

# From Trophoblast to Human Placenta

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### Glossary

amnion	the inner layer of the external membranes in direct contact with the amnionic fluid.
chorion	the outer layer of the external membranes composed of trophoblasts and extracellular matrix in direct contact with the uterus.
chorionic plate	the connective tissue that separates the amnionic fluid from the maternal blood on the fetal surface of the placenta.
chorionic villous	the final ramification of the fetal circulation within the placenta.
cytotrophoblast	a mononuclear cell which is the precursor cell of all other trophoblasts.
decidua	the transformed endometrium of pregnancy
intervillous space	the space in between the chorionic villi where the maternal blood circulates within the placenta
invasive trophoblast	the population of trophoblasts that leave the placenta, infiltrates the endo- and myometrium and penetrates the maternal spiral arteries, transforming them into low capacitance blood channels.

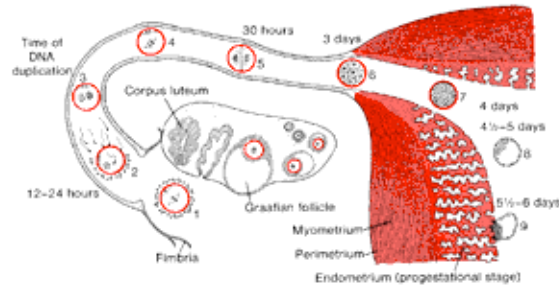
- junctional trophoblast the specialized trophoblast that keep the placenta and external membranes attached to the uterus.
- spiral arteries the maternal arteries that travel through the myo- and endometrium which deliver blood to the placenta.
- syncytiotrophoblast the multinucleated trophoblast that forms the outer layer of the chorionic villi responsible for nutrient exchange and hormone production.

## **I. Introduction**

The precursor cells of the human placenta—the trophoblasts—first appear four days after fertilization as the outer layer of cells of the blastocyst. These early blastocyst trophoblasts differentiate into all the other cell types found in the human placenta. When fully developed, the placenta serves as the interface between the mother and the developing fetus. The placental trophoblasts are critical for a successful pregnancy by mediating such critical steps as implantation, pregnancy hormone production, immune protection of the fetus, increase in maternal vascular blood flow into the placenta, and delivery.

## **II. Formation of the placenta**

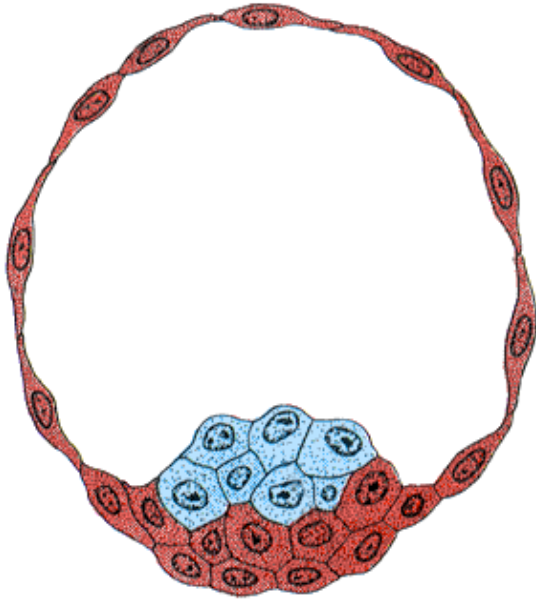
As early as three days after fertilization, the trophoblasts—the major cell type of the placenta—begin to make human chorionic gonadotropin (hCG), a hormone which insures that the endometrium will be receptive to the implanting embryo. Over the next few days, these same trophoblasts attach to and invade into the uterine lining, beginning the process of pregnancy ([Figure 1](#)). Over the next few weeks the placenta begins to make hormones which control the basic physiology of the mother in such a way that the fetus is supplied with the necessary nutrients and oxygen needed for successful growth. The placenta also protects the fetus from immune attack by the mother, removes waste products from the fetus, induces the mother to bring more blood to the placenta, and near the time of delivery, produces hormones that matures the fetal organs in preparation for life outside of the uterus.



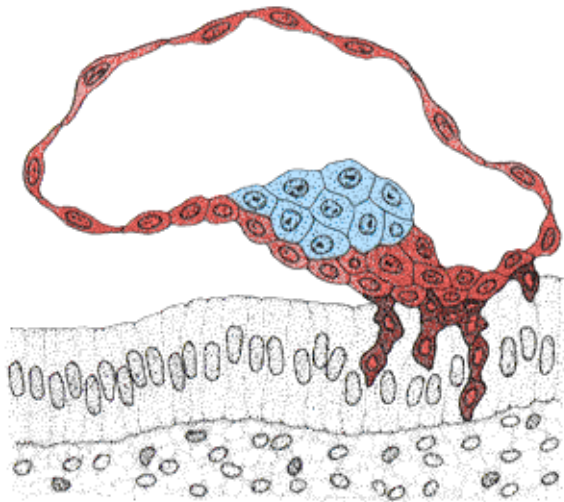
**Figure 1. From ovulation to implantation.** Ovulation occurs around day 14 of the menstrual cycle, followed by fertilization within 24 hours. The first three days of development occur within the fallopian tube. Upon arrival within the uterus the conceptus has developed into a blastocyst (see figure 2) and has already begun to make mRNA for human chorionic gonadotropin, the first hormone signal from the early embryo. By day 6 after fertilization the blastocyst has initiated implantation into the maternal endometrium. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

#### *A. Early Development*

Within a few days of fertilization the embryo develops into a blastocyst, a spherical structure composed on the outside of trophoblasts and on the inside of a group of cells called the inner cell mass (Figure 2). The inner cell mass will develop into the fetus and ultimately, the baby. In addition to making hCG, the trophoblasts mediate the implantation process by attaching to, and eventually invading into the endometrium (Figure 3).



