicles that are containing high-quality oocytes with a high probability of recuperating, fertilizing, cleaving, and implanting. The 3D ultrasound makes it also possible to visualize cumulus oophorus (Fig. 6).

Fertilization is the end of the complicated process of conjunction of gametes by entering one spermatozoon into the oocyte. The gametes, ovum and sperm, contain half of the number of the chromosomes (haploid) compared with the number present in somatic cells (diploid). They obtain haploid number of chromosomes through the process of meiosis during the gametogenesis (4). The oocyte is approximately 120 μm in diameter and has a thick membrane known as zona pellucida. The spermatozoon moves using the flagellum or tail, and the total length of the spermatozoon including the tail is 60 μm . Both oocyte and spermatozoon are highly specialized sex cells. Oocytes are produced in the ovary and expelled from it during the ovulation after which fimbriae of the fallopian tube sweep the oocyte into the ampulla where it can be fertilized. Sperms are produced in seminiferous tubules of the testes and stored in epididymis and ductus deferens. Ejaculation of semen results in the deposit of million sperms in the vagina around the external uterine os. They pass through the uterus and tubes and in the ampulla they surround the

secondary oocyte if it is present. When the sperm enters the oocyte, the nucleus of the mature oocyte consists of the female pronucleus and the head of the sperm separates from the tail and enlarges to become male pronucleus. Fertilization is complete when the pronuclei unite and the maternal and paternal chromosomes intermingle during metaphase of the first mitotic division of the zygote. That cell is the primordium of the human being. The inification of the pronuclei restores a haploid number of chromosomes and completes the fertilization. Therefore, fertilization is a distinct moment when the diploid cell is formed and the development of an individual human begins (3).

Transfer of the oocyte is facilitated by the changing endocrine milieu of the early luteal phase with its rising ratio of progesterone to estrogen, which affects the oviductal and uterine musculature and relaxes the isthmic sphincter. It is probable, however, that the cilia, rather than the musculature, are the primary active transporters of the conceptus. Thus, pharmacologic inhibition of oviductal muscles does not prevent transfer of the conceptus. Furthermore, if a segment of oviduct is excised, turned around, and replaced such that cilia beat away from the uterus, the conceptus moves to that part of the oviduct and then it stops (Figs. 7–9) (4).

Already few minutes after the ovulation, oocyte is located in the ampullar part of the fallopian tube. It is surrounded by the *zona pellucida*. On the surface of the zona pellucida, there are few rows of the granulosa cells that make *corona radiata*. At the periphery of the corona radiata are left cells of *cumulus oophorus*. Often, these three units are called *oocyte-corona-cumulus complex*. Capability for the fertilization is limited: oocyte can be fertilized only 6 to 12 hours after the ovulation. Sperms are capable for fertilization 48 to 72 hours, until they are movable. Fertilization occurs in the ampullar part of the fallopian tube. The process of fertilization begins with conditioning of the spermatozoon in the male and female reproductive tracts. Thereafter, fertilization involves not only the egg itself but also the various investments that surround the egg at the time it is released from the ovary follicle. Fertilization, therefore, is not an event; it is a complex biochemical process requiring a minimum of 24 hours to complete syngamy (formation of a diploid set of chromosomes). During that process, there is no commingling of maternal and paternal chromosomes within a single nuclear membrane (pre-zygote); after this process, the paternal chromosome material is commingled (zygote). The most important activity of this new cell is the recognition of the new genome that presents the principal information center for the development of the human being and for all its further activities.

Figure 1 Visualization of morphology and vascularity of preovulatory ovarian follicle on the 10th and 11th day of menstrual cycle.

Figure 2 Developmental stages of fresh corpus luteum displayed by transvaginal color Doppler.

Early Development of the Embryo

After syngamy, the zygote undergoes mitotic cell division as it moves down the fallopian tube toward the uterus. Shortly after gene activation, conceptus shows marked quantitative increase in its biosynthetic capacity. Net synthesis of mRNA and protein increases, transport of amino acids and nucleotides into the cells rises, and changes occur in the synthetic patterns of phospholipids and cholesterol. The growth and metabolic activity of the preimplantation conceptus in vitro has been shown to be stimulated by a number of growth factors that vary between species and include insulin-like growth factors (IGF-1 and 2), transforming growth factors alpha (TGF-alpha) and beta (TGF beta 1 and 2), epidermal growth factor (EGF), and platelet-derived factor A. Receptors of those factors have been identified on human conceptus. Synthesis of many of

those factors has been detected in early conceptus itself or in maternal tissues; therefore, it is reasonable to conclude that these factors act as *autocrine* or *paracrine* agents that promote the early development (3–5).

