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Chapter 1

FERTILIZATION, IMPLANTATION AND ORGANOGENESIS

Pregnancy is a long road to a new life; it begins at the moment when the spermatozoon fertilizes the ovum, and ends with delivery. This period, which is of paramount importance both for the mother and child, can be divided into the following main stages of antenatal (pre-delivery) development.

- **Embryonic stage** starting at the moment of fertilization (at two weeks of pregnancy)[1] to full 10 weeks of pregnancy (full 8 weeks after fertilization) sees the following events:
  – 3–8 weeks: formation of rudiment organs in the fetus;
  – 2–4 weeks: formation of the heart and vessels;
  – 4–5 weeks: beginning of lung formation, early development of the nervous system;
  – 7–8 weeks: kidney formation.
- **Fetal (syn.: antenatal) stage** lasting from 11th-week of pregnancy until birth (8–38 full weeks from fertilization or 10–40 weeks from the first day of the last menstrual period):
  – 8–12 weeks: sex differentiation;
  – 15–20 weeks: intensive growth and maturation of the cerebral cortex;
  – 20–24 weeks: formation of major functional systems of the fetus.

Embryology (from Greek εμβρυον — fetus, embryo, *logos* — study) developed as a study of embryogenesis, intrauterine development from the moment of conception until birth.

The first notions of the child's intrauterine development emerged in ancient times; they were propounded in the works of philosophers and doctors of Ancient India, Egypt and Greece (Hippocratic Corpus). Some of them (like Anaxagoras in the 5th cent. BC) thought that the paternal or maternal semen contains in miniature all the parts of the future fetus (Fig. 1.1).

Thus there should exist a small human being not discernible by the eye; its development means that it merely grows in size (the **idea of premorphism** from Latin *praeformare*, to form in advance).

Aristotle (384–322 BC) was the first to challenge these notions (Fig. 1.2). He stated that the organs of the future fetus develop from the fertilized egg by way of consecutive transformations (the **idea of epigenesis** from Latin *epi-*, above and *genesis*, origin). This thesis by Aristotle persisted in science without essential modifications until the 17th century. For a long time, the ideas of premorphism and epigenesis

[1] Henceforward the gestational age is determined starting from the first day of the last menstrual period, although conception takes place on day 10–18 of the menstrual cycle (2 weeks after the first day of the last menstrual period, on average).

existed side by side, with premorphism holding the predominant position, especially in the 17–18th centuries.

The rise of embryology as a science is associated with the name of William Harvey (1578–1657), English physician who made seminal contributions to Anatomy and Physiology (Fig. 1.3).

In 1651 he published his work Experiments Concerning Animal Generation (*Exercitationes de generatione animalium*) which saw countless later editions. Having studied the development of chicken and some mammals, Harvey contested the idea of self generation and put forward closely reasoned arguments against the premorphism doctrine. He generalized the notion of the egg as a source of development for all animals. However, due to the inadequacy of microscopy equipment, Harvey had no opportunity of studying the egg of mammals.

Regnier de Graaf (1641–1673), Dutch anatomist and physiologist, came close to the discovery of the ovum (Fig. 1.4).

De Graaf was the first to study testicular tubules and defined them as 'semen-producing vessels'. In 1672 he described the follicles in female sex glands which he mistakenly took for eggs, *ova*, hence the word 'ovarium'.

Only a hundred and fifty years later it became possible to establish the truth: using more advanced microscopy equipment, K.M. Baer showed that Graafian follicles are merely cavities where ova are formed and from where they are released as a result of ovulation (from late Latin *ovulum*, small egg, a diminutive word for ovum).

Karl Ernst von Baer (1792–1876), Academician of the St Petersburg Academy of Sciences and its honorary member, holds a special place among the founders of embryology (Fig. 1.5).

He discovered the main laws of embryogenesis and made important theoretical generalizations. K.M. Baer was the first to see and describe the ovum of mammals and humans (1827); he discovered the blastula, explored and described the development of all major organ systems from the germinal layer in vertebrates.

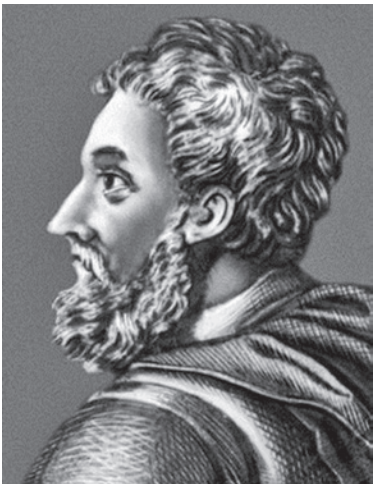


Fig. 1.1. Anaxagoras

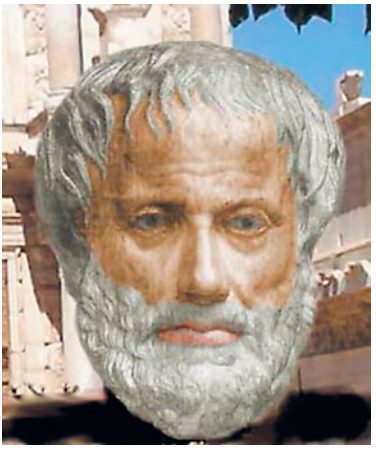


Fig. 1.2. Aristotle

Fig. 1.3. William Harvey

Fig. 1.4. Regnier de Graaf

Fig. 1.5. Karl Ernst von Baer

Having established the law of similarity between embryos of different calluses of vertebrates he showed that during the intrauterine development the first characteristics to become manifest are the characteristics of the phyllum followed by those of the class, order and so on; specific and individual characteristics develop at later stages of embryogenesis. He also noted that the human embryo develops in a fashion similar to that of all vertebrates.

1.1. FERTILIZATION

1.1.1. Germ cells

The male germ cell (spermatozoon) (Fig. 1.6) is a filamentous cell consisting of the head, neck and tail. The nucleus contains 23 chromosomes, a half of spermatozoa carry X-chromosome; their mass is greater than that of the spermatozoa carrying Y-chromosome. The spermatozoa carrying X-chromosome are less motile. The ejaculate is a jelly-like mass containing a mixture of secret from the testes, prostate, Kupffer cells and seminal vesicles. Normally the volume of ejaculate is 3–5 ml depending on the man's age, nutrition and the intensity of his sex life.

Ejaculate normally contains 200–500 million spermatozoa (no less than 150 million); their concentration per 1 ml of sperm exceeds 6 million.

Spermatogenesis. Sperm is produced inside coiled seminiferous tubules that take up over 97% of the volume of testes. Spermatozoa develop to become mature over 72 hours (Fig. 1.7).