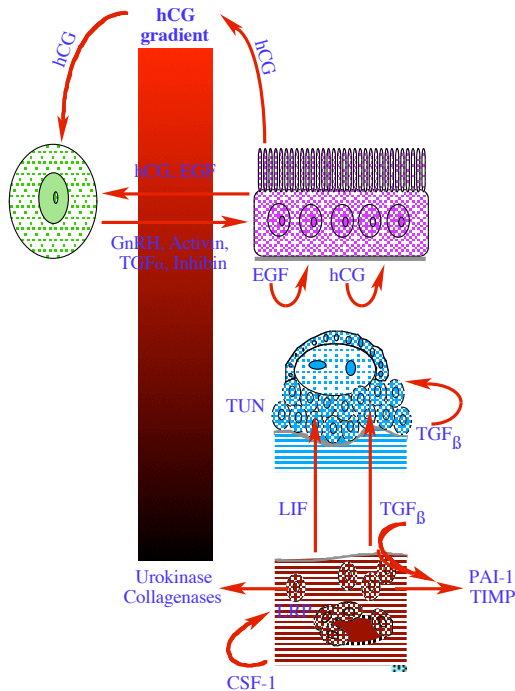
**Figure 2. Blastocyst.** By 4-5 days after fertilization the embryo has differentiated into two distinct cell types: inner cell mass (lighter cells)— which will develop into the fetus and eventually become the newborn and trophoblasts (darker cells)— which will develop into the placenta and external membranes. Even by this stage the trophoblasts have begun to make their hallmark hormone: human chorionic gonadotropin (hCG)— the hormone of pregnancy-test fame. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)



**Figure 3. Implantation.** The most difficult hurdle for the floating blastocyst is to become attached to the uterine lining (endometrium). Like trying to dock a tanker coming into port, the blastocyst is first slowed down by long molecules that extend from the endometrium (mucins), followed by a cascade of molecules that bring the trophoblasts into closer and closer contact with the endometrium. Once intimate contact is made the trophoblasts begin to invade into the endometrium, beginning the process of placentation. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

Implantation is regulated by a complex interplay between trophoblasts and endometrium. On the one hand trophoblasts have a potent invasive capacity and if allowed to invade unchecked, would spread throughout the uterus. The endometrium, on the other hand, controls trophoblast invasion by secreting locally acting factors (cytokines and protease inhibitors), which modulate

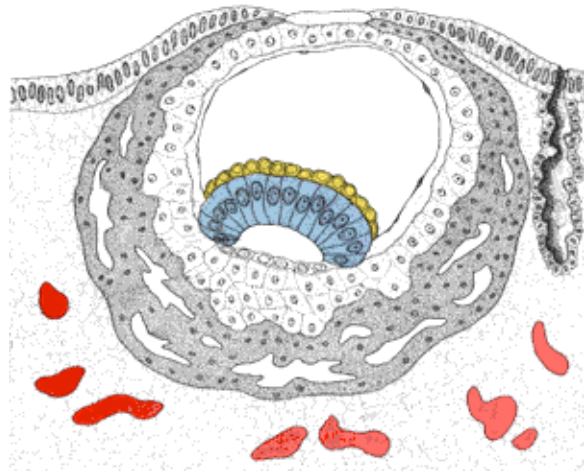
trophoblast invasion. Ultimately, normal implantation and placentation is a balance between regulatory gradients created by both the trophoblasts and endometrium (**Figure 4**).



**Figure 4. Regulation of trophoblast invasion by an hCG gradient.** Within the placenta the syncytiotrophoblasts generate high levels of hCG which shifts cytotrophoblast differentiation towards a non-invasive hormone secreting villous-type trophoblast. The closer the trophoblasts are to the endometrium the less hCG is made, allowing the trophoblasts to differentiate into anchoring type cells which make the placental glue protein trophouteronectin. Trophoblasts that leave the placenta and migrate within the endo and myometrium are induced to make proteases and protease inhibitors, presumably to facilitate trophoblast invasion into the maternal tissues.

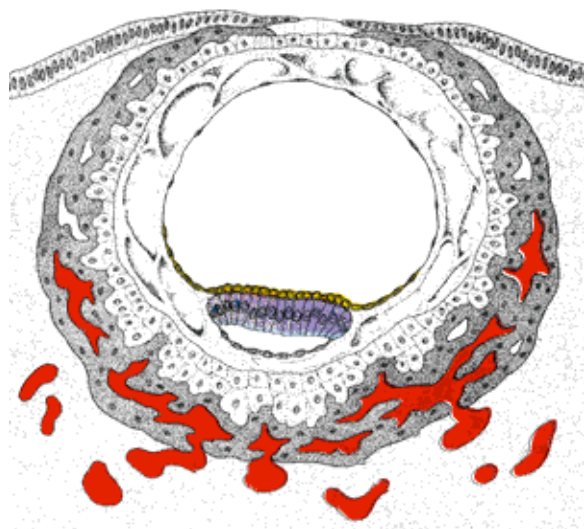
### *B. Formation of the early placenta*

Once firmly attached to the endometrium the developing conceptus grows and continues to expand into the endometrium. One of the basic paradigms which is established even within the first week of gestation is that the embryonic/fetal cells are always separated from maternal tissues and blood by a layer of cytotrophoblasts (mononuclear trophoblasts) and syncytiotrophoblasts (multinucleated trophoblasts) (**Figure 5**). This is critical not only for nutrient exchange, but to protect the developing fetus from maternal immunologic attack.