A series of mitotic divisions lead to the development of the *pre-embryo*. The newly divided cells are called blastomeres. 24 hours after fertilization from single-cell zygote two-celled embryo is developed. 2 days after the fertilization embryo consists of 6 to 8 blastomeres. Blastomeres form cellular aggregates of distinct, totipotent, undifferentiated cells that retain the capacity to develop independently into normal pre-embryos. After every division, blastomeres become half of their previous size keeping the total size the same as in the beginning when it was just one cell. All up to eight-cell phase, blastomeres are cluster of cells inside the zona pellucida.

Figure 3 Improved visualization of perifollicular blood flow by three-dimensional power Doppler.

Figure 4 Vascularization of nonpregnant uterus.

But after the third division, they lose their round shape and they impact one to the other making compact round cluster of cells that are tightly connected by tight junctions. With that change, which is called *compaction*, cells from the outside start to differentiate from those inside. Third day after the fertilization, cells of the compact embryo divide again and *morula* arises. It contains 16 cells, outer part from which trophoblast will appear and inner cells from which embryo will appear.

Blastocyst enters the uterine cavity. In most species, it appears at the 32- to 64-cell stage. Inner part of cells is then called embryoblast and it is located on one side of the blastocyst. Outer part of cells is trophoblast and it makes epithelial surface of the blastocyst. The expansion of the blastocoele is stimulated by growth factors EGF and TGF- α (4). Throughout the development, from fertilization to the blastocyst, conceptus remains enclosed in zona pellucida. Zona pellucida has two functions. First, it prevents blastomeres of the conceptus from falling apart during early cleavage period prior to compaction. If conceptus does divide into two distinct groups of cells at this time, monozygotic twins result. Second, it prevents two genetically distinct conceptuses from sticking together to make a single chimeric conceptus (4). Free-living

Figure 5 Richly vascularized thickened endometrium at the day of embryo transfer.

Figure 6 Surface of ovary of a young patient. Follicular cyst and follicle with intrafollicular structure visualized by three-dimensional sonography.

blastocyst is bathed in uterine secretions from which it draws the oxygen and metabolic substrates important for growth and survival. There is a limit of the size that free-living conceptus can attain before such exchanges become inadequate. Before that critical stage is reached, conceptus grows its own blood vascular system. It also grows highly vascularized zones at its extraembryonic surface through which the interchange of materials with maternal tissues is particularly facilitated. During the pre-, peri-, and postimplantation stages of pregnancy, conceptus utilizes maternal juices almost exclusively. That sort of nutrition is called *histiotropic*. However, this source becomes progressively inadequate and so with the successful conclusion of implantation, in maternal endometrial tissue, develops a corresponding specialized and vascularized region. That region will become a placenta, and this sort of nutrition is called *hemotropic*.

When zona pellucida disappears, it makes it possible for blastocyst to implant into the endometrium (Fig. 10).

Once entering the uterus, the conceptus engages in an elaborate interaction with the mother in which several messages are transmitted in both directions. First, conceptus establishes physical and nutritional contact with the maternal endometrium at the implantation. Failure to do so would

Classification of perifollicular vascularity according to the percentage of color by power doppler:

- A) Grade I, when color mapping takes up less than 25% of the follicular circumference;
- B) Grade II, when color mapping covers between 25 and 50%;
- C) Grade III, if color signal appears between 50 and 75%; and
- D) Grade IV, color mapping between 75 and 100% of the follicular perimeter.

Figure 7 Power Doppler display of vascularization of periovulatory follicle.

deprive the conceptus of essential nutritional substrates and arrest in growth. Second, conceptus signals its presence to maternal pituitary-ovarian axis; somehow it must convert the whole female reproductive system from cyclic pattern to a noncyclic in which progesterone dominates.