The entire process of spermatogenesis can be broken up into four distinct stages: reproduction, growth, maturation and formation.

Female germ cells (oocytes) (Fig. 1.8). Formation of the ovaries begins at 8 weeks of intrauterine development. By the moment of birth, about 1 million of primary follicles are contained in the cortical layer of the ovary, each of them carrying one (seldom two) germ cell. Each primary follicle can achieve complete development and produce an ovum ready to be fertilized. The store of follicles (oocyte pool, ovarian reserve) cannot be replenished during the woman's life; this is an individual amount

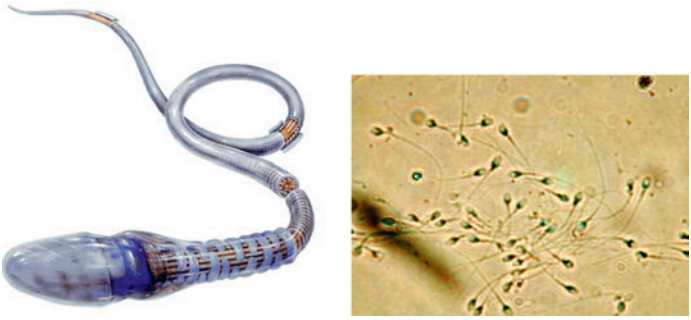


Fig. 1.6. Spermatozoa

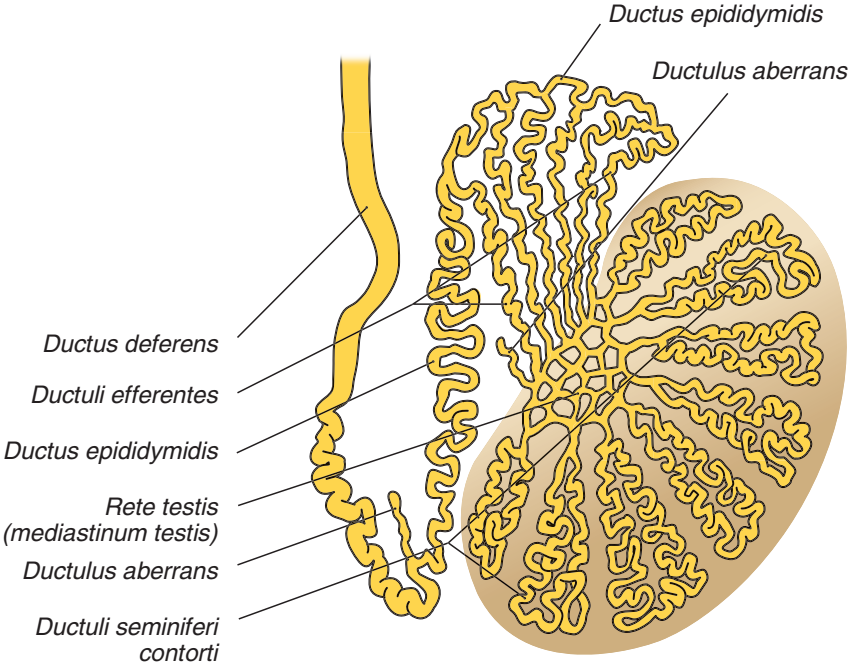


Fig. 1.7. Structure of testis

that reaches its peak values by 3–4 months of gestation (about seven million follicles). As the ovary ages, the number of primordial follicles decreases due to the process of apoptosis from 1 million at birth to 250,000–300,000 by the time of menarche. Over the whole span of the woman's sexual maturity only 400–500 follicles reach maturation, other primary follicles die.

NB! The mean lifespan of a spermatozoon after ejaculation is 48 hours.

The development of follicles starts with mitosis of follicular cells and their transformation from initially flat ones to cuboid-shaped and then to highly prismatic. The reproducing cells now called **granulosa** fill the whole follicle. In the course of further development the fluid secreted by granulosa cells begins to push the cells aside ousting