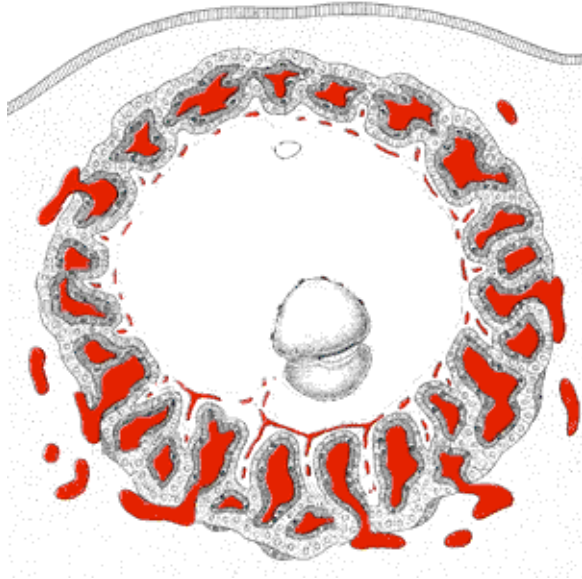
**Figure 5. Day 9 implantation site.** By nine days the embryo is surrounded by two layers of trophoblasts: the inner mononuclear cytotrophoblasts and the outer multinucleated syncytiotrophoblast layer. This arrangement of embryo, trophoblasts and maternal tissue remains the paradigm throughout gestation. This trophoblast interface not only serves as the means to extract nutrients from the mother, but protects the embryo and fetus from maternal immunologic attack. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

Within the first 2 weeks of development, the invading front of trophoblasts has penetrated the endometrial blood vessels, forming intertrophoblastic maternal blood filled sinuses (Figure 6). The trophoblastic shell continues to grow and develop until by 3 weeks after fertilization the earliest evidence of fetal circulation can be identified, and with that, the earliest evidence of the chorionic villi formation (Figure 7).



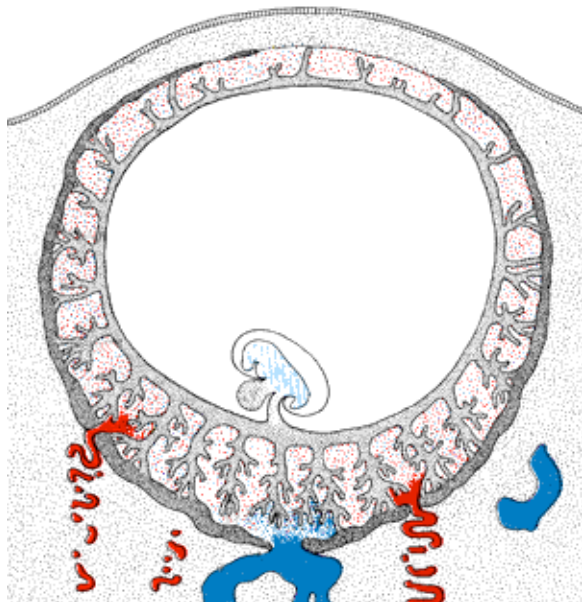
**Figure 6. Day 12 implantation site.** The invading trophoblasts have penetrated the maternal vessels, forming pools of maternal blood which surround the growing trophoblasts. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)





**Figure 7. 3 weeks implantation site.** Still barely perceptible to the naked eye, the embryo has already begun to make an early circulatory system. Again, embryonic tissue and maternal blood are separated by a layer of cytotrophoblasts and syncytiotrophoblasts. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

In spite of the fact that the entire conceptus is less than 2 cm in diameter at four weeks, the basic structure of the mature placenta has been laid out: a fetal circulation that terminates in capillary loops within chorionic villi which penetrate a maternal blood-filled intervillous space which is supplied by spiral arteries and drained by uterine veins (Figure 8).



**Figure 8. 4 weeks implantation site.** The basic structure of the placenta has been formed with maternal blood being delivered to the forming placenta via spiral arteries while being drained away via uterine veins. Like the roots of a tree, the developing chorionic villi remain immersed in a space filled with the nutrient rich maternal blood. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

The chorionic villi closest to the maternal blood supply will continue to develop and expand into a mass of chorionic tissue which we identify as the placenta. The chorionic villi farthest

away from the maternal blood supply are slowly pushed into the uterine cavity by the expanding amniotic sac which surrounds the embryo. These villi eventually degenerate and form the chorionic layer of the external membranes. At around 20 weeks of gestation the combined amnion–chorion membrane makes contact with the opposite side of the uterus, where it fuses with the decidualized maternal endometrium, forming the complete external membrane consisting of amnion, chorion and decidua layers.

### III. Structure and function of the placenta

#### A. Basic structure

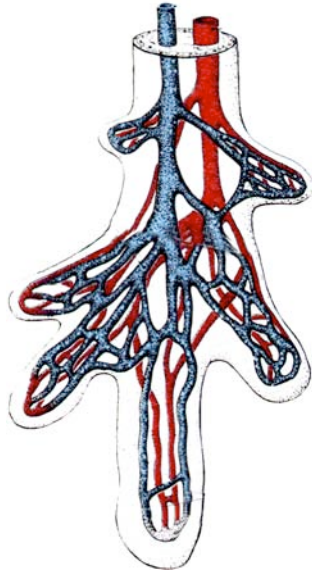
The placenta is the fetus' extension into the mother, where it functions as the interface between the two. Like the radiator of a car—which is a heat exchanger—the placenta is a nutrient and waste exchanger. The fetal circulation enters the placenta much like the water of an automobile engine enters the radiator—via the umbilical arteries embedded within the umbilical cord. Once in the placenta, the fetal circulation branches into units called cotyledons, structures similar to inverted trees (Figure 9). The finest branches of the fetal circulation are made up of capillary loops within the chorionic villi (Figure 10). Once nutrients have been absorbed and waste products released, the fetal blood ultimately collects into the umbilical vein, where it returns to the fetus via the umbilical cord.



**Figure 9. Maternal and fetal circulations within the placenta.** Maternal blood is fountained into the placenta through the uterine spiral arteries where it circulates around the chorionic villi—much like ocean water circulating around sea anemone. The fetus pumps blood into the placenta via two umbilical arteries that branch over the fetal surface of the placenta. The fetal arteries then dive into the placental mass, continuously branching until the blood reaches the capillary loops of the chorionic villi—much like a branching tree with leaves. (Modified from Moore KL, The developing human,



5th edition, WB Saunders, 1993, with permission.)