At the time when blastocyst is prepared for implantation, in the middle of the luteal phase of cycle, endometrium is about 10 to 14mm thick. It is prepared for the implantation by various factors: EGF, IGF-I, and local paracrine and autocrine factors. From day 16 to 22, the receptivity of the endometrium is the highest, which is called "implantation window." At that time, a large number of pinopodes (outgrowth of cytoplasm of endometrial cells) occur, and help blastocyst to implant. When zona pellucida disappears, it makes it possible for conceptus to make contact with decidual endometrium. Implantation consists of three phases: apposition, adhesion, and invasion. Apposition and adhesion are assisted with adhesive molecules of endometrium like laminin, fibronectin, and receptors like integrins. Conceptus produces signals that make change in mother's blood flow. It produces early pregnancy factor, which has immunosuppressive effect. By the beginning of the implantation, proteases and matrix metalloproteinases are activated. Conceptus that is stuck to the surface of the endometrium begins its invasion.

Enzymes of the trophoblast invade mother's circulation and build hemochorial placentation.

In some species, implantation is invasive (humans) in difference to some where it is noninvasive. Invasive conceptus tends to be smaller at attachment and only a few trophoblast cells are involved in making contact with the maternal endometrium. Within few hours, increased vascular permeability is observed. This is associated with edema and a progressive sprouting and ingrowth of capillaries. The stromal reaction is particularly marked in primates where it is called *primary decidualization reaction*. The uterine glandular and decidual tissue adjacent to the invading trophoblast of the conceptus is destroyed. It releases primary metabolic substrates: lipids, carbohydrates, nucleic acids, and proteins, which are taken by the growing conceptus. Decidual tissue thus functions like a large yolk reservoir.

The invasiveness of the conceptus is influenced by the effectiveness of the decidual response. The depth of the invasion varies between species. Those species in which the conceptus invades the stroma so deeply that the surface epithelium becomes restored over it are called to be implanted *interstitially*.

Endometrium that occurs in the attachment process involves leukemia inhibitory factor (LIF). It is produced by

Figure 8 Endometrial thickness and rich vascularization indicating good uterine receptivity.

the cells of the endometrial glands under the influence of estrogen. Receptors for LIF also appear in both the epithelium and the stroma of the endometrium around the time of implantation. Seems like LIF promotes endometrial receptivity to attachment and/or subsequent decidualization.

In the presence of the estrogen, either the conceptus produces the signal or the local endometrium becomes sensitive to a signal being already sent. The nature of this putative signal is unknown, but it induces the HB-EGF production locally and the conceptus in turn responds in shedding its zona, attaching and invading (4).

The invasiveness of the trophoblast depends of the expression and regulation of the different proteolytic enzymes. TGF-beta promotes decidualization and controls trophoblastic invasion. Histamine and prostaglandins are involved in the stromal response: inhibition of either one of them reduces the decidualization.

An important feature of decidual response is increase in vascular permeability and growth of new capillaries (*angiogenesis*).

As implantation proceeds, trophoblast differentiates into two layers: inner cytotrophoblast and outer syncytiotrophoblast. Around day 9, syncytiotrophoblast cells begin to produce human chorionic gonadotropin (hCG). It maintains the thick endometrium and can be detected in maternal blood or urine, which is used for early pregnancy testing (3).

A small intradecidual gestation sac can be seen by transvaginal sonography between 32 and 34 days (Figs. 11 and 12). The

secondary yolk sac is the earliest extraembryonic structure normally seen within the gestational sac at the beginning of the 5th gestational week (Fig. 13). Yolk sac volume increases from week 5 to 10. When it reaches its maximum at week 10, it would have already started to degenerate, which can be seen by the significant reduction in visualization rates of the yolk sac vascularity. The start of embryo-chorionic circulation changes the source of nourishment to all intraembryonic tissues. Development of the embryo becomes dependent on the circulation of embryonic blood. In the embryo, there are three distinct blood vascular systems: vitelline circulation (from yolk sac to embryo), intraembryonic circulation, and feto-placental circulation. It is possible to visualize and assess them virtually from conception.