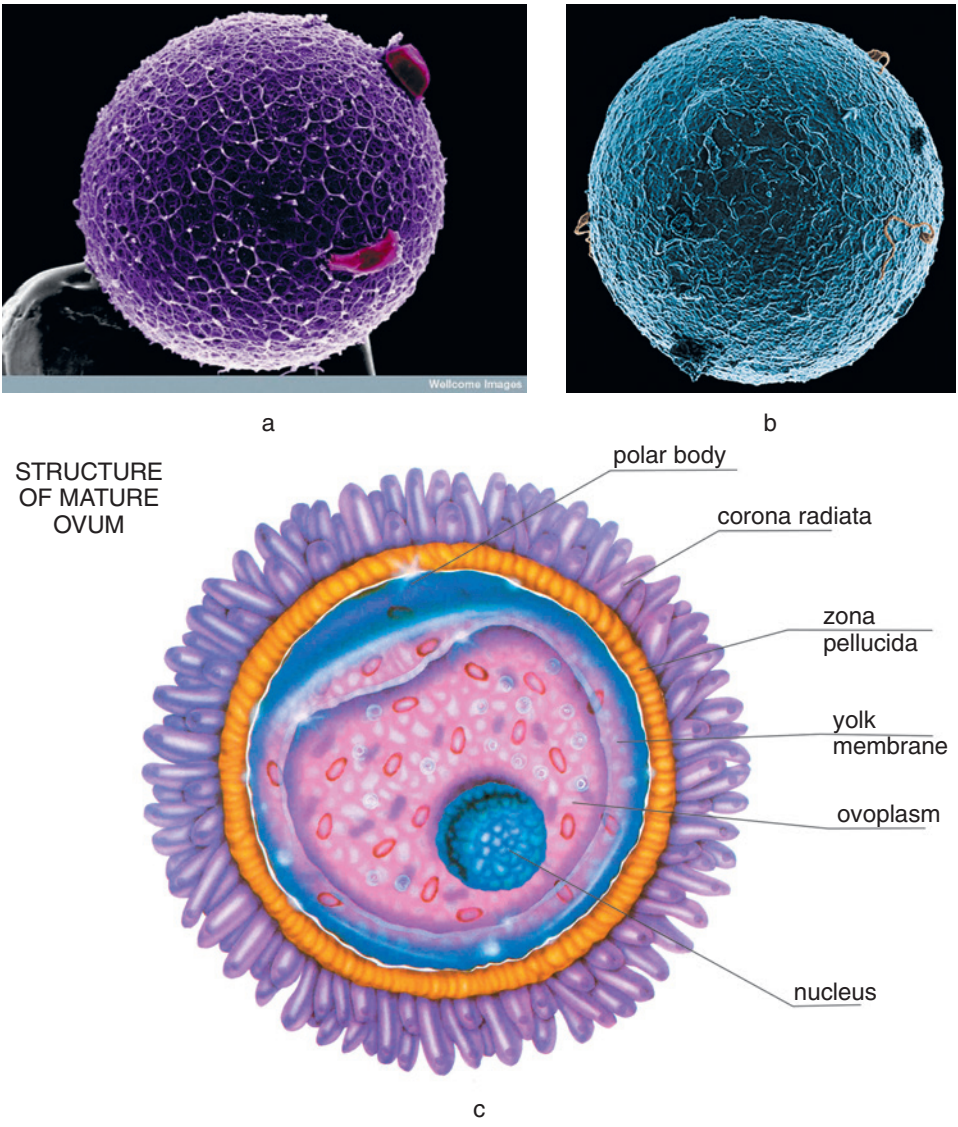


Fig. 1.8. Ovum: a, b — photomicrography; c — ovum structure

them to the peripheral follicular layers. A cavity filled with **follicular fluid** is formed inside the follicle. The follicle itself grows larger, distends; from compact it becomes hollow, and now it is called a **mature follicle** (syn.: Graafian follicle, Graafian vesicle). A developing follicle suppresses the maturation of other follicles. Other follicles that have begun to mature undergo atresia.

NB! The amount of follicles is not restored during a human lifetime; it is the pool of oocytes that emerged at the eighth week of intrauterine life that is spent.

NB! Atresia of follicles means their destruction

In atresia the oocyte dies first, then follicular epithelium undergoes adipose transformation and vacuolization; the fluid of the follicle is absorbed, its cavity becomes hollow and gets obliterated by the ingrowing connective tissue.

A follicle is considered dominant once its diameter reaches 18 mm (Fig. 1.9).

On the outside it is surrounded with fibrous connective tissue consisting of two layers: the external thin layer of dense connective tissue, and the internal layer rich in vessels and consisting of large connective tissue cells.

The inner layer of the dominant follicle is represented by several layers of epithelial cells forming the *membrana granulosa*. At one point of the mature follicle the cells of *membrana granulosa* are collected into a mass which projects into the cavity of the follicle; this formation is called **cumulus oophorus**, the ovum is contained inside it. The ovum is surrounded by three coats:

- yolk membrane which is the superficial layer of the ovum cytoplasm;
- a thick luminous coat of the follicular epithelium, *zona pellucida*;
- radiate crown (*corona radiata*) — granulosa cells placed in a radial fashion in 2–3 layers; they adhere to the ovum immediately.

Granulosa cells are of great importance for the ovum nourishment. The follicular fluid that is formed induces the Graafian vesicle to get larger leading to extreme distention at its pole extending above the ovarian surface. The protein coat above it gets overdistended, exsanguinated, thinned out.

The Graafian vesicle opens, under pressure the follicular fluid flows to the place of rupture (**stigma**) and carries the ovum with it. The release of a mature ovum from the follicle into the abdominal cavity is called **ovulation**.

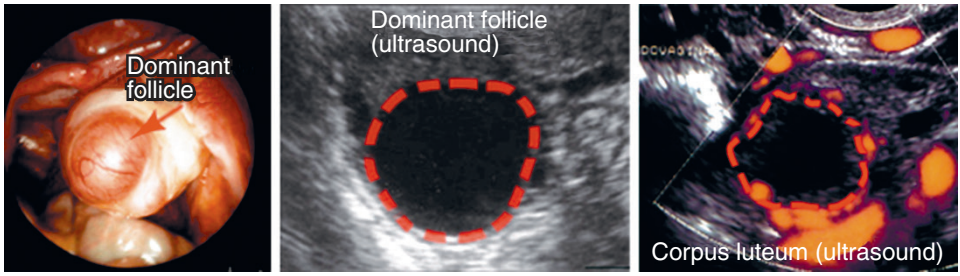
Having left the follicle the ovum begins its extrafollicular life.

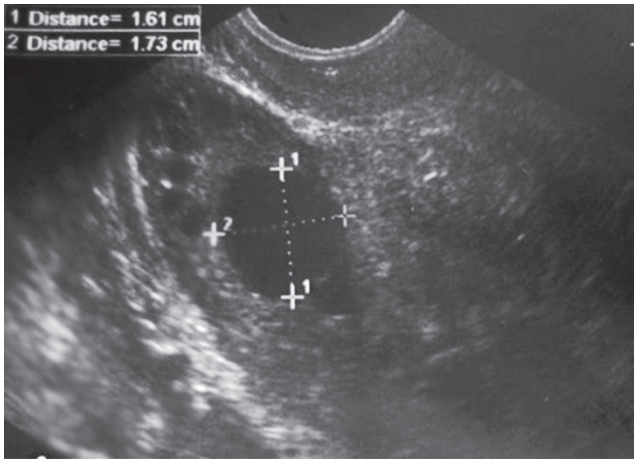
NB! The lifespan of extrafollicular existence of an ovum is no longer than 24 hours

This premise is of fundamental importance in dealing with practical issues of fertility and conception (for instance, when determining when to withdraw the ovum in the procedure of in vitro fertilization and embryo transfer or in prevention of unwanted pregnancy when determining the day most likely for conception considering the lifespan of spermatozoa and the ovum, which is no more than three days!)

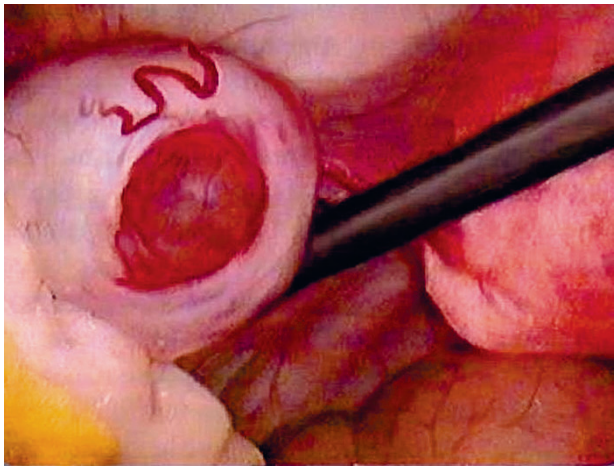
The ovum released from the follicle finds itself in the abdominal cavity where it has the opportunity to find its way into the lumen of the Fallopian tube and move along into the uterine cavity.