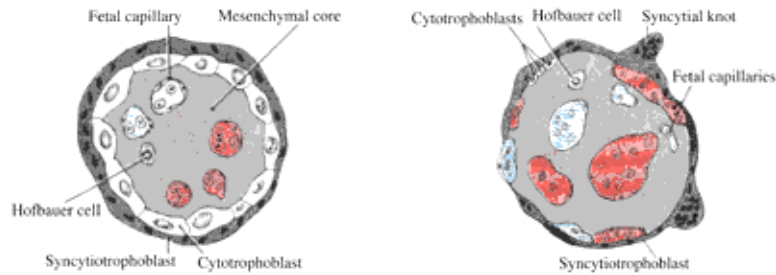


**Figure 10. Terminal chorionic villous.** The fetal circulation branches until it reaches the capillaries of the chorionic villi (Latin for leaf) where exchange of nutrients takes place between the mother and fetus. Like a growing branch, new villous branches bud off of the larger villi to increase the mass and exchange surface area of the placenta. (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

If the fetal circulation is analogous to the circulating water in an engine, the maternal circulation is analogous to the cool air rushing by the fine fins of the radiator. The maternal blood enters the placenta via the spiral arteries of the uterus. At the point where the spiral arteries make contact with the placenta, they end in open channels, fountaining maternal blood into the intervillous space (Figure 9). The intervillous blood is returned to the maternal circulation via drain-like uterine veins. In order to support the developing fetus, especially at term, up to 35% of the maternal blood flow courses through the intervillous space.

The finger-like chorionic villi are the main functional units of the placenta (Figure 10)—mediating nutrient absorption, waste elimination and generating the bulk of the hormones produced by the placenta during pregnancy. Cross sectioning a chorionic villous reveals the basic components of this part of the placenta (Figure 11). Towards the end of the first trimester a cross section of a chorionic villous reveals a central mesenchymal core with embedded fetal capillaries surrounded by a layer of cytotrophoblasts and syncytiotrophoblasts (Figure 11A). At term a chorionic villous cross section reveals the same basic structure with some noteworthy differences (Figure 11B). A term chorionic villous exhibits increased numbers of fetal capillaries, some of which are very close to the outer edge of the villous to facilitate nutrient exchange. The syncytiotrophoblast layer, a flat multinucleated cell sheet for most of pregnancy, develops grape-

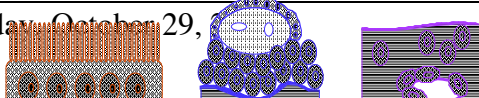
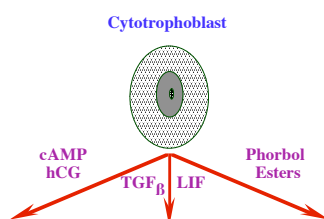
like nucleated clusters within its cytoplasm called syncytial knots near term. Although there are fewer cytotrophoblasts visible at term, they are still present, as they have been throughout gestation, functioning as the precursor cells for all the other trophoblast types.



**Figure 11. Diagrammatic cross sections of first (A) and third (B) trimester chorionic villi.** The villous core of villi from all gestational ages beyond 5 weeks of gestation contains fetal capillaries embedded in a loose matrix which contains fibroblasts and macrophages (also called Hofbauer cells). In the first trimester a villous cross section (A) reveals two distinct trophoblast layers, the outer syncytiotrophoblast layer which is in direct contact with maternal blood and the inner cytotrophoblast layer, the source of new trophoblasts. A cross section of a third trimester villous still exhibits a distinct syncytiotrophoblast layer, but fewer cytotrophoblasts can be identified, although they persist throughout pregnancy. (See also figures 13 and 14). (Modified from Sadler TW, Langman's Medical Embryology, 5th edition, Williams & Wilkins, 1985, with permission.)

### B. Trophoblast differentiation pathways

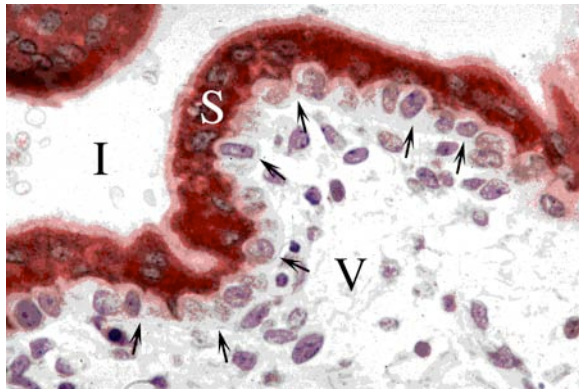
Concomitant with the overall development of placental architecture is the differentiation of three distinct trophoblast types. Depending on their subsequent function *in vivo*, undifferentiated cytotrophoblasts can develop into 1) hormonally active villous syncytiotrophoblasts, 2) extravillous anchoring trophoblastic cell columns, or 3) invasive intermediate trophoblasts (Figure 12). Within the villi of the human placenta—at all gestational ages—there always exists a population of cytotrophoblasts which remain undifferentiated and available for differentiation as necessary.



**Figure 12. Pathways of trophoblast differentiation.** Just as the undifferentiated basal layer of the skin gives rise to differentiated keratinocytes, the cytotrophoblast—the stem cell of the placenta—gives rise to the differentiated forms of trophoblasts. Left) Within the chorionic villi, cytotrophoblasts fuse to form the overlying syncytiotrophoblast. The villous syncytiotrophoblast makes the majority of the placental hormones, the most studied being hCG. Cyclic AMP and its analogs, and more recently hCG itself, have been shown to direct cytotrophoblast differentiation towards a hormonally active syncytiotrophoblast phenotype. Center) At the point where chorionic villi make contact with external extracellular matrix (decidual stromal ECM in the case of intrauterine pregnancies), a population of trophoblasts proliferates from the cytotrophoblast layer to form the second type of trophoblast—the junctional trophoblast. The junctional trophoblasts make a unique fibronectin—trophouteronectin (TUN)—that appears to mediate the attachment of the placenta to the uterus. TGF $\beta$ , and more recently, leukemia inhibitory factor (LIF), have been shown to downregulate hCG synthesis and upregulate TUN secretion. Right) Finally, a third type of trophoblast differentiates towards an invasive phenotype and leaves the placenta entirely—the invasive intermediate trophoblast. In addition to making human placental lactogen, these cells also make urokinase-type plasminogen activator (u-PA) and type 1 plasminogen activator inhibitor (PAI-1). Phorbol esters have been shown to increase trophoblast invasiveness in *in vitro* model systems and to upregulate PAI-1 in cultured trophoblasts.