At 5 weeks it is possible to obtain a 3D image of the developing intervillous circulation in the maternal site of the placenta (Fig.14). Doppler shows intense vascular activity surrounding the chorionic shell. At 7 weeks 3D power Doppler images depict aortic and umbilical blood flow.

DEVELOPMENT OF EMBRYO THROUGH FIRST 4 WEEKS

Along with the implantation process, changes occur in the embryoblast to produce a bilaminar embryonic disc, composed of the epiblast and the hypoblast. Early in the 2nd week, the amniotic cavity appears as a space lined with amnioblasts derived from the epiblast. By the end of the 2nd week, the embryonic disc becomes oval in shape. Along the median line

Echographic characteristics of implantation and early placentation

Endometrium - secretory phase (3–4 weeks)

- The “triple – line” disappears and is replaced by an **echogenic pattern**. At this stage the endometrium is at the maximum thickness as it reaches upto **14mm**.

Figure 9 Vascularization is very obvious in the secretory phase of menstrual cycle.

in the posterior region of the embryonic disc, a thickening of the epiblast called the *primitive streak* appears, and it defines the longitudinal axis of the embryo. During the 3rd week, lateral epiblast cells migrate medially, enter the primitive streak, and then converge to form the primitive groove.

During the latter half of the 3rd week, drastic changes for morphogenesis (development of the body form) begin. The embryonic disc elongates craniocaudally to become pear-shaped and then slipper like. Cranial to the primitive node, a thickening of the ectoderm appears bilaterally and is called the neural plate because it will eventually develop into the neural tissue. Between the bilateral neural plates, a longitudinal neural groove develops in the midline, flanked by the neural folds bilaterally. Around day 20, the bilateral neural folds begin to fuse with each other to form the neural tube, which is the primordium of the central nervous system (CNS; brain and spinal cord). The rostral (anterior) neuropore normally closes on days 24 to 26, and the caudal (posterior) neuropore closes by 28 days. When the neuropores close completely, three brain vesicles (forebrain or prosencephalon, mesencephalon or midbrain, and rhombencephalon) have already appeared. Rarely the neural tube fails to close completely, resulting in a variety of neural tube closure defects such as anencephaly, encephalocele, myeloschisis, and myelomeningocele.

By the end of the 3rd week, the paraxial mesoderm condenses segmentally along the midline to form somite pairs. First the somites are recognized as conspicuous surface elevations and gradually increase in number craniocaudally. The somites give rise to most of the axial skeleton, trunk and limb musculature, and dermis of the skin. The formation and specification of somite segments are controlled by a unique combination of Hox genes (Hox code). Concurrent with somite differentiation, the intraembryonic coelom arises as isolated spaces between the layers of the lateral plate mesoderm, which is the primordium of body cavities. The coelomic cavities subsequently coalesce to form a single, horseshoe-shaped cavity that eventually gives rise to the pericardial, pleural, and peritoneal cavities.

At the beginning of the 4th week, the embryo is almost straight. During the 4th and 5th weeks, the embryo body undergoes a drastic shape change as a result of embryonic folding. Embryonic folding occurs in both the longitudinal and the horizontal planes, resulting from the rapid growth of the embryo, especially the brain and spinal cord, as well as the expansion of the amniotic cavity. Folding of the embryo in the horizontal plane rolls the lateral parts of the embryonic disc ventrally and forms a cylindrical embryo.

Figure 10 Schematic illustration of the process of implantation.

Echographic characteristics of implantation and early placentation

Gestational sac (5th week)

Figure 11 Gestational sac at 5 weeks visualized by two-dimensional sonography.

Another important developmental event that occurs during the 4th week is the emergence of limb buds. Forelimb buds appear by 28 days and hindlimb buds about 2 days later. By the end of the 4th week, the embryo is in a C-shape.

During the 5th week, limb buds elongate and their distal ends become paddle shaped. The second pharyngeal arch overgrows the third and fourth arches and forms an ectodermal depression known as the cervical sinus. Ultra-sonography reveals some primitive spontaneous movements of the embryo, such as twitching of the trunk, in the 7th week.