After rupture of the Graafian vesicle its inner wall deflates forming folds, the cavity gets filled with blood. At the same time, an intensive development and reproduction of granulosa cells begins; now they are **lutein** cells producing progesterone. The lutein cells appearing in the place of the ruptured follicle give rise to a temporary endocrine structure, the **corpus luteum**. It grows in size fast; its fate depends on what happens to the ovum. If the ovum is fertilized, the corpus luteum develops and is active until





a



b

Fig. 1.9. Dominant follicle. Ultrasound (a) and laparoscopy (b) images

16 weeks gestation (corpus luteum of pregnancy). If fertilization does not occur, the process ends in regression of the corpus luteum within 12–15 days. A connective tissue adhesion, *corpus albicans*, remains in its place. It persists in the ovary for several days and then resolves and disappears (Fig. 1.10).

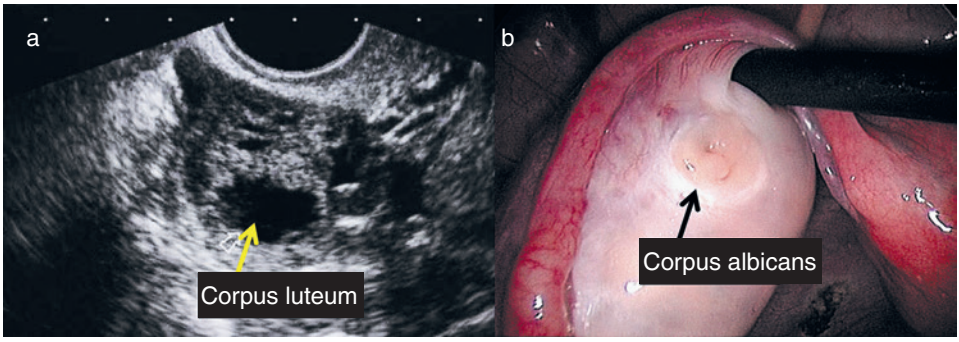


Fig. 1.10. Corpus luteum (a) and albicans (b) in the ovary

1.1.2. Fertilization

NB! **Fertilization** (conception) is a process when the male and female sex cells unite and assimilate each other to produce a new organism with a unique genetic makeup

After the intercourse the ejaculate finds itself in the woman's vagina, in the posterior fornix predominantly; that is where the vaginal part of the uterine cervix faces when the uterus is in *anteflexio-anteversio* position. After a normal ejaculation 100 million spermatozoa on average remain in the vagina.

A portion of the ejaculate may leak from the vagina, but even in the remaining portion all the spermatozoa can hardly retain their vitality intact: under the impact of the acidic medium most spermatozoa either die or lose their motility. Having overcome the obstacle in the form of cervical plug the remaining sperm finds itself in the uterine cavity within half an hour, in 1–2 hours — in the lumen of the uterine tube. In this case the acidic vaginal medium, the cervix of uterus and the isthmus of the uterine tube act as a selective filter and reservoir of spermatozoa: the population of spermatozoa which reached the ampullar region of the uterine tube contains healthier spermatozoa than the ejaculate (Fig. 1.11).

Up to the present day it has not been established where fertilization takes place. Some researchers believe that the fusion occurs in the ampullar region of the uterine tube, others state that this happens in the abdominal cavity followed by capture: the fertilized ovum is captured by the fimbriae of the uterine tube. It was well established that a woman with one ovary and one uterine tube on the opposite side can become pregnant as in this case there is an opportunity for capture of the ovum from the abdominal cavity.

In uterine tubes the sperm become active under the impact of mucous secretion of tubal epithelium, which leads to **capacitation** (binding) of spermatozoon to the ovum surface. An acrosomal reaction follows: lysosome-like bodies contained in the spermatozoon head dissolve the ovum coat and the spermatozoon can penetrate inside (Fig. 1.12).

It is supposed that in fertilization lysosomes disintegrate releasing their own enzymes as well as a number of enzymes of other organelles that activate biochemical reactions in the ovum. Once a spermatozoon (head, neck and the intermediate part) penetrates the cytoplasm, the tail is discarded. Cortical granules form the **fertilization membrane** — a new membrane resistant to such influence and preventing the penetration of other spermatozoa.

The nuclei of the female and male germ cells transform into **pronuclei**. Upon their contact the **syncaryon stage** (fusion of two nuclei) begins, thus a **zygote** (from Greek *zygote*, joined together) appears — beginning of a new life.

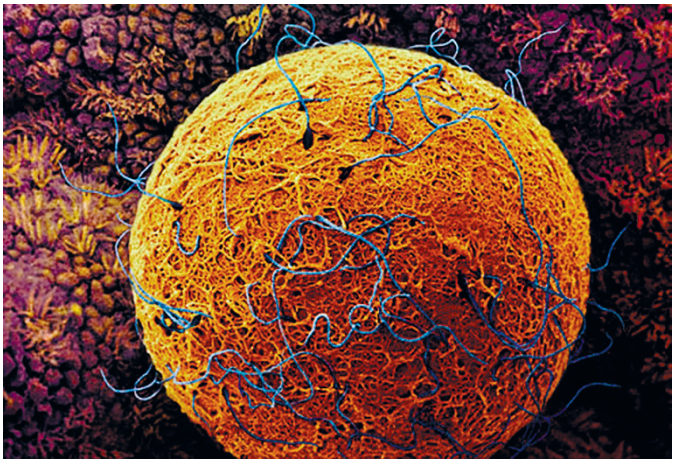


Fig. 1.11. Spermatozoa attempting to penetrate the ovum (photomicrography)

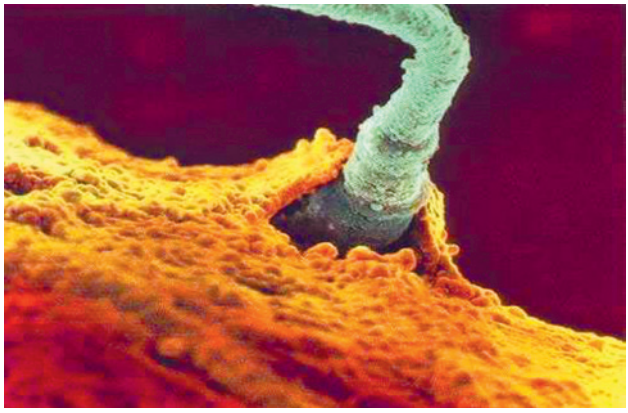


Fig. 1.12. Spermatozoon penetrating the ovum (photo Lenart Nilsson)

Division of the zygote begins by the first day of its existence. Thus the fertilized ovum begins to divide while it is in the uterine tube. During the period from ovulation to implantation, which is within about one week, the fertilized ovum persists in a free suspended state. Its transition along the whole length of the uterine tube takes 3–4 days on average.

1.2. EARLY PREGNANCY (FIRST TRIMESTER)

Cleavage of the zygote into blastomeres (spheres of division) follows a strongly defined genetic program starting 24 hours after fertilization spanning over the next three days. Cleavage of a human zygote is complete, asymmetrical, and asynchronous. The first cleavage takes place over 24 hours, others — every 12 hours.