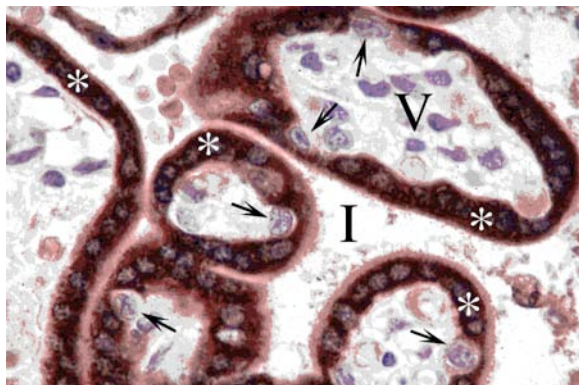
### 1. Villous syncytiotrophoblast

Within the chorionic villi, cytotrophoblasts fuse to form the overlying syncytiotrophoblast. The villous syncytiotrophoblast makes the majority of the placental hormones, the most studied being human chorionic gonadotropin. Human chorionic gonadotropin (hCG) (Figure 13) is critical to pregnancy since it rescues the corpus luteum from involution, thus maintaining progesterone secretion by the ovarian granulosa cells. Its usefulness as a diagnostic marker of pregnancy stems from the fact that it may be one of the earliest secreted product of the conceptus. Researchers have demonstrated by *in situ* hybridization that  $\beta$ -hCG transcripts are present in human blastocyst trophoblasts prior to implantation. Placental production of hCG peaks during the tenth to the twelfth week of gestation, and tends to plateau at a lower level for the remainder of pregnancy.



**Figure 13. Immunohistochemical identification of hCG in first trimester chorionic villi.** The villous core (V) is covered by two layers of trophoblasts. The intensely stained syncytiotrophoblast layer (S) is in direct contact with the intervillous space (I) which contains the maternal blood. Underlying the syncytiotrophoblast layer is a continuous layer of cytotrophoblasts (arrows), the proliferative cell type of the placenta.

Human placental lactogen (hPL) is a potent glycoprotein made throughout gestation, increasing progressively until the 36th week, where it can be found in the maternal serum at a concentration of 5-15  $\mu\text{g/ml}$ , the highest concentration of any known protein hormone. The major source of hPL appears to be the villous syncytiotrophoblasts, where it is made at a constant level throughout gestation. In addition to the villous syncytiotrophoblast, hPL has been identified in invasive trophoblasts during the first trimester. hPL appears to regulate the lipid and carbohydrate metabolism of the mother (Figure 14). Placental researchers have also demonstrated by immunohistochemical studies that villous syncytiotrophoblasts contain prolactin, relaxin and chorionic adrenocorticotropin, an ACTH-like protein. The physiological role of placental ACTH is unclear, but as with other placental hormones, all of these hormones may represent a shift from maternal to placental control.



**Figure 14. Immunohistochemical identification of hPL in third trimester chorionic villi.** The villous cores (V) are surrounded by a continuous layer of syncytiotrophoblasts (\*) which are in direct contact with the intervillous space. Underlying the syncytiotrophoblasts are scattered cytotrophoblasts (arrows). Although fewer in number during the third trimester, cytotrophoblasts are still easily identified, especially when the overlying syncytium

is stained so as to highlight the less differentiated, hormonally negative cytotrophoblasts.

The significance of placental elaboration of progesterone was revealed when it was demonstrated that bilateral oophorectomy between 7 and 10 weeks of gestation had little impact on the conceptus or urinary pregnanediol levels. More recently, researchers have been able to directly demonstrate progesterone secretion by cultured term trophoblasts. The placenta does not have all the necessary enzymes to make estrogens from cholesterol, or even progesterone. Human trophoblasts lack  $17\alpha$ -hydroxylase and therefore can not convert  $C_{21}$ -steroids to  $C_{19}$ -steroids, the immediate precursors of estrogen. To bypass this deficit, dehydroisandrosterone sulfate from fetal adrenal is converted to estradiol- $17\beta$  by trophoblasts. Not surprisingly, trophoblasts contain the necessary enzymes to make this conversion, namely sulphatase,  $3\beta$ -hydroxysteroid dehydrogenase/ $\Delta^5 \rightarrow 4$ -isomerase ( $3\beta$ HSD), and aromatase.

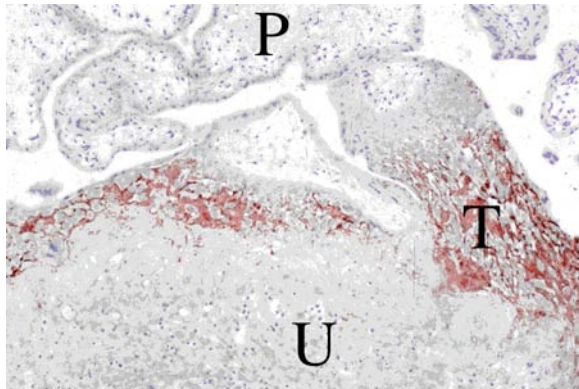
The placenta also appears to produce a number of hypothalamic-pituitary hormones, including GnRH and CRH. GnRH was first identified within villous cytotrophoblasts by immunochemical staining of intact placentae. Corticotropin releasing hormone (CRH) is also made and secreted by cultured trophoblasts and that glucocorticoids stimulate this secretion. In addition to the hypothalamic factors GnRH and CRH, pituitary growth hormone is synthesized and secreted by first and third trimester cultured trophoblasts. It appears from these studies that the placenta, in addition to replacing much of the women's pituitary function during pregnancy, also replaces critical hypothalamic functions so as to maintain control and feedback loop mechanisms close to the conceptus.