During the 6th week, the upper limbs undergo rapid morphologic differentiation, and digital rays, indicating that the future digits are recognizable. By the end of this week, the elbow and wrist regions become clearly identifiable. Retinal pigment is visible in the eyes. On the first and second pharyngeal arches, six swellings develop as the primordia of the auricle of the external ear. As a result of the rapid growth of the brain, the head is larger relative to the trunk and starts to lie over the cardiac prominence (1–6).

Figure 12 The same sac shown by three-dimensional sonography.

Figure 13 The first visible structure at 5 weeks is yolk sac (YS).

Establishment of intervillous circulation (IVS).

Figure 14 Development of intervillous circulation visualized by three-dimensional power Doppler (left) and conventional Doppler (right).

ULTRASONIC VISUALIZATION OF EARLY HUMAN DEVELOPMENT

Gestational sac can be visualized from the middle of the 5th week of amenorrhea as a small spherical anechoic structure placed inside one of the endometrial leafs (7). Planar mode tomograms are helpful in distinguishing early intraendometrial gestational sac from collection of free fluid between the endometrial leafs (pseudogestational sac). The 3D sonography enables precise measurement of exponentially expanding gestational sac volume during the first trimester. At the beginning of the 5th week, small secondary yolk sac is visible as the earliest sign of the developing embryo (Fig. 15). Adjacent to the yolk sac, embryo can be seen as a small straight line when it reaches 2 to 3 mm in length at the end of the 5th week.

Six Weeks

Typical characteristics of the 6th week embryo include a rounded bulky head and a thinner body. The head is prominent due to the developing forebrain. Limb buds are rarely visible in this stage of pregnancy. However, umbilical cord and vitelline duct are always clearly visible. At this stage of pregnancy, the planar mode will allow for more accurate assessment of multiple pregnancies.

Seven Weeks

During the 7th week, fast development of rhombencephalon (hindbrain) takes place. This process gives even more prominence to the head. By the use of planar mode developing vesicles of the brain can be depicted as anechoic structure inside the head. The biggest and usually the only visible part is rhombencephalon placed on the top of the head (vertex). Diencephalon and its cavity become visible few days later. The head is strongly flexed anteriorly being in the contact with the chest. Limb buds are often visible laterally to the body.

Eight Weeks

During the 8th week of pregnancy, there is expansion of the ventricular system of the brain (lateral, third, and midbrain ventricles). The shape of the face is becoming apparent, but the flexion of the cranial pole still makes it difficult to view the face entirely (Fig. 15). The vertex is now located over the position of the midbrain. Structures of the viscerocranium are not visible due to the small size. Arms and feet are clearly visible. Insertion of the umbilical cord is visible on the anterior abdominal wall. During the 8th and 9th weeks, developing intestine is being herniated into the proximal umbilical cord. The amnion can be clearly seen and the extraembryonic coelom is still larger in volume than the amniotic sac.

Figure 15 Normal morphologic development from 5 to 10 weeks of gestation.

The extraembryonic space will appear more echogenic than the amniotic sac. The ventricular cavities are characteristically cystic, particularly the rhombencephalon. This should not be confused with an early diagnosis of Dandy Walker cyst.

Nine to Ten Weeks

Cerebral hemispheres continue with development during the 9th and 10th weeks of pregnancy. Visible are lateral ventricles containing hyperechoic choroid plexuses. The head is clearly divided from the body by the neck. External ear is sometimes depicted in the 3D surface image. Herniation of the midgut is present. Dorsal column, the early spine, can be examined in its whole length. The arms with elbow and legs with knee are now visible. Feet can be seen approaching the midline (Fig. 15).

Eleven to Twelve Weeks

During the last phases of the end of the first trimester, striking changes in the development of the head and neck continue. Details of the face such as the nose, orbits, maxilla, and mandible are visible. Herniated midgut returns to the abdominal cavity.

Planar mode enables detailed analysis of embryonic body with visualization of the stomach and urinary bladder. Kidneys are also often visible. Arms and legs continue with development. Long bones can be visualized as hyperechoic elongated structures inside upper and lower extremities. Fingers and toes are visible (Fig. 16). Detailed 3D analysis of fetal spine, chest, and limbs is obtainable by using the transparent X-ray-like mode.

Figure 16 Fetus at 11 weeks showing movement of upper and lower extremities (A–D).