The first two blastomeres differ from each other: one is dark, smaller in size; the other one is larger and lighter. The light blastomeres cleave faster growing in one layer over the dark ones. Within 40 hours the number of blastomeres becomes 4; on day 4 the embryo consists of 7–12 blastomeres; 50–60 hours after conception a **morula** is formed (a diminutive word from Latin *morum*, mulberry) (Fig. 1.13).

For three days the morula persists in the uterine tube. Depending on the number of blastomeres the stage of morula development can be defined as first or second. For 4 days, as the outer layer of blstomeres grows thicker, an inner cavity, **blastocoel**, forms in the morula. This is the way the blastocyst, or the third presomite stage, develops. The superficial light cells of the blastocyst give rise to an external thin layer thus forming the **primary trophoblast** while the dark cells give rise to the **embryoblast**.

NB! **The primary trophoblast** is a stem cell for most placental cells and tissues; **the embryoblast** is a source of all fetal cells, tissues and related membranes

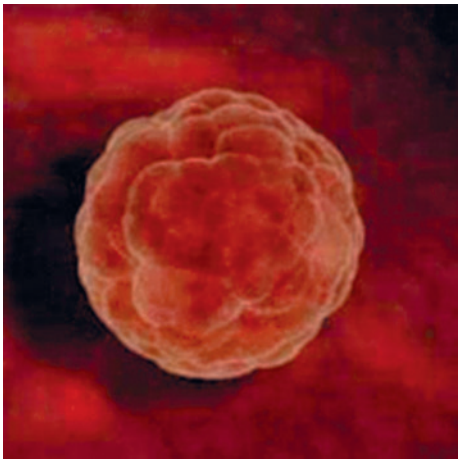


Fig. 1.13. Morula 50–60 hours after fertilization

The embryoblast presented as an aggregation of a number of cells (**inner cell mass**) attaches itself to the trophoblast on the inside. As such the blastocyst enters the uterus on day 4, and for another 24 hours it remains there in a free state (free blastocyst stage) (Fig. 1.14).

From day 6–7, once the blastocyst hatches out of the partially dissolved *zona pellucida*, its **implantation** (**nidation**, from Latin *nidus*, nest) takes place.

Implantation proceeds in two stages: **adhesion** and **invasion**. First the trophoblast adheres to the endometrium and starts differentiating into **cytotrophoblast** still connected to the embryo wall, and **syncytiotrophoblast**, a peripheral layer in the form of cytoplasm mass with several nuclei without borders, that is, a typical symplast. They are referred to as primitive, or prechoroid forms. During the invasion the lysosomes of these primitive villi of syncytiotrophoblast release proteolytic, glycolytic and other enzymes that 'melt down' the tissues of the uterine mucosa (Fig. 1.15).

NB! **Blastogenesis** includes the stage of free blastocyst and implantation process

The blastocyst invades energetically between epithelial cells of the endometrium and finally positions itself inside the stroma. This process is accompanied by specific morphological and metabolic changes promoting cell growth and differentiation.

It was established that the cytotrophoblast cells show the greatest enzymatic activity; the primary trophoblast produces proteolytic enzymes vigorously (**proteolysis**).

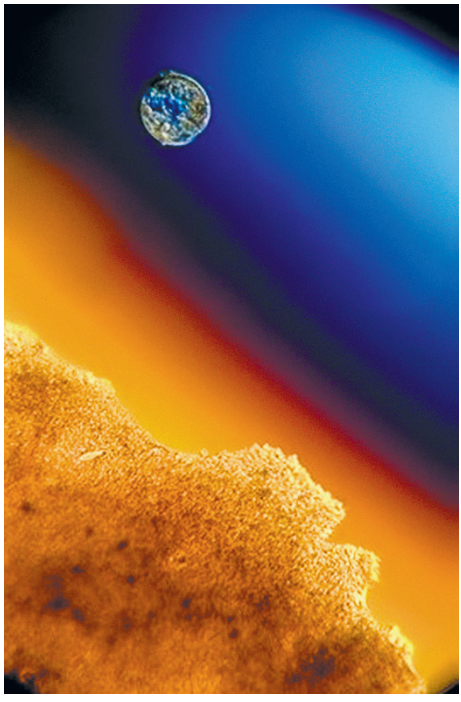


Fig. 1.14. Blastocyst in the uterine cavity 5–6 days after fertilization, free blastocyst stage (photo Lenart Nilsson)

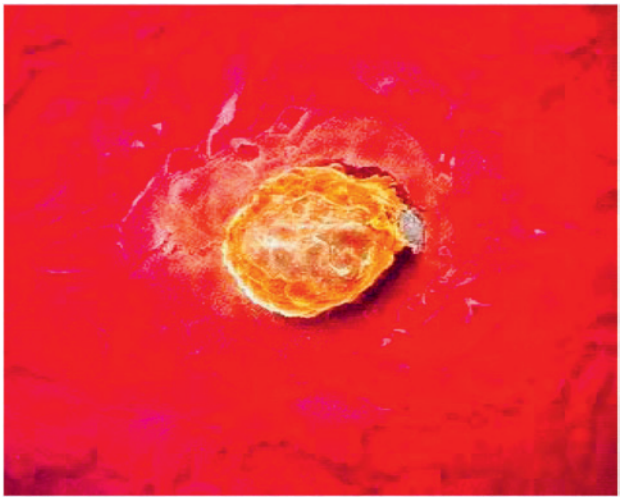


Fig. 1.15. Implantation complete: day 8 (photo Lenart Nilsson)

The depth and extent of invasion depend mostly on the lytic ability of the trophoblast. Autolysis of the uterine epithelium itself is of no small importance in this process; it proceeds under the control of progesterone (endometrial sensitivity). Blastocysts are necessary in that they provide for a specific molecular environment.

NB! Implantation is only possible when endometrium is ready for autolysis and the blastocyst is ready for proteolysis

If blastocysts start interacting with insensitive (unprepared) endometrium, they do not get implanted. Based on this assertion, a theory of implantation window was elaborated. In humans the implantation window is limited to day 19–22 of the menstrual cycle, as a rule. This is the time when the uterine endometrium is ready for implantation, which is manifested by production of a great number of biologically active substances. The endometrial ability to receive the blastocyst ceases completely on the 22nd day.

What factors determine the endometrial sensitivity to implantation?

- First, it is the physiological changes in the endometrium that take place in the second phase of the menstrual cycle.
- Second, it is the rise of adhesion molecules on the endometrial surface; they interact with ligands on the corresponding epithelium of the blastocyst (same as in leukocyte adhesion to the vascular wall).
- Third, it is the rise of large cytoplasmatic formations (**pinopodes**) in endometrial cells, which takes place exactly from the 19th to the 22nd day of the menstrual cycle, that is, while the implantation window is open.