## 2. Anchoring trophoblasts

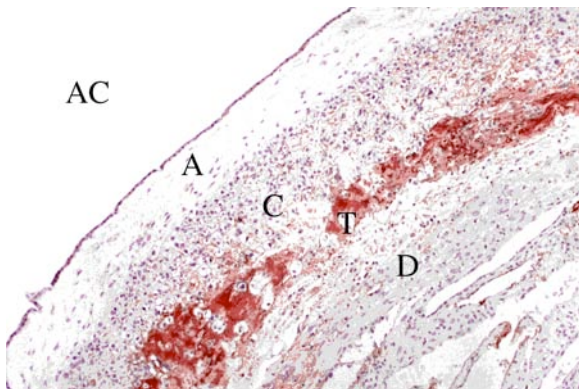
At the point where chorionic villi make contact with the uterus, a population of trophoblasts proliferates from the cytotrophoblast layer to form the second type of trophoblast—the junctional trophoblast. Like the pylons of a bridge that attach to a river bed, these cells form the anchoring cell columns that can be seen at the junction of the placenta and endometrium throughout gestation (Figure 15). Similar trophoblasts can be seen at the junction of the chorion layer of the external membranes and the decidua (Figure 16). The junctional trophoblasts make a unique fibronectin—trophouteronectin (TUN)—that appears to mediate the attachment of the placenta to



the uterus. Transforming growth factor beta ( $TGF_{\beta}$ ), and more recently, leukemia inhibitory factor (LIF), have been shown to downregulate hCG synthesis and upregulate TUN secretion. The premature loss of attachment of the developing conceptus or placenta to the uterus can terminate the gestation. Therefore, the anchoring trophoblast cell columns and the extracellular matrix proteins that promote this attachment are critical to the developing pregnancy.



**Figure 15.** Trophouteronectin (pregnancy glue) expression by the junctional trophoblasts (T) that mediate the attachment of the placenta (P) to the uterus (U).



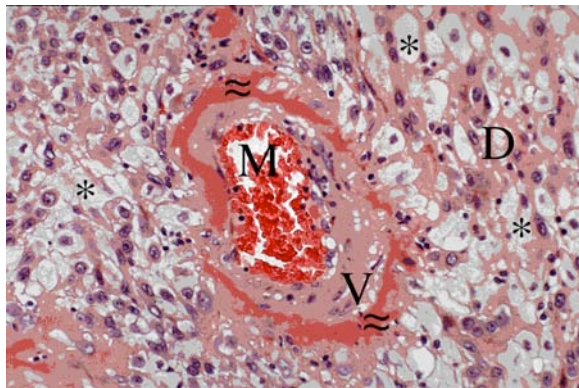
**Figure 16.** Trophouteronectin (pregnancy glue) expression by membrane trophoblasts (T). The external membranes consist of two layers: amnion epithelium and its underlying connective tissue (A) and chorion trophoblasts (C). Where the chorionic trophoblasts make contact with the maternal decidua (D), a layer of trophouteronectin can be identified (T). Amniotic cavity (AC).

### 3. Invading trophoblasts

Finally, a third type of trophoblast differentiates towards an invasive phenotype and leaves the placenta entirely—the invasive intermediate trophoblast. Studies using human specimens three to four weeks after implantation show that as gestation progresses, these invasive populations of extravillous trophoblasts attach to and interdigitate through the extracellular spaces of the endo- and myometrium. The endpoint for this invasive behavior is penetration of maternal spiral arteries within the uterus. Histologically, trophoblast invasion of maternal blood



vessels results in disruption of extracellular matrix components and development of dilated capacitance vessels within the uteroplacental vasculature (Figure 17). Biologically, trophoblast-mediated vascular remodeling within the placental bed allows for marked distensibility of the uteroplacental vessels, thus accommodating the increased blood flow needed during gestation. Abnormalities in this invasive process have been correlated with early and mid-trimester pregnancy loss, preeclampsia and eclampsia, and intrauterine growth retardation.



**Figure 17. Invasive trophoblasts.** Uterine spiral artery (V) containing maternal blood (M) from a four week pregnancy. The maternal endometrium (D) has become decidualized, meaning that the stromal cells have been transformed into large, pale cells (\*). Infiltrating between these decidual cells are the invasive trophoblasts (some examples are highlighted by arrows) which have begun to modify the vessel wall (≈).

#### IV. Complications of pregnancy related to trophoblasts and the placenta

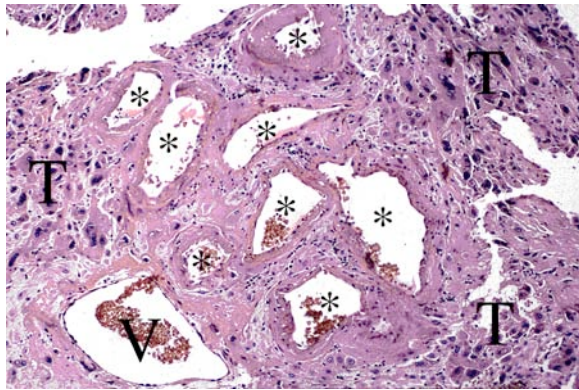
As in any complicated system, problems can arise. It is not possible to discuss all of these pathologic states of the placenta, but the three most important complications of pregnancy related to the placenta will be outlined below.

##### *A. Diseases of trophoblast invasion: preeclampsia and gestational trophoblastic neoplasia*

Preeclampsia, the clinical state prior to full blown eclampsia (seizures), is one of the ‘toxemias’ of pregnancy. The basic clinical definition is a “pregnancy-specific condition of increased blood pressure accompanied by proteinuria, edema, or both.” In spite of the simplicity of this description of these clinical signs and symptoms, the etiology of the disease has remained elusive. In fact, a seal over the University of Chicago Lying-in-Hospital remains blank awaiting the name of the person(s) who elucidate the cause of this disease. Many phenomena have been investigated, but the recurring theme appears to be an abnormally low blood flow into the

placenta. One of the difficulties has been to distinguish between primary cause and secondary effects. Part of this difficulty may be attributable to the fact that the common end result of low uteroplacental blood flow may be caused by many primary defects. Possibly, therefore, preeclampsia/eclampsia is not a disease, but a syndrome with many causes. Significantly, one of the most frequent findings in preeclampsia is decreased or absent trophoblast invasion of the maternal spiral arteries.