Excellent visualization has been achieved by the 3D and 4D ultrasound combined with the transvaginal approach (1,6,8,10,11). Demonstration of small embryo less than 10mm is still difficult. Figure 17 shows 18.3mm-CRL (crown-rump length) embryo at 8 weeks of gestation and macrographic pictures of an aborted specimen at the same gestational age.

Maximum mode of 3D ultrasound can clearly demonstrate fetal bony structure. Rapid ossification of craniofacial bones dramatically occurs during the first trimester. Craniofacial bony structure can be demonstrated from 10 weeks of gestation by ultrasound. Figures 18 and 19 show premature structure of craniofacial bony structure visualized by using maximum mode of 3D ultrasound at 11 and 14 weeks of gestation respectively.

Difference of frontal bone appearance between 11 and 14 weeks indicates membranous ossification of cranium in this stage. Figure 20 demonstrates bony structure of the occipital region of head at 13 weeks of gestation.

During early embryonal period, the CNS structure rapidly changes its appearance. The 3D ultrasound produced

high-resolucional images of embryos in early stage by tomographic ultrasound imaging and three-orthogonal imaging technologies (Figs. 21–26).

Indeed, advances described deserve the adjective “breath-taking,” in particular 4D parallel study of the structural and functional early human development (12–23).

Those 3D neuron-sono-embryologic imaging in utero may change embryonal staging in vivo in future, from “gestational age based on menstrual period or measurement of CRL” into “morphologic staging system” such as Carnegie staging in embryology. Indeed, improvements in the visualization deserve the adjective “breath-taking” in the better inside into the early human development (1,6,24–29).

THE ASSESSMENT OF VASCULARITY BY 3D POWER AND COLOR DOPPLER SONOGRAPHY

By adding colored blood flow signals to the gray scale image or extracting just the color-coded signals, it is possible to depict 3D image of vascular features of early pregnancy (Fig. 27).

This enables research of longitudinal changes in embryonic and early uteroplacental circulation. It has been found

Figure 17 Three-dimensional ultrasound images of the embryo at 8 weeks of gestation (left) and macroscopic pictures of aborted fetus at the same gestation (right). Normal 8-week embryo. Lateral view (upper left) and occipital view (lower left). Occipital view shows the premature spinal cord that is seen in the right picture. Source: From Ref. 6.

Figure 18 Three-dimensional maximum mode image of normal craniofacial structure at 11 weeks of gestation. Premature bony structure of frontal bone (F), parietal bone (P), nasal bone (NB), maxilla, and mandible are recognizable. *Source:* From Ref. 6.

Figure 19 Three-dimensional maximum mode image of normal craniofacial structure at 14 weeks of gestation. (Left) oblique view. (Right) frontal view. Anterior fontanelle (AF), sphenoidal fontanelle (SF), frontal suture (FS), coronal suture (CS), nasal bone (NB), maxilla, and mandible are gradually formed according to cranial bony development. *Source:* From Ref. 6.

Figure 20 Three-dimensional maximum mode image of occipital view at 13 weeks of gestation. Note the premature occipital bone appearance. Midline crack is demonstrated. Abbreviations: S, Sagittal suture; P, parietal bone; PF, posterior fontanelle; O, occipital bone; Cla, clavicle; Sca, scapula; LS, lambdoid suture. Source: From Ref. 6.

Figure 21 Tomographic sagittal imaging of normal fetus at the beginning of 8 weeks of gestation. Source: From Ref. 6.

that 3D power Doppler can clearly depict the full 3D continuation of early embryonic and umbilico-placental circulation. By adding the pulsed Doppler, pulsations from the embryonic aorta and umbilical artery can be identified as early as the 6th week of gestation. Umbilical artery is characterized by pulsations with absent end-diastolic

velocities. Between the 11th and 14th weeks, diastolic velocities gradually emerge (24). Placental branches of umbilical artery are easily depicted by 3D power Doppler (12,30–39).

Intracranial circulation becomes visible from the 8th week of gestation (Fig. 28).

Figure 22 Three orthogonal image and thick slice of three-dimensional reconstructed image (right lower) of normal brain at the end of 8 weeks of gestation. The development of premature ventricular system is seen. Note the different appearance from the beginning of 8 weeks of gestation. Source: From Ref. 6.