NB! Implantation can only occur during the implantation window, a period of time limited to day 19–22 of the period when the endometrium is sensitive to the blastocyst to the utmost

Implantation occurs on the posterior wall in the superior uterine segment exactly where a blood vessel runs close to the endometrial surface. This spot will later become the uteroplacental area. The notion of **uteroplacental area** includes the zone of immediate contact between the anchoring villi of the placenta with the endometrium and the adjacent myometrial layers. Apart from this term, other names are used depending on the gestational age. Thus, at the stage of implantation the zone of immediate contact between the embryo and the endometrium and adjacent myometrial layers is called implantation site or **cytotrophoblast shield**. Later, along with placenta formation, the underlying endometrium is defined as *decidua basalis* as opposed to *decidua parietalis* that is part of fetal membranes. When the placenta is born, its rupture line passes roughly in the middle of *d. basalis* (from Latin *decidere*, fall off) that is divided into two parts: the severed fragments on the maternal placental surface called basal plate, and the remaining endometrial portions with the adjacent myometrial layer — **placental bed**, or uterine bed.

During implantation the process of cleavage continues in the trophoblast: cytoplasmatic excrescences (**primary villi**) develop; the inner cell mass transforms into **embryonic disc** (**embryonic shield**). The cleavage of cells in the trophoblast and in the inner cell mass proceeds independently from each other. This stage is called **epiblastula**. The embryonic shield has a compact structure; two types of cells are distinguished in it: the **ectoblast** (**ectoderm**) and the **endoblast** (**endoderm**). The embryonic disc and the cavities surrounding it take up only a smaller part of the chorionic sac cavity; the greater part is taken up by loosely placed cells of the extraembryonic mesenchyma (**mesoderm**) and the fluid contained in it; together they form the **extracoelomic cavity**.

The embryo grows fast which is due to both cell division and the inflow of protein-containing fluid coming through the trophoblast and accumulating inside. As a result, the epiblastula transforms into the embryonic sac. Later a groove emerges circling the embryonic sac; becoming deeper it forms a pedicle that connects the abdominal part of the embryo with the rest of the embryonic sac. This portion of the embryonic sac is called the **yolk sac**, the pedicle — the **vitelline duct**. As the nutrients supplied by the yolk sac begin to run low, its walls deflate and get atrophied. Simultaneously with the yolk sac formation the ectoderm and the parietal plate of the mesoderm rise forming a fold around the protruding dorsal surface of the embryo. Growing in all directions these folds meet over the embryo's back; thus the embryo finds itself enclosed in two sacs.

In the period between day 17 and 19 the chorionic sac continues to grow in diameter later differentiating into two portions: the bald chorion (its thinning-out segment protruding into the uterine cavity), and the bushy chorion where the mesenchyma continues to invade the distal segments of cellular columns forming typical mesenchymal villi with a continuous coat of cyto- and syncytiotrophoblast. Lateral branches emerge there thus expanding the intervillous space, whereas, in contrast, there is no arborization of villi in the area of the bald chorion.

As the cytotrophoblast moves on in an alien antigen medium, muscular elements and endothelium get lysed around the spiral artery wall, and the arterial lumen grows considerably wider. The process that consists in lysis of muscular-elastic elements and endothelium in the spiral arteries with their replacement by fibrinoid, drastic

enlargement of the lumen and formation of an ostium opening into the intervillous placental space is called **gestational modification of spiral arteries into uteroplacental arteries**. Gestational modification of spiral arteries, dissolution of their end portions and formation of ostia opening into the intervillous space takes place 4–6 weeks after fertilization, that is, at week 6–8 of obstetric gestational age thus giving rise to uteroplacental blood circulation.

Towards the end of the first trimester, after emergence of 20–30 uteroplacental arteries, the cytotrophoblastic invasion runs low as most cells of the interstitial cytotrophoblast become gigantic multinuclear cells concentrating at the border between the endometrium and myometrium. The initial extent of uteroplacental circulation that developed due to the first wave of cytotrophoblastic invasion now becomes insufficient for further fetal development. That is why after a «rest period» lasting several weeks there emerges a discrepancy between the inflow of arterial maternal blood and the demands of the developing placenta and intensive organogenesis of the fetus (another hypoxia stimulus). As a result, a second wave of cytotrophoblastic invasion is launched at week 15 reaching its maximum degree at week 16–18. This wave expands to include mostly the arteries of the adjacent myometrium as well as those of peripheral placenta affecting new spiral arteries of the nearby parietal endometrium. In this way the placenta develops along with the uteroplacental bed enlarging both their total area and the depth of penetration into the myometrium.

The waves of cytrophoblastic invasion into the endometrium, walls of arterioles and arteries of the uteroplacental area are key processes that to a great extent determine the blastocyst implantation, formation of uteroplacental circulation and the rate of its growth depending on metabolic requirements of the embryo and fetus.

1.3. SECOND TRIMESTER

1.3.1. Changes in the fetus and extraembryonic structures

The transition from first to second trimester is marked by an emergence of new significant changes in the fetus and extraembryonic structures.

- First, the rudiments of major organs consistently build up their functions requiring additional nourishment, more active metabolic processes, more intensive placentofetal circulation.
  - The development of blood circulation system outstrips other organs: there emerges the fetal type of circulation with three bypasses (venous duct, foramen ovale, arterial duct) where arterial blood from the umbilical vein mixes with venous blood.
  - The liver is active as the main organ of erythropoiesis.
  - Lymphoid organs (the thymus, spleen, lymph nodes and others) gradually begin their function.
- Second, the cytotrophoblastic invasion livens up after a period of certain attenuation. It is aimed mainly at myometrial segments of uteroplacental arteries;

this provides for the inflow of maternal arterial blood into the intervillous space of placenta, which is necessary for fetal development.

- Third, a consistent transformation of extraembryonic organs takes place: the allantois disappears having «flickered» for a brief period of time in the tenth somite stage (its function was to help the embryo's blood vessels move along and connect with the capillaries in the wall of the chorionic sac, that is, to help the formation of embryoplacental blood circulation). The yolk sac disappears too; the amniotic cavity continues to grow as well; it closes ranks with the wall of the chorionic sac and, together with *decidua capsularis*, gradually forms typical fetal membranes. A second wave of cytotrophoblast invasion follows: the development of the placenta, umbilical cord and formation of the amniotic space.

The second wave of cytotrophoblast invasion. At 16–18 weeks of gestation the second wave of cytotrophoblast invasion begins due to a discrepancy between the inflow of arterial maternal blood and the requirements of the developing placenta and intensive organogenesis.