Decreased or absent trophoblast invasion may be a consequence of primary defects in the invasive trophoblasts or in the environment that the trophoblasts are attempting to invade. Studies have shown that in some cases of preeclampsia there are abnormalities in trophoblast function, including, but not limited to: integrin expression, glycogen metabolism, and decreased galactose alpha 1-3 galactose expression. In addition, preeclampsia has been associated with trisomy 13, the chromosome that carries the gene for type IV collagen. Placental bed biopsy in a case of preeclampsia in a multiparous woman carrying a trisomy 13 fetus showed lack of trophoblast invasion of maternal spiral arteries. These trophoblasts may have had difficulty invading through the maternal ECM because of increased type IV collagen production. In addition to primary trophoblast defects, many cases of preeclampsia appear to be related to maternal immunologic reaction against the invading trophoblasts. A common clinical finding in these cases is that the invasive trophoblasts have reached the vicinity of the spiral arteries, but have not penetrated them. In addition, the unconverted arteries are often surrounded by lymphocytes, presumably attacking the foreign-appearing invasive trophoblasts. As can be seen from a placental bed biopsy in a typical case of preeclampsia, the invasive trophoblasts have invaded through the endo- and myometrium, but have failed to complete their journey into the spiral arteries (Fig. 18). Failure to convert the maternal spiral arteries into low-resistance channels can induce the placenta to secrete vasoactive substances that leads to maternal hypertension. If the maternal blood pressure rises significantly, the spiral arteries can be damaged and may even become occluded, leading to placental infarction.



**Figure 18. Failure of invasive trophoblasts to penetrate the maternal spiral arteries.** Normally the invasive trophoblasts (T) infiltrate through the endo- and myometrium, reach the spiral arteries (\*) and convert their muscular walls into pliant channels. In cases of preeclampsia, the trophoblasts often do not complete the final arterial penetration, possibly due to the maternal lymphocytes that often surround the spiral arteries. Compensatory maternal hypertension can lead to spiral artery damage or even occlusion. Maternal uterine vein (V).

In contrast to the clinical syndrome of decreased trophoblast invasion, gestational trophoblastic disease (GTD) represents increased and uncontrolled trophoblast invasion. Expanded trophoblast invasion ranges from an exaggerated placental site with increased numbers of benign intermediate trophoblasts, to placental site trophoblastic tumors, to invasive moles, to frank choriocarcinoma. Morphologic distinction between these forms of trophoblast proliferation can be difficult, but it appears that the normal mechanisms that stop trophoblast invasion are defective in choriocarcinoma cell lines. Normal cytotrophoblast differentiation can be shifted towards a villous syncytiotrophoblast and away from an invasive trophoblast phenotype by cAMP analogues, while this treatment does not affect choriocarcinoma invasiveness, suggesting a primary defect in differentiation-signalling in the malignantly invasive trophoblast.

### *B. Infection*

More than a third of all preterm births are associated with labor initiated by acute chorioamnionitis (inflammatory infiltrates in the chorionic plate and chorion and amnion layers of the external membranes). Not only does chorioamnionitis have severe consequences for the fetus through the initiation of preterm delivery, but chorioamnionitis increases the risk for cerebral palsy by a factor of at least four. Elucidation of the role of cytokines in controlling the inflammatory process during pregnancy has facilitated the development of diagnostic tests which

can detect the earliest phases of chorioamnionitis as well as pointing the way towards effective treatment modalities for this disease process.

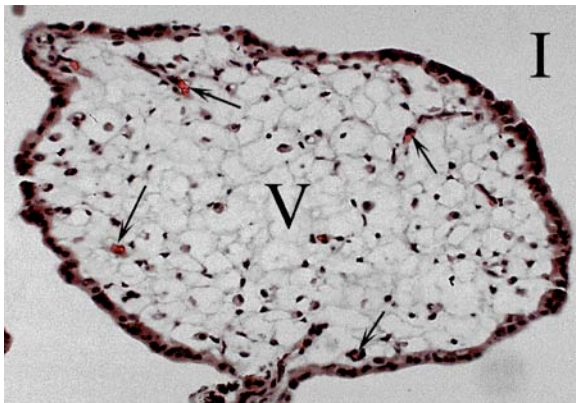
The Collaborative Perinatal Study (CPS) of the National Institute of Neurological and Communicative Disorders and Stroke followed the course of over 56,000 pregnancies in the United States between 1959 and 1966. In the CPS, more than a third of all preterm births were associated with labor initiated by acute chorioamnionitis. This study also revealed that acute chorioamnionitis was the most frequent cause of stillbirth and neonatal death. Chorioamnionitis does not only have severe consequences for the fetus through the initiation of preterm delivery but may—through the initiation of the inflammatory cascade in the placenta and decidua—have direct deleterious effects on the fetus. The CPS showed clearly that acute chorioamnionitis was followed by a 20% greater-than-expected frequency of neurologic abnormalities at 7 years of age.

Infections of the amniotic fluid arise by a variety of routes—including from the abdominal cavity through the fallopian tube, via the maternal blood stream through the placenta, or iatrogenically following amniocentesis or funipuncture—but the most common route is an ascending infection through the cervix. It is not surprising, therefore, that the most common organisms cultured from amniotic fluid are commonly found in the vagina. There are clinical reports, however, of a wide variety of organisms found to cause intrauterine infections, including: Group B streptococci, *Listeria monocytogenes*, *Morganella morganii*, *Ureaplasma urealyticum*, Herpes simplex virus, parvovirus, *Chlamydia* species, *Capnocytophaga*, adeno-associated virus, and human immunodeficiency virus.

Once bacteria enter the amniotic fluid, they quickly multiply. Within 30 hours of the initiation of the infection, maternal neutrophils begin to be chemoattracted from the maternal circulation towards the amniotic cavity to fight off the bacterial infection. The extent to which these maternal neutrophils migrate towards the amniotic fluid has been used to estimate the severity and timing of intraamniotic infections.