Figure 23 Sagittal section and coronal sections of 10-week fetus. CP, choroid plexus of the lateral ventricles. Source: From Ref. 6.

Figure 24 Normal brain at 10 weeks. Tomographic ultrasound imaging of sagittal (left upper), coronal (right upper), and axial (lower) sections. Choroid plexus (CP) occupies most of the lateral ventricles. Source: From Ref. 6.

Figure 25 Normal brain at 12 weeks. Tomographic ultrasound imaging of sagittal (left upper), coronal (right upper), and axial (lower) sections. Choroid plexus (CP) still occupies most of the lateral ventricles. Source: From Ref. 6.

Figure 26 Normal brain development on the midsagittal section between 8 and 12 weeks of gestation. Source: From Ref. 6.

The 3D power Doppler image presents early vascular anatomy on the base of the skull with branches evolving laterally to the mesencephalon and cephalic flexure. Pulsed Doppler signals are initially characterized by the absent end-diastolic velocities. Continuous pan-diastolic flow emerges at the gestational age of 9 to 10 weeks. A significant decrease in impedance was observed in intracranial vessels with advancing gestational age, being more pronounced than that in the other parts of the fetal circulation (12,22).

From the maternal side of placenta, it is possible to obtain the simultaneous 3D imaging of the developing intervillous circulation during the first trimester of pregnancy (Fig. 32A and 32B).

The 3D power Doppler reveals intensive vascular activity surrounding the chorionic shell starting from the first

sonographic evidence of the developing pregnancy during the 5th week of gestation.

Hyperechoic chorionic ring is interrupted by color-coded sprouts that penetrate its limits. These are areas of developing intervillous circulation. Pulsed Doppler reveals blood flow of two types in these areas: a pulsatile arterial like and a continuous venous like (27–29). All were in concordance with clear histomorphology evidence that spiral arteries began to open directly into the intervillous space during the second month of gestation (29).

FUNCTIONAL DEVELOPMENT OF CNS

The structural and functional development of the human CNS begins in the early embryonic period and proceeds through a sequence of very complicated processes long

Figure 27 On 4th and 5th weeks of the normal pregnancy, endometrium more (A) or less (B) vascularized, but it is always possible to distinguish a three-dimensional color signal penetrating to gestational sac. We named this finding the “comet sign” and it indicates the implantation site.

8 Weeks

Figure 28 Embryo at 8 weeks. Left picture showing clear morphology with normal appearances of head, limbs, and body on the three-dimensional picture. Right part of picture is made by three-dimensional power Doppler technology visualizing fetal aorta and earliest appearance of middle cerebral artery.

9 Weeks

Figure 29 Three-dimensional (3D) static visualization of morphology of early fetus at 9 weeks (left) and 3D power Doppler visualization of umbilical cord vascularity, fetal aorta, and maternal part of circulation (right).

Figure 30 Morphology and vascularization of fetus at 10 weeks of gestation.

Figure 31 Illustrative examples of the anatomy of fetus and both parts of placental circulation: fetal (with bifurcation) and maternal uterine wall.

Figure 32 Uterochorionic, intervillous, and fetal circulation at 8th (A) and 10th (B) weeks of pregnancy. Abbreviations: UCV, uterochorionic (spiral and radial) vessels; IVF, intervillous blood flow; UV, umbilical vessels; H, heart; DV, ductus venosus; CV, cerebral vessels; ACV, antichorionic vessels.

after delivery (40). 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. 33) (41).

The earliest interneuronal connections, the synapses, can be detected in the spinal cord shortly before the onset of embryonic motility, at 6 to 7 weeks of gestation (42). Therefore, the neural activity leading to the first detectable movements is considered to originate from the spinal motoneurons (43). 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

Figure 33 The main events in the development of the central nervous system (CNS) and motor development during the first 8 weeks of pregnancy. Source: From Ref. 40.

contract as soon as they are innervated by motoneurons (44). 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 33. 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 (45). These, so-called vermicular movements appear in irregular sequences (46). Simultaneous 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 (47). 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. This pattern has been interpreted as the first sign of a supraspinal control on motor activity (48,49) and can be recognized from 8 to 9 weeks of gestation onward (49,50).