The main mechanism of the second wave consists in penetration of interstitial cytotrophoblast through the walls of endometrial arterial segments, its egress through the damaged endothelium into radial uterine arteries and further intravascular migration; from this moment on it is referred to as **intravascular cytotrophoblast**. The way the intravascular proliferation and cytotrophoblast migration (the latter slowly moves against the flow of maternal blood in proximal direction) are regulated on the molecular level is not understood well enough.

The main physiological purpose of the second wave is to boost the inflow of maternal blood into the intervillous space by way of gestational remodeling of walls and enlarging the lumen of radial arteries, or rather, their myometrial segments. In this way adequate hemodynamic conditions for a faster fetal growth in comparison with less intensive accretion of placental mass are created.

NB! Reaching its peak at 16–18 weeks, the second wave of cytotrophoblast invasion occurs due to an imbalance between the inflow of maternal arterial blood, the demands of the developing placenta, and intensive fetal organogenesis

As a result, the cytotrophoblast invasion, taken all round, is a unique short-term tumor-like growth of specialized placental cells: first it is the growth of symplastic complexes, and then — of invasive cytotrophoblast (both interstitial and intravascular).

The wave-like and limited nature of the cytotrophoblastic invasion is regulated by the hypoxia stimulus as well as by para- and autocrine molecular signals passing between:

- the blastocyst and the adjacent endometrium;
- proliferate of villous cytotrophoblast from anchoring villi and components of the surrounding matrix of spiral arterial walls within the endometrium (the first wave during the somite and postsomite stages of embryonic development);
- intravascular cytotrophoblast and the endothelium of myometrial segments in uterine arteries (the second wave of cytotrophoblast invasion).

1.3.2. Development of placenta

By the end of the first trimester the anatomic formation of the placenta is roughly complete; it is now ready to meet the growing requirements of the developing fetus. Thus by the age of 12 weeks the placental mass amounts to 26 g while the embryo weighs only 17 g (Fig. 1.16).

During weeks 13–16 the development of the placenta corresponds to the beginning of fetalization. The villi are mostly of the **intermediate immature type** with characteristic stromal channels and loose-lying Hofbauer cells (placental macrophages) in their lumen. Another structural characteristic of the stroma of these villi is the immediate adjacency of capillaries to the epithelial coat; due to this fact the distance between the epithelium and capillaries is shortened. The villous epithelial coat is 2–12 μm thick (9.6 μm on average), the area of capillaries and other fetal vessels amounts to 6% of the total villi area, and the total area of the villous tree amounts to 0.544 $m^{2.}$

NB! In regards to its structure, the placenta meets the demands of constantly increasing uteroplacental circulation and those of the quickly increasing fetal weight

Thus **during weeks 13–16** the placenta increases its mass from 30 to 65 g, the fetus—from 23 to 80 g, which means that at the boundary between weeks 14–15 the mass of the placenta and fetus level out, from now on the mass of the fetus always exceeds that of the placenta. This is reciprocated by a considerable growth rate of the main fetal organs.

During weeks 17–20, as the second wave of cytotrophoblast invasion unfolds; the intermediate immature villi with an extensive network of stromal channels and numerous Hofbauer cells retain their dominant role; stromal channels exercise the function of collectors for placental macrophages. The first **intermediate mature or differentiated villi** emerge; their main distinction is that stromal channels have disappeared as a result of fibroblast proliferation and strengthening of the collagen base of the stroma. The average size of villi decreases to 150 μm while the total area of the villous tree increases to 1.48 m^2, and the average epithelium-capillary distance amounts to 22.4 μm. The outcome of the second wave of cytotrophoblast invasion is

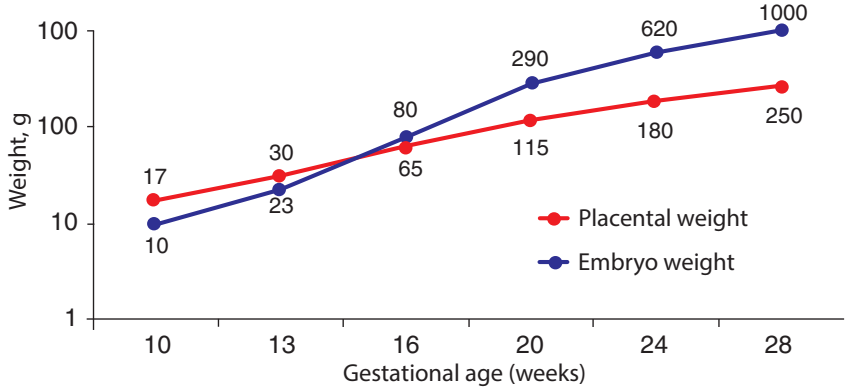


Fig. 1.16. The mass of the placenta and embryo at different gestation ages

that the fetus has almost doubled its mass (130 g at 17 weeks, and 250 g at 20 weeks) while the gain in placental mass is rather small (80 g at 17 weeks, 115 g at 20 weeks).

Weeks 21–24 of gestation are marked by a rapid gain in fetal weight from 300 to 600 g while the placental weight gain is insignificant: from 150 to 180 g. Progressive longitudinal growth of stem and intermediate differentiated villi is noted; the number of small villi 50–80 μm in diameter is not great. However, the average epithelial-capillary distance becomes 20 times shorter than at the previous gestational age (from 0.6 to 0.89 μm). Besides, the total area of villi surface continues to grow and amounts to 2.81 m^2, which undeniably affects the diffusion ability of the placenta and promotes the intensive increase in the size and weight of the main fetal organs.

During weeks 25–28 the placental weight increases from 110 to 250 g. **Intermediate differentiated branches** predominate where small **terminal villi** appear. The size of intermediate villi is 80–100 μm; that of terminal villi — only 40–60 μm. The capillary network is represented by wide sinusoids incorporated into the epithelial layer; in small areas they adjoin the thinned-out denucleated syncytiotrophoblast. In this way the first fragments of true placental barrier are formed. Thus, by the end of the second trimester (28 weeks):

- weight of placenta is 250 g;
- fetal weight is four times greater than the placental weight (1100 g);
- the villous tree is represented by a ramified system of supporting and intermediate differentiated villi and the first generations of terminal branches;
- the high synthetic activity of syncytiotrophoblast persists;
- the aggregate area of villi is on the increase.

1.4. PLACENTAL PHYSIOLOGY

The placenta provides the fetus with the required nutrition, fluid and oxygen. It also eliminates fetal waste; it produces a host of proteins and steroid hormones needed in pregnancy. It is quite apparent that the placenta is not just a passive viaduct for passage of various substances; it is an organ that plays the key role in providing for fetal growth and development.