The basic paradigm for inflammation induced cytokine release and leukocytic chemoattraction has been elucidated in a number of organs, and is being actively studied in the placenta. Breakdown products of the growing bacteria—especially the lipopolysaccharides (LPS) of the bacterial cell walls (endotoxin)—appear to play an important role in the initiation of this process. LPS initiates the inflammatory response by triggering the release of a cascade of

cytokines from a variety of cell types which mediate the physiological process of inflammation. Components of this process include the chemotaxis and activation of inflammatory cells—like the neutrophil—and the leaking of fluid from vascular spaces into the extracellular space—possibly in response to tumor necrosis factor alpha (TNF $\alpha$ ). In experimental models, peak TNF $\alpha$  production occurs within 1 h of LPS treatment. TNF $\alpha$  then induces the prolonged production of IL-8, a neutrophil chemoattractant and activator. In addition to chemoattracting leukocytes to the focus of inflammation, this cytokine cascade—as is succinctly described in Celsus’s first century phrase *rubor, tumor, calor, dolor*—also results in swelling due to edema. In the placenta this swelling is manifested by villous edema—fluid accumulation within the mesenchymal cores of chorionic villi of the placenta. It has been hypothesized that villous edema makes the fetus hypoxic by compressing fetal blood vessels inside the villi and by increasing the diffusion barrier for oxygen between the maternal and fetal circulations (Fig. 19). These effects make villous edema the most frequent cause of stillbirth, neonatal death and neonatal morbidity, especially cerebral palsy, in children born before 28 weeks.



**Figure 19. Severe villous edema following induction of intrauterine inflammation.** Cross section of a markedly edematous chorionic villous 16 hours after the initiation of an intrauterine infection. Note the very pale, fluid filled villous core (V). The edema fluid has compressed the fetal vessels (arrows) so severely that only a one or two erythrocyte cross sections can be seen in each capillary. Intervillous space (I).

### *C. Immunologic rejection*

In spite of the fact that the placenta and fetus are ‘foreign’ to the mother, most pregnancies show no evidence of ‘immunologic rejection.’ When immunologic reactions do occur, they can be against any of the components of the gestation (placenta and fetus). These reactions can occur at all stages of pregnancy, and can occur repeatedly, pregnancy after pregnancy.

Examination of hematoxylin and eosin stained histologic sections of placental, abortion or dilation and curettage material can often reveal evidence of immune damage. However, more specialized techniques are needed to reveal the specific immune cells involved in these reactions. Immunohistochemistry of formalin-fixed, paraffin-embedded tissues can be used to identify specific cell types involved in immunologic reactions of pregnancy.

In approximately 1-2% of all gestations, mononuclear cells can be seen infiltrating into the chorionic villi of the placenta. Until the work of Redline and Patterson, however, the origin of these cells had been controversial, some arguing for a fetal origin, some for a maternal origin. Since immunochemistry alone could not answer this question they utilized *in situ* hybridization for Y and X markers in male gestations to demonstrate that the lymphocytes present in cases of chronic villitis are maternally derived, allowing us to focus on the causes of this apparent maternal immunologic reaction against trophoblast and/or villous antigens. Immunohistochemistry of such cases has shown that the cells within the villous core are T-cells and macrophages.

Occasionally placentas manifest an intervillous space that is filled with mononuclear cells. When immunohistochemically stained, these cells are revealed to be monocytic/macrophage in origin. This monocytic intervillositis has been associated with IUGR, preeclampsia, recurrent pregnancy loss and intrauterine fetal demise.

Dizygotic twins offer a unique opportunity to examine maternal immunologic reactions against the placenta and fetus. Examination of dizygotic twins can sometimes reveal discordant chronic villitis between the two placentas, with concomitant discrepancies in fetal growth. Detailed analysis of each of such twins' HLA expression and cytokine expression may help to elucidate the causes of these immunologic reactions.

Although most cases of first trimester pregnancy loss are the result of genetic defects in the fetus and/or placenta, some patients have recurrent pregnancy loss due to repeated maternal immunologic reactions. These reactions can be directed against villous core antigens, against antigens of the syncytiotrophoblast surface—manifested as an intervillositis, or against invasive intermediate trophoblasts. Some have suggested a variety of therapies for these conditions, including treatment with intravenous immunoglobulins, immunization with paternal or allogenic leukocytes, or exposure to semen through vaginal or rectal suppositories. However, the scientific



basis of many of these approaches remains controversial and the efficacy of the therapies proposed have been questioned.

**Further Reading**

- Benirschke K, Kaufmann P. (1995) Pathology of the human placenta. Third edition. Springer-Verlag, New York.
- Boyd JD, Hamilton WJ. (1970) The human placenta. W Heffer & Sons, Cambridge, England.
- Feinberg R, Kliman H, Lockwood C. (1991) Oncofetal fibronectin: A trophoblast “glue” for human implantation? *American Journal of Pathology* 138:537-543
- Feinberg RF, Kliman HJ, Cohen AW. (1991) Preeclampsia, trisomy 13, and the placental bed. *Obstet Gynecol* 78:505-8
- Kliman H. (1994) Trophoblast infiltration. *Reproductive Medicine Review* 3:137-157
- Kliman H. (1994) Placental hormones. *Infertility and Reproductive Medicine Clinics of North America* 5:591-610
- Kliman H, Nestler J, Sermasi E, Sanger J, Strauss J. (1986) Purification and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology* 118:1567-82
- Kliman HJ. (1993) The Placenta Revealed. *Amer J Pathol* 143:332-336
- Kliman HJ, Feinberg RF. (1990) Human Trophoblast-Extracellular Matrix (ECM) Interactions Invitro - ECM Thickness Modulates Morphology and Proteolytic Activity. *Proceedings Of the National Academy Of Scien* 87:3057-3061
- Kurman RJ, Main CS, Chen HC. (1984) Intermediate trophoblast: a distinctive form of trophoblast with specific morphological, biochemical and functional features. *Placenta* 5:349-69
- Moore KL. (1993) The developing human. Fifth edition. WB Saunders, Philadelphia.
- Naeye RL. (1992) Disorders of the placenta, fetus, and neonate: Diagnosis and clinical significance. Mosby Year Book, St. Louis.

Redline RW, Patterson P. (1993) Villitis of unknown etiology is associated with major infiltration of fetal tissue by maternal inflammatory cells. *Am J Pathol* 143:473-9

Sadler TW. (1990) *Langman's medical embryology*. Sixth edition. Williams & Wilkins, Baltimore.