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 (51). The interconnected neurons generate patterned activity because of

endogenous properties of the neurons (52). Recent investigations have shown that neurons are able to communicate through non-synaptic mechanisms even before the onset of synaptogenesis (53–55).

The brain stem is fashioned around the 7th week of gestation (48) and basic structures of the diencephalon and cerebral hemispheres are formed by the end of the 8th gestational week (56). 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 (53,57).

The brain stem consists of the medulla oblongata, pons, and midbrain (Fig. 34).

It forms and matures in a caudal to rostral direction. That means that the phylogenetically 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 7th to 8th week of gestation and are completely matured by 7 months of gestation (48). In addition to its many subnuclei, the medulla gives rise to a variety of descending spinal motor tracts that 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. 34) 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

Figure 34 The main events in the development of the central nervous system (CNS) and motor development during the last 6 weeks of the first trimester. Source: From Ref. 40.

Figure 35 Image of the fetus recorded by three-dimensional/four-dimensional sonography, showing isolated finger movements.

10 Weeks

Figure 36 Fetal movement at 10 weeks and 6 days. Active arm movements (A–E) are associated with active body and lower limb movements. Despite active movements of glenohumeral and elbow joints at this stage, wrist and finger movements were not visualized in most cases.

11 Weeks

Figure 37 Fetus at 11 weeks still opens the palms but five digits are no longer on the same layer (A). Mild adduction of the thumb and atonic fingers were observed and the palm appearance was clearly different from that in 9 and 10 weeks (B).

12 Weeks

Figure 38 Clench (A) and unclench (B) of the fist at 12 weeks. At this stage, fetuses start to clench and unclench their fists.

structures of the pons include the V to VIII cranial nerves (vestibular nuclei of the nerve VIII) and the medial longitudinal fasciculus, 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 to 11 weeks.

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 (58,59). From 10 weeks onward, the number and frequency of fetal movements increase and the repertoire of movements begins to expand. Qualitative changes in general movements can also be observed. These movements, which are slow and of limited amplitude during 8 and 9 weeks, become more forceful at 10 to 12 weeks. After the 12th week, they become more variable in speed and amplitude (60). The isolated limb movements seen at the 9th week of gestation are followed by the appearance of 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 to 13 weeks. Finally, at 13 to 14 weeks, isolated finger movements can be observed, as well as increases in the activity and strength of the hand/finger movements (61) (Fig. 35). Using 4D sonography, Kurjak and collaborators have found that from 13 gestational weeks onward, a “goal orientation” of hand movements appears and a target point can be recognized for each hand movement (62).

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 4D 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 (63). Using 4D sonography, general movements were found to be the most frequent movement pattern between 9 and 14 weeks of gestation (64). Furthermore, the advanced ultrasonic techniques, 3D and 4D sonography, significantly improve the assessment of structural and functional development of embryonic and fetal CNS (65–67). Some illustrative examples are shown on Figures 36, 37, and 38.

The development of CNS begins rather early in the embryonic period and proceeds throughout the gestation and continues long after birth.

Although some histogenetic processes are not finished until puberty, the neurodevelopmental events are very active in the early pregnancy (56). The earliest signs of functional development of CNS, such as motility, can be registered as early as the late embryonic period (62,68).

The sequential appearance of specific movement patterns, first described by de Vries et al., is presented in Table 1 (68).

Despite the rapid development and a fascinating variety of movements during this early period of gestation, dynamic

Table 1 Developmental Sequence of Fetal Behavioral Patterns in the First Trimester of Pregnancy (68)

Type of movements	Postconceptional weeks				
	7	8	9	10	11
Sideway bending	+	+	+	+	+
General movement		+	+	+	+
Startle		+	+	+	+
Isolated arm movement			+	+	+
Isolated leg movements			+	+	+
Hiccup			+	+	+
Suckling			+	+	+
Swallowing			+	+	+
Hand to face contact				+	+
Head retroflexion				+	+
Head antelexion				+	+
Head rotation				+	+
Breathing movement				+	+
Stretching				+	+
Jaw opening				+	+
Yawning					+

pattern of neuronal production and migration, as well as the immature cerebral circuits, is considered too immature for the cerebral involvement in the motor behavior (69).

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