Placental blood flow. The placenta transports gases and solutions of many substances in both directions achieving the needed concentrations in the intervillous space (maternal blood flow) and in fetal capillary blood. The blood flow velocity in these two systems determines how well the fetus is supplied with oxygen and nutrients.

Uterine blood flow. Apart from the myometrium and endometrium, the uterine artery supplies blood to the placenta in pregnancy, which takes up about 90% of the total uterine blood flow in a term pregnancy. In uncomplicated singlet pregnancy the maternal blood flow increases more than 50 times.

Placental metabolism. This very special role of the placenta is due to its intensive metabolism. For instance, the placenta on the whole consumes as much oxygen as the fetus does, and in terms of total weight it consumes much more [10 ml/(min × kg)]. At the age of 22–36 weeks placental weight increases 4–5 times. **Glucose** is the main component of oxidation processes occurring in placental tissues. The placenta consumes up to 70% of total glucose coming from the mother. Besides, a consider-

able portion of glucose comes to the placenta from the fetus. Despite the fact that 1/3 of placental glucose can transform to free lactate, placental metabolism is not of the anaerobic type. Most probably this lactate is fetal energy material. The factors responsible for rapid changes in the consumption of oxygen and glucose by the placenta have not been studied well enough; the same can be said about our understanding of the mechanism regulating the placenta development. Lately there was an important breakthrough in the study of gene influence on the processes of development and differentiation of placental tissues. In late pregnancy there is an increase in cellular mass of the trophoblast, which exceeds the increase in the mass of endothelial cells in the capillaries of the villous tree. However it is not yet known whether the proliferation of trophoblast cells is primary or it depends on proliferation of endothelial cells. In term pregnancy the mass of the placenta amounts to 500 g which accounts for 1/7 of the mass of a term fetus. In some clinical studies it was noted that there is an association between decreased oxygen saturation of tissues and increased placental weight. This means that a large placenta mostly occurs in pregnancy complicated with maternal anemia, in fetal hemolytic disease, in fetal hydrops due to fetal α-thalassemia. A large placenta is also seen in diabetes mellitus, perhaps resulting from insulin stimulating the mitotic activity or from enhanced angiogenesis. Enlarged placenta is noted in cloned animals. It is believed that this is due to defective expression of specific imprinted genes. An association between a large placenta and increased morbidity in the neonatal period and later life was noted in humans.

Receptors for many protein hormones responsible for insulin sensitivity were found in placental tissues: insulin-like growth factors IGF-I, IGF-II, and epidermal growth factor.

Alongside with long-term changes of maternal blood flow, the maternal cardiac output volume doubles and the maternal circulating blood volume increases by 40%. Such a considerable increase in maternal blood flow occurs on the account of two factors: development of the placenta and expansion of maternal arterial bed. Alongside the growth of the fetus and placenta, the volume of intervillous space almost triples at 22–36 weeks gestation. Thus alongside increased placental diffusion capacity there is a marked development of maternal placental vascular bed. Second, enhancement of blood flow proceeds partially due to estrogen-driven direct induction of vasodilation in the uterine blood flow system. This effect is induced by the action of nitric oxide. As a result of these cumulative effects, uterine blood flow in term pregnancy amounts to 750 ml/min, which amounts to 10–15% of maternal cardiac output.

The uterine artery is nearly always in the state of almost complete relaxation. However, it is susceptible to some short-term regulatory influences, too. For instance, systemic administration of vasodilator drugs can lead to a decrease in maternal blood flow.

1.5. UMBILICAL CORD DEVELOPMENT

The umbilical cord provides connection between the fetus and the placenta; the umbilical cord develops side by side with the growth of the placenta. Early development of the body stalk proceeds in close cooperation with the yolk sac, allantois and their vessels, that is why at the end of the first trimester and at the beginning of the

second trimester the fetus still retains partly reduced extraembryonic ducts — vitelline and allantoic ducts, urachal remnants — in the proximal segment at the umbilical ring. The allantoic duct is placed between two umbilical arteries; it is lined with a layer of epithelial cells on a thin basal layer without the surrounding muscular coat. Sometimes signs of epitheliocyte secretion are noted, but in most cases the allantoic epithelium is at the stage of structural involution. Obliteration of the vitelline duct is complete by 10 weeks of pregnancy as a rule, obliteration of the allantoic duct — by the age of 5–6 months of pregnancy. Accessory vessels of a small size are noted occasionally in the abdominal part of the umbilical cord; these vessels are of venous or capillary type, they belong to the rudiments of the now-defunct vascular system of the yolk sac. Starting at 9 weeks the umbilical cord becomes tortuous; it grows in length quite rapidly (Fig. 1.17).

NB! In the second trimester the umbilical cord shows the typical structure of a wire covered by simple epithelium and having a stroma represented by Wharton's jelly.
Two arteries and a vein run along the cord

One can measure the umbilical length as early as in the first trimester of pregnancy when the size of the embryo is small compared with the amniotic cavity volume. The umbilical length at this stage is comparable with the crown-rump length that can be measured by ultrasound.

Surprisingly enough Leonardo da Vinci stated that «... the length of the umbilical cord equals the fetus length at the given gestational age». This statement is true with a minor adjustment: the umbilical cord is somewhat longer than the fetus (Table 1.1).

The diameter of umbilical vessels prior to 30–32 weeks of gestation grows in a uniform, linear fashion, and then the growth practically stops. Starting at the age of 34–35 weeks the umbilical diameter decreases progressively, and at 41–42 weeks of gestation the average umbilical diameter is the same as at the gestational age of 27 weeks (15.5 and 15.0 mm, correspondingly). The progressive decrease in umbili-

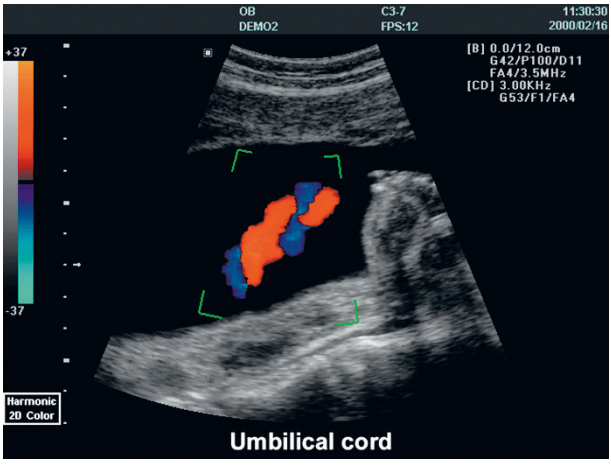


Fig. 1.17. Twisting of umbilical vessels. Ultrasound color flow mapping